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The effect of environmental factors on the physiology, yield and oil composition of safflower (Carthamus tinctorius L.)

Mohamed, Shiren Jalal

THE EFFECT OF ENVIRONMENTAL FACTORS ON THE PHYSIOLOGY, YIELD AND OIL COMPOSITION OF SAFFLOWER (CARTHAMUS TINCTORIUS L.)

by

SHIREN JALAL MOHAMED

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Copyright statement

Abstract

The effect of environmental factors on the physiology,

Yield and oil composition of safflower (*Carthamus tinctorius* L.)

This study investigated the effects of drought, nitrogen fertilizer and elevated CO_2 and its interaction with nitrogen fertilizer on the physiology, growth, and production of the oil crop safflower (*Carthamus tinctorius* L.) in a semi-controlled glasshouse environment.

Three levels of water stress were imposed: rosette (mid-season drought), stem elongation (terminal drought) and rosette to maturity (mid-season + terminal drought). Results indicated that all drought treatments imposed reduced stomatal conductance, but after the relief of mid-season drought plants recovered and as a result there were no significant differences from control in terms of yield components (branch and capital number) and seed number. Terminal drought and mid–season + terminal drought induced significant reductions in branch number (48% and 50%), in capitula number (33% and 67%), in seed number (89% and 92%), in above ground dry weight (30% and 54%) and in individual fresh seed weight (90% and 94%) respectively. However, water stress treatments had no significant effect on the maximum quantum efficiency of PSII (Fv/Fm) in dark adapted leaves compared with the control.

Levels nitrogen fertilizer was studied equivalent to 0, 25, 50, 75, 100, 125, 150, 175 kg N ha^{-1} were evaluated. Safflower responded incrementally to increasing nitrogen applied in a curvilinear asymptotic fashion. Assimilation rate (42%), transpiration rate (32%), stomatal conductance (52%) and LAI (42%) increased

up to 100 kg N ha^{-1} compared with the control. The above ground dry weight and seed yield associated with WUE continued to increase with each increment in nitrogen rate and above ground dry weight (42%), individual seed fresh weight (76%) and WUE (41%) increased up to 175 kg N ha^{-1} compared with the control.

The effect of elevated CO_2, (1000 µmol mol^{-1}) significantly increased assimilation rate (27%) reduced stomatal conductance (29%) and transpiration rate (18%), increased LAI (28%) and above ground dry weight (51%) when measured at anthesis compared with ambient (400 µmol mol^{-1}). At the same time plant organ N content was reduced. At harvest, elevated CO_2 increased above ground dry weight (42%) and individual fresh seed weight (49%).

The interaction effect of elevated CO_2 with nitrogen input was investigated using four nitrogen levels equivalent to 25, 75,125 and 175 kg ha^{-1}. The nitrogen response rate was raised by elevated CO_2 equally at each nitrogen application rate so that there was no significant interaction effect between the two for most parameters measured. In this way both CO_2 and nitrogen were acting as “fertilizers”.

Overall the results showed that despite being put forward as a drought resistant crop for low input agricultural systems safflower is capable of responding positively to well irrigate and well fertilized conditions. Furthermore under conditions of elevated CO_2 it can be expected to increase its yield potential but to achieve this will require a higher degree of nitrogen fertilization. CO_2 is capable of substituting for up to 100 kg N ha^{-1} without a decline in yield and this shows that CO_2 is the primary limiting factor in safflower assimilation.

Seed oil content and its fatty acid profile appeared to be relatively stable and were not affected drastically by either nitrogen fertilization or elevated CO_2.This demonstrated the integrity of the oil filling process during seed fill and emphasized that this is primarily under genetic control with relatively little influence from environmental parameters.

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Dedication

I dedicate this thesis to the light which inspired me to complet my study (my family) and my country Kurdistan/Iraq

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Author's Declaration

At no time during the registration for the degree of Doctor of philosophy has the author been registered for any other University award.

I declare that the work submitted in this thesis is the results of my own investigations except where reference is made to published literature and where assistance where acknowledged.

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Candidate

Director of Studies

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Presentation and conferences attended

- Oral presentation PhD Project presentation in biological symposium at University of Plymouth 9th May 2010.
- Presented a poster entitled (the effect of Varing levels of nitrogen fertilizer on safflower physiology, growth and seed yield) at the Annual meeting of the society of experimental Biology (SEB) 1- 4th July 2011. Glasgow, P187.
- Oral presentation, CARS Symposium (Centre for Agriculture and Rural Sustainability) symposium, Duchy College, Cornwall, 9th of July 2012.
- Presented a poster at Plymouth society short conference, entitled (The effect of drought on the physiology, growth, yield and seed oil content of safflower) on 18th November 2010. And awarded second prize.

Module attended

- BIO5124 Research Skills in Biological Science.

Generic skills development courses attended

- Attended the 4 week UOP academic pre- sessional English language course (from 18th August to 12th September).
- Training session- advanced Endnote 7th November 2008.
- Introduction to electronic resource on 26th November 2008.
- Presentation skill part 1 on 9th February 2009.
- Preparing effective poster 12th February 2009.
- The transfer process n 18th February 2009.
- Visualising data on 11th March 2009.
- Power Point 2007 creating presentation 18th March 2009.
- Learning teaching for general teaching association (GTA). An oral presentation of the research project was presented.
- ENV101 Laboratory basic teaching method from 30th of November to 4th December 2009.
- Fatty acid in peanut practice (3 sessions).
- Preparing effective poster presentation on 8th February 2010.
- Excel 2007 Pivot Tables& Macro on 26th March 2009.
- Preparing for the viva on 5th March 2009.

Other Activities

- Attended the seminar (critical factor informing local Regional and Global climate on 12th November at Plymouth University.
- Attended Annual General Meeting by UK Controlled Environment User's Group. Rothamsted Research Centre 15th September 2009.
- Attended a conference on Agrivision (agriculture business) 2020 Royal Showground, Wade Bridge, and Cornwall, sponsored by Clydesdale Bank and Mole Valley Farmers Ltd. 3rd April 2009.

List of Abbreviations

SRES:	Scenarios report on emissions scenarios
IPPC:	Intergovernmental panel on climate change
A:	Assimilation rate
gs:	Stomatal conductance
E:	Transpiration rate
Ci:	Intercellular CO_2 concentration (substomatal conductance)
WUE:	Water use efficiency
NUE:	Nitrogen use efficiency
PNUE:	Photosynthetic nitrogen use efficiency
ABA:	Abscisic Acid
FWC:	Field water capacity
UV-B:	Ultra-violet B Radiation
RWC:	Relative water capacity
ATP:	Adenosine triphosphate
Fv/Fm:	Variable to maximum fluorescence ratio
Vc,max:	Maximum carboxylation velocity
J max:	Maximum rate of electron transport
RuBP:	Ribulose bisphosphate
Rubisco:	Ribulose 1, 5 bisphosphate
PED:	Photon flux density
H:	Plant height
AGB:	Above ground biomass
NC:	Number of capitula
NS:	Number of seeds
FSW:	Fresh seed weight
OC:	Oil content
LNC:	Leaf nitrogen concentration
TCC:	Total chlorophyll content
LAI:	Leaf area index
SY:	Seed yield
pH:	Acidity/Alkalinity
NiR:	Nitrate reductase
GDH:	Glutamate dehydrogenase

Chapter 1

General introduction

Over the last century, atmospheric carbon dioxide has increased due to the anthropogenic activities and will continue to increase and this rise may contribute to a change in other environmental factors such as temperature and rainfall pattern (Change, 2007). Therefore, in this study the C_3 plant safflower (*Carthamus tinctorius* L.) physiology and growth response was investigated under the effect of drought, nitrogen and elevated CO_2. The following section gives a summary of reviewing literature on the impact of these factors on C_3 plant species.

1.1. Introduction

According to the scenarios report on emissions scenarios (SRES) in The Fourth Assessment of the Intergovernmental panel on climate change (IPCC) working group III " Mitigation of Climate Change" in 2007, the concentration of CO_2 could reach 850 ppm by the year 2090 a 3 fold rise pre industrial revolution times (Change, 2007). Such increases in the atmospheric CO_2 levels are likely to contribute in a direct and indirect global climate changes and have profound effects on agriculture and crop production worldwide (Reddy *et al*., 2010). This increase in CO_2 concentration and other gases in trace concentration (methane CH_4, nitrous oxide N_2O, halocarbons, ozone O_3, water vapour and aerosols) is released to the atmosphere due to anthropogenic activities involved in the burning of fossil fuel sources and absorb and reflex infrared radiation (so called long wave radiation) released from the earth's surface. The increased transit time of these radiation frequencies in the atmosphere leads to greater observance of their energy by atmospheric gases and a consequent rise in temperature which eventually will result in an increase in global temperature.

Many models of global climate change predict a rise in the mean global temperature of up to 0.07 ^{0}C per decade (Caporn and Bridget, 2009; Shi *et al*., 2010) leading to a rise of over 2 ^{0}C by 2050. As a result of rising temperature atmospheric water vapour carrying capacity of air increases and changes in annual precipitation patterns have been projected. Patterns of precipitation in eastern parts of North and South America, northern Europe and northern Asia have already been recorded over the period from 1900 to 2005 (Change, 2007). Since the 1970s wide areas, particularly in the tropics and sub-tropics, have been faced with extreme drought. Furthermore in many regions like the Mediterranean, southern Africa and parts of southern Asia long term drought has been observed (Change, 2007).

Climate change prediction and its impact on agriculture have stimulated many research studies involving scientists, economists and ecologists. Some have concluded that global food security is extremely threatened because they predict the negative impact of climate change on agriculture (Nelson, 2009) However, Mall et *al*., (2006) reported the positive impact of climate change on agriculture in some agro climatic regions such as India. Also Olesen and Bindi, (2002) stated that a positive effect of climate change might be through the introduction of new crops in northern European.

Over the past decade many system tools have been used to assess how terrestrial plants will respond or adjust to climate change for example, Free-air CO_2 (FACE) and enclosure technology have been used for studying the effect of elevated CO_2 on crop vegetation and natural ecosystem involving many C_3 and C_4 species (Ainsworth *et al*., 2002; Kimball *et al*., 2002; Poorter, 1993). It has been reported in particular that future crops will be affected by elevated CO_2,

generally in a positive way in a phenomenon referred to as carbon fertilization. Present research challenges are quantifying the magnitude of crop yield response to increasing CO_2 (Ainsworth and McGrath, 2010).

Most of the long term experiments on the climate change effect under controlled environmental conditions have been performed on temperate plant species (Garcia *et al*., 1998; Vu *et al*., 1989; Vu *et al*., 2001). Few studies have investigated the impacts of elevated CO_2 and its interaction with other climatic factors on the subtropical and tropical crops especially oil crops such as peanut (*Arachis hypogaea* L.) (Vu, 2005).

The following section describes in more detail the effect of elevated CO_2, water stress, nitrogen fertilizer, and the interaction effect of CO_2 in conjunction with these other factors on plant physiology, growth and productivity.

1.2. The effect of CO_2 on plant growth, productivity and quality

CO_2 is a fundamental input to photosynthesis giving rise to the term carbon assimilation. Plants take up CO_2 through their stomata into the leaves (Simpson and Ogorzaly, 2001) and CO_2 enters the leaves of plants due to a steep gradient of CO_2 between the atmosphere and the leaf interior. Inside the leaves by using light with water and the photosynthetic apparatus in the chloroplasts plants convert CO_2 into five carbon sugars (pentose) and polymerized and accumulated as hoaxes or carbohydrate (glucose) which is considered the most abundant mono-saccharides in nature (Eichhorn, 1999; Taiz and Zeiger, 2002). The primary enzyme in fixing CO_2 in C_3 plants is ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) which has two distinct functions:

carboxylation and oxidation. The state of the active binding site of Rubisco (i.e. whether CO_2 or O_2 is bound) depends on the relative abundance of CO_2 and O_2 and the ratio between CO_2:O_2 can favour either photosynthesis or photorespiration (Taiz and Zeiger, 2002). Therefore, it is often reported that CO_2 enrichment increased CO_2 at the carboxylation binding site of Rubisco and hence an inhibited photorespiration occurs (Andrews *et al*., 1995; Leakey *et al*., 2009). Reduced stomatal conductance due to either partial stomatal closure (Ainsworth and Rogers, 2007; Drake and Leadley, 1991; Wheeler *et al.,* 1999) or developmentally, from decreases in stomatal density (Shaw et al., 2005) is a secondary response of the plant to elevated CO_2. This is in turn leads to reduced transpiration and enhanced water use efficiency (WUE) (Bowes, 2004) the ratio of the amount of biomass produced to the total amount of water consumed through transpiration (Hsiao and Jackson, 1999a; Hsiao and Jackson, 1999b). As a result of these biochemical and physiological changes in plant growth is expected in almost all cases to increase, but the magnitude of this response differs between different crops (Ainsworth and Long, 2005; Poorter, 1993).

A meta-analysis of free-air CO_2 experimental (FACE) results concluded that elevated CO_2 enhanced light-saturated photosynthesis (in C_3 plants increased by an average of 31% and reduced stomatal conductance by 22% (Ainsworth and Rogers, 2007). As an example, Garcia *et al*., (1998) reported that for spring wheat grown from emergence to grain maturity under elevated CO_2 using FACE. Elevated CO_2 increased the seasonal midday photosynthesis by an average of 28%, and the seasonal average of the daily essential photosynthesis by 21% compared to ambient CO_2 and reduced stomatal conductance by 36% at

midday and throughout the growth period. Also little evidence of acclimatory loss of leaf photosynthesis was observed with elevated CO_2.

Among the agricultural crops, C_4 crop species show less response to changes in atmospheric CO_2 than C_3 crop species (Kimball *et al*., 2002) because with the C_4 pathway of photosynthesis the CO_2 initially combines phosphoenol pyruvate to form malate or aspartic acid (4-carbon acid) which are translocated to bundle sheath cells, where carboxylation occurs again. As a consequence low concentrations of CO_2 saturate photosynthesis in C_4 plants (Allen Jr, 1990). Furthermore C_4 species have developed a mechanism that lead to a higher CO_2 concentration at the carboxylation site of Rubisco and overcomes photorespiration (Ehleringer, 2005). Kimball *et al*., (2002) by using the reports from experiments on several C_3 and C_4 crops concluded that elevated CO_2 increased the photosynthesis and consequently, increased the plant biomass and economic yield significantly in C_3 plants but little in C_4 plants. In both species however stomatal conductance increased with elevated CO_2 and as a result water use efficiency (WUE) markedly improved in all the crops they studied.

In addition, among the C_3 species herbaceous dicotyledons show a larger response than monocotyledons (Poorter, 1993) and inherently fast growing species are generally more responsive than slow growing species (Poorter and Perez-Soba, 2002). Furthermore, plants with strong sink capacity such us crop and competitive non-crop species have the greatest response to CO_2 enrichment with average 30-40% biomass stimulation (Bowes, 1996).

In addition, reduced transpiration at the canopy results from reduced stomatal closure under elevated CO_2 and leads to an increase in leaf internal CO_2 concentration on one hand, and under long term exposure higher root biomass is possible, and this contributes to higher water availability and by these mechanisms the plant water status and leaf water potential under elevated CO_2 improves and leads to higher rate of net assimilation rate and leaf growth (Grossman-Clarke *et al*., 2001). Moreover, the decrease in leaf level stomatal conductance in response to elevated CO_2 allows plants to maintain (WUE). Recently, Prior *et al*., (2010) reported that the long term exposure to 750 µmol mol^{-1} CO_2 increased photosynthesis in soybean (*Glycine max*) by 50% and in sorghum by 15% as a result of increasing water use efficiency due to reduced transpiration rate.

In contrast, although there is a reduction in transpiration rate resulting from partial stomatal closure in response to doubling CO_2 it also leads to a rise in leaf temperature and ultimately leads to an increase in transpiration rate offsetting the effects of stomatal closure (Allen, 1998).

Whole plant photosynthetic rate is strongly related to LAI, as LAI determines the amount of light intercepted (Gastal and Lemaire, 2002) and both leaf area and specific leaf area ratios change with rising CO_2. Many recent studies suggest that canopy photosynthesis shows a significant increase with increasing LAI under elevated CO_2 (Campbell *et al*., 2001; Rodriguez *et al*., 2001). However, in some cases elevated CO_2 decreased leaf area (Ainsworth and long, 2005) or there was no difference in leaf area and LAI between ambient and elevated CO_2 (Yoon *et al*, 2009). An increase in LAI at the canopy level at elevated CO_2 also provides a greater surface for transpiration and increased LAI should lead to

increased assimilation rate but at the expense of transpired water i.e. decreased water use efficiency (Allen Jr, 1999) However, it has been frequently reported that elevated CO_2 increased both LAI and water use efficiency (WUE) and led to an increase in photosynthesis rate as a consequence biomass and productivity increased (Carlson and Bazzaz,1980; Lawlor and Mitchell, 1991) Pooter and Perez-Soba (2002) and Warrick (1988) reported that plant dry matter production dramatically increased due to enhancing photosynthetic rate and reducing transpiration. Ultimately, as result of increased crop growth and biomass allocated towards the sink the grain yield increased at elevated CO_2 (Hogy and Fangmeir, 2008; Wu *et al*., 2004). Jablonski *et al*., (2002) synthesized data from 79 crop and wild species reports and found across all species at elevated CO_2 (from 500-800 µmol mol^{-1}) CO_2 resulted in producing more flowers (+19%), fruits (+18%) more seeds (+16%) greater individual seed weight (+4%) and greater total seed yield (+25%).

Alongside increasing crop growth and yield at elevated CO_2, it has been often reported that elevated CO_2 reduced N: C ratio in vegetative tissues and as a result crop grain quality was altered (Hogy and Fangmeir, 2008; Högy et al., 2011; Lieffering *et al*., 2004). Due to drop in nitrogen concentration protein concentration decreased (Wu *et al*., 2004) and non-structural carbohydrate (Hogy et al., 2009) and lipid increased in cereal crops (Sator, 1999). However, the seed oil content was not affected in oilseed rape at elevated CO_2 (Franzaring *et al*., 2008) but Hogy et al., (2010) reported that fatty acid composition slightly changed in the same species at elevated CO_2. Taub *et al*., (2008) used meta-analysis techniques to investigate the effect of elevated CO_2 on protein concentration of major food crops from data from 228 experimental

data recorded on wheat, barley, rice, soybean and potato and concluded that each crop had lower protein concentration when exposed to elevated CO_2 (540-958 µmol mol^{-1}) compared with ambient (315-400 µmol mol^{-1}). Also Jablonski *et al*., (2002) conducted a meta-analysis on 79 crops and wild species observation and reported that elevated CO_2 (500-800 µmol mol^{-1}) led to significant reduction in seed nitrogen concentration by 19% in most non-legumes but the seed nitrogen was not affected by doubling CO_2 as seed yield also increased.

According to Long (1991) and Stit and Karpp (1999) the photosynthetic rate in response to elevated CO_2 increased when there was sufficient sink strength for additional photo assimilates. In support of this hypothesis, most long term exposure to elevated CO_2 demonstrated photosynthesis down regulation known as acclimation in C_3 species attributed to, the source to sink imbalance. The magnitude of acclimation depends on the functional groups and other environmental factors (Ainsworth and Long, 2005). Where some other factor is severely limiting, such as low nitrogen availability (Kanemoto *et al*., 2009; Le Roux *et al*., 2001) low temperature (Long, 1991), high temperature (Fageria *et al*., 2010) or growing plant in pots where both root growth and nutrient availability are limited (Ainsworth *et al*., 2002; Arp, 1991) in these situations the imbalances within the photosynthetic system occurred and led to accumulation of non-structural carbohydrate which may act as the feedback mechanism of the photosynthetic process.

1.3. The effect of nitrogen on plant growth, productivity and quality

Nitrogen is one of the most important mineral nutrients for plant growth and yield and plants need nitrogen in larger quantities compared with other mineral

nutrients **(**Forde *et al*., 1999). However what determines a plant's demand for nitrogen and the amount of nitrogen they need for optimum growth and yield needs to be determined empirically. Knowledge of the factors concerning nitrogen demand is essential in order to anticipate the needs of crops under a wide range of conditions (Abrol and Raghuram, 2007; Grindlay, 1997). This is important both for economic reasons and because of the risks to the environment and human health that might arise from an over application of nitrogen fertilizer (Addiscott, 2005; Hatfield and Follett, 2008).

The optimization of nitrogen supply is strongly related to the plant's nitrogen use efficiency (NUE) (Lawlor *et al.,* 2001) which is the product of seed dry weight per unit of nitrogen accumulated. NUE is used as an indicator of the amount of nitrogen required for each crop to produce an optimum yield. Some plants are characterized by low NUE and they need high amounts of nitrogen to produce an economic seed yield (Rathke *et al*., 2006). NUE is based on; 1) Root nitrogen uptake efficiency; 2) Shoot incorporation efficiency; 3) Utilization efficiency dependent on nitrogen remobilization from the root to the shoot and other parts of plants; 4) Adequate levels of nitrogen in soil (Abrol and Raghuram, 2007).

For example, comparison studies on the response between safflower (*Carthamus tinctorius* L.) and sunflower (*Helianthus annus* L.) growth to nitrogen fertilizer in the form of ammonium nitrate concluded that the growth and yield increased for safflower with nitrogen supplied up to 1.0 g pot $^{-1}$ while 2.0 g pot $^{-1}$ was optimum for sunflower growth and yield because safflower was more efficient than sunflower in concentrating nitrogen in their shoots (Abbadi *et al*., 2008). Safflower therefore can be considered a more nitrogen use efficient

crop compared to sunflower (Abbadi and Gerendás, 2009). A positive correlation between seed yield and nitrogen use efficiency in safflower and a negative correlation with stem and leaf nitrogen accumulation at maturity was also observed by (Koutroubas *et al*., 2008) and most nitrogen in the early vegetative growth stage was found in stem and leaves but as the plant physiologically matured nitrogen shifted towards the seed (Abdurahman *et al*., 1999) typical of most seed crop plants.

Another widely used approach to determine nitrogen demand is to express nitrogen content on a plant dry matter basis. It has been shown that the instantaneous rate of nitrogen taken up can be calculated by multiplying the plant material's nitrogen concentration by the growth, that is represent it as the percentage of nitrogen in the biomass (Gastal and Lemair, 2002). This nitrogen rate calculation is dependent on dry matter weight and requires a chemical analysis to estimate the nitrogen concentration in the plant tissues (Greenwood *et al*., 1991), and it is complicated because the nitrogen concentration basis on biomass varies with the age of plant, the leaf position in the canopy, the photosynthetic photon flux density (PFD) under which the plant is grown, nitrogen supply and the time of nitrogen application (Gregory *et al*., 1981). The critical nitrogen concentration is defined as the minimum nitrogen concentration which allows maximum growth rate. The relationship between critical nitrogen concentration and dry matter accumulation is similar within most C_3 and C_4 cultivated species over the growing period. This parameter is widely used in agronomy as the basis of crop nitrogen status diagnoses (Gastal and Lemair, 2002).

A key determinant of plant nitrogen demand has also been established on the growth of leaves since photosynthetic function of leaves requires a large nitrogen concentration compared to other tissues of the plant because of the high protein content of leaves (Novoa and Loomis, 1981). All of the photochemical and biochemical processes of photosynthesis require nitrogen (Givnish 1986). In particular the photosynthesis capacity of C_3 plants is limited by nitrogen per unit leaf area because proteins of the Calvin cycle (Rubisco) and thylakoids are related to leaf nitrogen content (Evans, 1989). The structures involved in the light harvesting in photosynthesis which capture the photon energy are chlorophyll: protein complex (Lawlor *et al*., 2001).

Another indicator of nitrogen demand has been based on leaf chlorophyll content which tends to vary with variation in leaf nitrogen content and is hence correlated with the rate of leaf photosynthesis (Cabrera-Bosquet *et al*., 2009) but Evans (1989) suggested that the increased chlorophyll content effect on capturing energy is very small, except under extreme shade. For example, a two year field study on two safflower hybrids (CW9048 and CW9050) at three levels of nitrogen (0, 100 and 200 kg N ha^{-1}) was conducted to determine the effect of nitrogen on yield, yield components, chlorophyll content, photosynthetic characteristics and water use efficiency under rain fed conditions. The results concluded that the nitrogen fertilizer increased the photosynthetic rate by an average of 51%, stomatal conductance by 27%, water use efficiency by 60%, seed yield by 19%, seed weight per plant by 60%, seed weight per head by 18%, the number of heads per plant by 32% and the number of seeds per plant by 41% compared with the control (Dordas and Sioulas, 2007; Dordas and Sioulas, 2008; Dordas, 2009). In addition, a field experiment concluded that

from seven levels of nitrogen (0, 30, 60, 80, 120, 150, and 180 kg ha $^{-1}$) tested on safflower growth and yield component, 120 kg ha $^{-1}$ prolonged the time to maturity (172 days) and significantly increased number of branches, seed weight index and seed yield. Further increases in N up to 180 kg ha $^{-1}$ produced the same yield and thus, 120 kg ha $^{-1}$ was considered the most economic rate for safflower (Siddiqui and Oad, 2006).

The above experiments also showed, unsurprisingly, that leaf area index (LAI) increased through increasing the number of cells and their size by doubling nitrogen supply (Lea *et al*., 2001). As a result the amount of light intercepted, radiation use efficiency and leaf nitrogen content increased and photosynthetic efficiency was maintained in plants (Gastal and Lemair, 2002). Moreover, increases in photosynthetic rate was attributed to increases in chlorophyll content and maximization of Rubisco of both canopy and individual leaves due to increased leaf area (Cabrera-Bosquet *et al*., (2009). Consequently, plant growth increased as total plant dry matter accumulation and grain yield increased as did the harvest index (the ratio of grain weight to total above ground biomass) under nitrogen fertilizer (Sinclair, 1998).

Some researchers have been interested in investigating the effect of nitrogen fertilizer on seed oil content and fatty acid composition because among the oil crops in addition to the crop productivity parameters, the oil quantity and quality is also important. For example, the oil content of safflower was improved by nitrogen application up to the recommended rate (40 N + 30 P_2O_2 kg ha^{-1}) (Eksilinge *et al*., 1993). However, Bassil *et al*., (2002) indicated that safflower seed oil content was not affected by nitrogen fertilizer but Zaman (1988) found

that nitrogen fertilizer up to 60 kg N ha $^{-1}$did increase seed oil content in safflower.

A study of field grown oilseed rape (canola) concluded that none of the levels of nitrogen fertilizer (50, 100, 150 and 200 kg N ha^{-1}) had significant effects on seed oil content and fatty acid composition (Starner *et al*., 1999). By contrast, Rathke *et al*., (2006) found a positive effect of nitrogen fertilizer on the seed yield of winter oilseed rape. The oil content tended to reduce as nitrogen rate increased and this inverse correlation might be due to reduction in carbohydrate availability for generating oil at high nitrogen supply. Steer and Seiler, (1990) using four glasshouse and two field experiments and five cultivars of sunflower (*Helianthus annuus* L.) found variable composition in individual fatty acids with time of nitrogen application. The percentage of palmitic (16:0) and linoleic (18:2) acids increased significantly when nitrogen was applied before floret initiation while the stearic (18:0) and oleic (18:1) acids decreased and only stearic acid responded when the nitrogen applied between floret initiation and anthesis. After anthesis nitrogen application increased the ratio of oleic/linoleic acid. Also the results differed between the glasshouse and the field but the same result was recorded in both environment for fatty acid composition when was nitrogen supplied after anthesis. Recently, many researchers drew the conclusion from the chemical analysis of seed from different oil crops including safflower, sunflower, oilseed rape and soybean that the effects of genotype, environment condition (location) and planting date were more important on seed quality rather than nitrogen fertiliser (Izquierdo *et al*., 2006; Kumar *et al*., 1994; Omidi *et al*., 2010; Samancı and Özkaynak, 2003).

Many studies have been carried out in an attempt to explain how plants respond to nitrogen fertilizer under different interacting conditions, for instance, varying light intensity, temperature, irrigation and other different stresses. Work to date has established that light intensity changes the crop response depending on the nitrogen applied. Three Brassica species were hydroponically grown in a greenhouse at three levels of nitrogen fertilizer (100% NH_4, 100% NO_3, 50% NO_3 + 50% NH_4) with three levels of photosynthetically active radiation (PAR) (low 50 µmol m^{-2} s^{-1}, medium 680 µmol m^{-2} s^{-1} and high 900 µmol m^{-2} s^{-1}) and it was concluded that leaf area index was similar with all forms of nitrogen supplied. The lowest value of leaf area and leaf number was recorded in plants under 100% NH_4 at low and medium level of radiation. No interaction effect between light and nitrogen type was found (Fallovo *et al.*, 2009).

Plant response to nitrogen at both warm and cool temperature was studied, for example, the impact of nitrogen on radiation use efficiency and photosynthesis in peanut (*Arachis hypogaea* L.) canopy grown at warm and cool environments was examined by (Wright and Hammer, 1996) and concluded that the radiation use efficiency was higher by 33% in warm condition than cool condition.

1.4. The effect of drought on plant growth, productivity and quality

At the whole plant level, water stress impacts on crop yield mainly by reducing rate, duration and number of leaves produced. As a result of reducing leaf expansion the rate of radiant energy interception is reduced. Drought also reduces light conversion into dry matter and partitioning of assimilate (Jefferies, 1995; Prasad *et al*., 2008). Physiologically, water stress is considered to be a limiting factor for a wide range of physiological processes in plants (McDonald

and Davies, 1996) and Abscisic Acid (ABA) is now considered to be one of the first signalling chemicals for sensing drought (Prieto *et al*., 2009). Lowered relative water content (RWC) of leaves gradually reduces stomatal conductance and inhibits Rubisco due to the inhibition of ATP (Lawlor, 2002). In general, stomatal closure induced by drought reduced transpiration rate, limits CO_2 uptake (Comstock, 2002) and increases in Rubisco oxygenase activity (Cornic and Massacci, 2004; Flexas and Medrano, 2002a). As a consequence the main sink (acceptor) for photosynthetic electrons and O_2 uptake via photorespiratory activity entirely replaces CO_2 as an acceptor, thereby, the photosystem II (PS II) are protected during dehydration (Cornic and Fresneau, 2002b). However, it has been reported that the excess light energy absorbed is dissipated as heat and was superior in energy that had been used to drive photosynthetic metabolism under conditions of drought (Chaves *et al*., 2002). The light energy absorbed by chlorophyll can undergo one of three outcomes**:** it can be used to drive photosynthetic metabolism, it can create excess energy which can then be dissipated as heat or it can be re-emitted as light (chlorophyll fluorescence). Therefore, any increase in the efficiency of one will result in a decrease in the yield of the other two (Maxwell and Johnson, 2000). During moderate to severe drought, thermal dissipation is estimated to be increased by up to 70-90 % of the total absorbed light in C_3 plants (Flexas and Medrano, 2002b). As a consequence, the damage of the PSII centre has been revealed and this can be indicated by a drop in the Fv/Fm ratio under drought (Prieto *et al*., 2009). For instance, three experiments were carried out on cowpea (*Vigna unguiculata*) grown in 2.8 L pots filled with silica: vermiculite 1:2 inside a glass house where plants were watered with 250 mL of Hoagland solution twice a week and after

28- 38 days three water stress treatments were imposed by withholding water. It was reported that during the initial stage of drought photochemical activity of PSII was not affected and the decrease in assimilation rate was strongly related to stomatal closure which restricted transpiration rate and intercellular CO_2 concentration, but with prolonged water stress a down-regulation in the maximum yield of PSII was observed (Souza *et al*., 2004).

Water stress however had no significant effect on the variable to maximum fluorescence ratio (F_v/F_m) in sunflower cultivar indicating that water stress had no effect on primary photochemistry of PSII in a tolerant cultivar whilst this ratio decreased in a vulnerable cultivar (Subrahmanyam *et al*., 2006). Also these parameters showed no change under water deficit in other experiments (Cornic and Fresneau, 2002a; Pankovic *et al*., 1999). For example, two sunflower hybrids were exposed to drought from bud formation up to full flowering in the field under full sunlight (1500-2000 µmol m^{-2} s^{-1}), the results concluded that assimilation rate and stomatal conductance significantly decreased, but maximum quantum yield did not show significant change in severely droughted leaves. Also results revealed that Rubisco content under prolonged stress increased and a higher amount was found in the drought tolerant cultivar (Pankovic *et al*., 1999).

The effect of drought in combination with high temperature was more pronounced on physiological parameters either stresses alone (Shah and Paulsen, 2003) such that both stomatal conductance and variable fluorescence to maximum fluorescence ratio (Fv/Fm) decreased (Xu and Zhou, 2006). Conversely the combination between drought and high light showed results on photosynthetic parameters for example, a study of *Arabidopsis thaliana* grown

under a 6 h UV-B radiation each day and 12 days water stress, indicated that plants grown under UV-B radiation were more tolerant to drought than plants grown without UV-B radiation, as plants under both stress showed two times higher assimilation rate with a 12% increase in relative water content (RWC), smaller reduction in the quantum yield of PSII compared to plants grown under water stress alone, and results suggested that higher tolerance to drought under UV-B radiation was related to higher content of proline content and decreased stomatal conductance (Poulson *et al*., 2006).

In addition to the above, the mineral nutrient uptake by plant root and metabolism (Sardans *et al*., 2008) and chlorophyll a, b, total chlorophyll and carotenoid reduced under drought (Manivannan *et al*., 2007). Taken all together, drought led to a down regulation in CO_2 assimilation rate in C_3 plant species (Jaleel et al., 2009; Medrano *et al*., 2002). Eventually, this leads to a change in plant morphology and a decrease in dry matter accumulation, total leaf area, growth and development (Manivannan *et al.,* 2007) grain yield and harvest index (Kang *et al.,* 2002a). For example, ten genotypes of cowpea (*Vigna unguiculata* L.) were exposed to drought from flower bud formation until maturity (10 days), using growth chambers and reductions in biomass was related to reduce WUE and leaf photosynthesis rate and leaf area. In tolerant genotypes, drought improved WUE and induced stomatal closure and led to maintenance of relative water content but still reduction in leaf area (Anyia and Herzog, 2004) and an effect on seed composition for example, seed oil content in sunflower (*Helianthus annus* L.) (Reddy *et al.,* 2001), peanut (*Arachis hypogea* L.), soybean (*Glycine max* L.) (Dwivedi *et al.,* 1996) and canola (Brassica napus L.) reduced while the protein content increased (Aslam *et al.,* 2009). The degree of

drought (intensity), its duration, and the plant growth stage at which it is imposed (Aiken and Lamm, 2006) and the sensitivity of crop cultivars are all found to be determinants of yield reduction (Mafakheri *et al*., 2010).

In general, long term drought during vegetative growth and anthesis (flowering) are considered to be the worst for crops. The first because it leads to the crop failing to establish properly and the second partly because it occurs when the reproductive organs are formed from resources either recently acquired or previously stored by vegetative parts. Therefore, any environment stress that affects vegetative parts finally affects reproductive yield and partly because pollen and ovule fertility can be affected by acute drought during their critical development phases (Chiariello and Gulmon, 1991). The plant biomass and productivity of a wide range of crops has been shown to be reduced under drought and sunflower (Nezami *et al.,* 2008; Schittenhelm, 2010), peanut (Chapman *et al.,* 1993) and wheat (Kang *et al*., 2002) are affected most when drought is imposed during the critical stages of growth. Also this has been demonstrated in cotton during flowering and boll formation, during the vegetative stage in soybean, the yielding stage in sugar beet and sunflower, during flowering and grain filling in soybean (Kirda, 2002) and in the flowering stage in oil seed rape (Istanbulluoglu *et al.,* 2010) and beans (Acosta Gallegos and Kohashi Shibata, 1989; Boutraa and Sanders, 2001). Moreover, the highest seed yield in field grown safflower was obtained in fully irrigated control at three stages (vegetative, flowering and yield formation) and was higher for winter sowing than summer sowing (Istanbulluoglu *et al*., 2009).

1.5. Effect of elevated CO_2 in conjunction with other factors

1.5.1. The interaction of CO_2 with some of other anthropogenic, greenhouse gases and global warming

In climate change scenarios, temperature and other greenhouse gases (CH4, N_2O, SO_2, O_3, etc.) have been predicted to rise in conjunction with CO_2 (Caporn and Bridget, 2009).

Many impact assessment studies show how elevated CO_2 interacts with other environment factors and may influence plant growth. The response of many crop species to increased atmospheric carbon dioxide and various temperature regimes have been studied and reported that plant growth response to increased CO_2 was higher at optimum temperatures. While negative or no effect of both supra-optimum and suboptimal temperature interaction with elevated CO_2 have been found (Baker and Boote, 1996; Long, 1991). One explanation is that under an increase in air temperature above optimum the growing cycle of crops may be shortened and ageing may be accelerated in which case the advantages of increasing CO_2 may be offset (Streck, 2005). For example, Baker *et al*., (1989) reported soybean yield response to elevated CO_2 under three temperature regimes (26/19, 31/24 and 36/29 ^{0}C) with elevated CO_2 to 660 µmol mol^{-1} and seed yield decreased because the warmer temperature either at ambient or elevated CO_2 reduce the duration of grain filling and reduced the seed weight. Moreover, high temperature shortened the crop life cycle and in this way reduced the yield component (sink) which led to reduced grain yield (Fageria *et al*., 2010). In another study Wheeler et al., (1996) indicated that an increase in mean seasonal temperature of 1.0 - 1.8 ^{0}C in the UK may offset the

beneficial effect of elevated CO_2 in winter wheat (*Triticum aestivum* L.) grain yield.

Temperature has been shown to control seed set in many crops, and its effect could not be ameliorated by elevated CO_2. For example, in a study with peanut seed yield response to elevated CO_2 at four temperature regimes (32/22, 36/26, 40/30 and 44/34 ^{0}C) concluded that seed yield decreased by 14%, 59% and 90% and harvest index from 0.41 to 0.05 as temperature increased from 32/22 to 44/34 ^{0}C at either normal or 700 µmol mol^{-1} despite a marked increase in photosynthesis and vegetative growth above 32/22^{0}C and it was suggested that the decrease in seed yield was related to lower set due to poor pollen viability and smaller seed size due to reduced seed growth duration (Vara Prasad *et al*., 2003). Furthermore, Pooter and Perez- Soba (2002) and Brooks and Farquhar (1985) suggested that high temperature above optimum decreases solubility of CO_2 relative to O_2 in the cytosol, and reduces the Rubisco activity (Crafts-Brandner and Salvucci, 2000). Consequently, there is a rise in photorespiration rates regardless of CO_2 concentration thereby net photosynthesis decrease (Taiz and Zeiger, 2002).

Under lower than optimum temperature the elevated CO_2 stimulates less photosynthesis (acclimated) causing non-structural carbohydrates to accumulate and as a result growth is inhibited (Poorter and Perez-Soba, 2002).

However, in some cases elevated CO_2 attenuated the negative effect of temperature from 1.5 and 6.0 ^{0}C above ambient temperature and increased the leaf photosynthesis and reduced stomatal conductance and transpiration rate which improved the WUE (Vu, 2005).

The response to elevated CO_2 and high temperature is dependent on the environmental condition, Rosenzweig and Liverman (1992) stated that at elevated CO_2 high temperature in temperate regions led to the length of the plant growth duration (during season) and the possibility of growing crops successively in tropical region may have a negative impact. Polly (2002) indicated that rising CO_2 will enhance crop water use efficiency mainly by increasing photosynthesis and growth but yield may be most responsive when increasing CO_2 is coupled with increased temperature. Thus, leaf area, and seed dry weight increased significantly by 72%, while seed number was unaffected with an increase in temperature of only 1.0 ^{0}C to 1.8 ^{0}C for winter wheat grown under rising CO_2 and air temperature in the UK.

The damaging effect of ozone is strongly ameliorated by elevated CO_2. This is because rising CO_2 reduced stomatal conductance as a consequence the O_3 flux in to the leaf interior is reduced (Poorter and Perez-Soba, 2002). For example, a study on long–term of CO_2 and ozone (O_3) enrichment in FACE reported that elevated CO_2 induced net photosynthesis and reduced transpiration and led to improvement in water use efficiency as also found in closed chamber experiments but also decreased the damage effect of ozone on photosynthetic capacity during vegetative growth of spring wheat (*Triticum aestivum* L.) (Mulholland *et al.,* 1997). Recently, Bernacchi *et al.,* (2006) demonstrated that the physiological response of soybean grown in the FACE under the combined elevation of CO_2 and O_3 the plant produced a greater assimilation rate compared with CO_2 or O_3 alone.

Previous reports on plant physiological response and yield results from elevated CO_2 and SO_2 interaction experiments also suggested that increasing CO_2 mitigated the effect of SO_2 stress by altering plant physiology (Lee *et al*., 1997).

1.5.2. Interaction of CO_2 with water stress

Drought is one of the most important environmental factors limiting the growth and productivity of crop species worldwide, among of all the physical stresses in the global environment (Mooney, 1999) and therefore, changes in rainfall patterns will affect carbon fluxes, assimilation rates and transpiration rates are expected to increase as temperature increases (Heimann and Reichstein 2008). Shaw et al 2005 observed this phenomenon in numerous studies in semi-arid ecosystems where stomatal closure and decrease in stomatal density permit the possibility for plants to balance growth demand for substrate with water lost by transpiration. Also under severe water stress, the plants root growth/shoot growth increased because the limited water extracted by roots was mainly consumed by the root system itself and only a small amount of water was transported to shoot (Mardanov *et al*., 1998).

Since elevated CO_2 enhances a partial closure of stomata which reduces water use water stress is found to be offset in a majority of species under increased ambient carbon dioxide (Tyree and Alexandar 1993) and therefore under elevated CO_2 plants growing under water stress might flourish longer on a given water supply (Poorter and Perez-Soba, 2002). For example, Bunce (2008) recorded that elevated CO_2 reduced stomatal conductance for crop plants but the relative reduction is not constant, but is dependent on other factors (light, temperature and humidity) and different species showed different responses

with a doubling of CO_2 in the stomatal aperture which varied between 15% for some crop species to > 50% in others (Pospisilova and Catsky, 1999) also leading to enhanced WUE where measured (Beering, 2005; Hsiao *et al*., 1999). It has also been shown that under elevated CO_2 plant root dry matter accumulation and number of roots at all depth of the soil profile to 150 cm increase, and the root to shoot ratio consequently enables plants to reach and capture more water (Rogers *et al*., 1999). This increase was observed under water/or nutrient limiting conditions at elevated CO_2 (Stulen and Hertog, 1993). Shaw *et al*., (2005) demonstrated that elevated CO_2 and water stress altered root capacity to water transport through their xylem for example, elevated CO_2 reduced root hydraulic conductance by 26% in soybean and by 50% in sunflower compared to ambient CO_2.

Other studies have concluded that the plant response to elevated CO_2 under limited soil water availability is temperature dependent. If the increase in ambient CO_2 concentration is accompanied by an increase in air temperature, transpiration rates are expected to increase which may offset the advantages of an increasing CO_2 in limited water supply grown plants (Heimann and Reichstein, 2008). In a study in which air temperature was controlled the water use was reduced by about 10% in rice; in this way the plant drought resistance improved and growth and yield were maintained (Baker *et al*., 1997).

In contrast, Wu *et al*., (2004) suggested that plants may benefit more from elevated CO_2 when adequate water is supplied. They subjected spring wheat to two levels of CO_2 (350, 700 µmol mol^{-1}) and two levels of soil water 80, 40% of field water capacity (FWC), and concluded that wheat plant produced more biomass and grain yield with greater grain number and harvest index which

resulted from a significant increase in shoot dry weight under higher of 700 µmol mol^{-1} and 80% field capacity and water use efficiency significantly increased.

1.5.3. Interaction of CO_2 with nutrients

Elevated CO_2 increases plant growth through its effect on photoassimilation but this requires increased mineral absorption and nutrient use efficiency and the demand for mineral nutrients increases with long term growth in increasing CO_2 levels (Stitt and Krapp, 1999). It is often documented that the most comparative explanation for photosynthesis activity down regulation in C_3 plant species is related to low nitrogen concentration in the leaf under long term exposure to elevated CO_2 and this is highly pronounced under low nitrogen supply (Del Pozo *et al*., 2007; Nakano *et al*., 1997). For instance, long term (4 weeks) exposure of alfalfa (*Medicago sativa* L.) to two levels of CO_2 (400 and 700 µmol mol^{-1}) and three levels of nitrogen (0, 10 and 15 mM) in the form of NH_4NO_3 concluded that the photosynthetic rate decreased at elevated CO_2 and (0 mM) nitrogen in plants compared with ambient as a result of C:N imbalance, but plants under elevated CO_2 and supplied with 15 mM NH_4NO_3 maintained high photosynthetic rates as a result of superior C:N modification (Sanz-Sáez *et al*., 2010).

Other researchers have concluded that the reduction in stomatal conductance and transpiration rate of elevated CO_2 reduced the leaf nitrogen concentration and was responsible for photosynthesis down regulation (Kanemoto *et al*., 2009), as a reduction in transpiration rate may decrease the mass flow of the soil solution and the mobilization of nitrogen from the soil to the root, and hence limit nitrogen acquisition by the plant (McDonald *et al*., 2002).

The reduction in leaf nitrogen concentration is occasionally associated with the reduction in the amount of Rubisco and its activity due to limited nitrogen availability at elevated CO_2 (Ainsworth and Long, 2005; Ainsworth and Rogers, 2007; Rogers and Humphries, 2000). For example, three levels of CO_2 (350, 550 and 900 µmol mol $^{-1}$) and nitrogen rates ranging from very low to very high were tested on wheat and the results showed, that the shoot growth was 30% greater at 550 µmol mol^{-l} as compared with ambient at all nitrogen rates, but there was no significant increase in shoot growth under 900 µmol mol^{-1} and low nitrogen rate. Elevated CO_2 to 900 µmol mol^{-1} reduced leaf nitrogen concentration by 58% under low nitrogen supply and led to a reduction in Rubisco and nitrogen was allocated away from Rubisco into other soluble protein fractions (Rogers *et al*., 1996). Alternatively photosynthetic acclimation after prolonged exposure to elevated CO_2 could in part be due to diverting nitrogen away from the photosynthetic apparatus towards the growth of other organs (Wolfe *et al*., 1998). Also the total non-structural carbohydrate was increased at elevated CO_2 and low nitrogen (Booker *et al*., 2000) and the increment in non-structural carbohydrate is considered to have led to a dilution of tissue nitrogen concentration (Taub and Wang, 2008) and might have a feedback on photosynthetic rate (Baxter *et al*., 1995).

Moreover, the down-regulation in photosynthetic activity under long term elevated CO_2 and low nitrogen is mainly correlated with the balance between source-sink strength rather than the nitrogen concentration in plant tissues. For example Ainsworth *et al*., (2003) using 10 years FACE experiments growing perennial rye-grass (*Lolium perenne* L. CV. Bastion) at 600 µmol mol^{-1} CO_2 and two levels (low and high) of nitrogen, concluded that differences between

ambient and elevated CO_2 in the low nitrogen treatment, the assimilation rate in term of maximum carboxylation velocity ($V_{c,max}$) and the maximum rate of electron transport (J_{max}) was smaller after cutting and were significantly lower in elevated CO_2 as compared to ambient in low nitrogen without cutting.

However, it is often reported that elevated CO_2 attenuated the negative effect of nitrogen stress, and enhanced marked growth (Wong and Osmond, 1991). For example, at limited nitrogen supplied, cotton plants responded more to elevated CO_2, because a large amount of carbon was fixed in photorespiration and subsequently, photorespiration was suppressed resulting in a lower nitrogen requirement at elevated CO_2 (Rogers *et al*., 1993).

An another example, in hydroponically grown wheat showed that the photosynthesis had not acclimated in response to elevated CO_2 and low nitrogen, and the amount and activity of Rubisco and tissue nitrogen concentration was maintained (Farage *et al*., 1998).

The variability in response towards nitrogen availability of elevated CO_2, appears to depend on crop species and the degree of nitrogen deficiency that plants received (Stitt and Krapp, 1999). It has been shown that nitrogen fixing species has the ability to respond strongly to CO_2 increase when nitrogen is lower (Poorter and Navas, 2003) and more than non-fixing species because root nodules provide the plant with an adequate source of nitrogen (Hebeisen *et al*., 1997; Schenk *et al*., 1997). The growth and seed yield in non-nodulated soybean (*Glycine max* L.) plants substantially increased under (700 µmol mol^{-1}) CO_2 and most nitrogen levels used (0.05,1.0, 2.5, 5.0 and 10.0 mM KNO_3), but not under the lowest levels of (0.05 mM) (Cure *et al*., 1988).

To date, there is no literature published on the effect of elevated CO_2 and/or its interaction with other environmental factors (nutrients or water) for safflower in spite of its undoubted medical, pharmaceutical and economic importance (Berglund *et al*., 2007; Dajue and Mundle, 1996; Smith, 1996) and the fact that it is widely grown in arid or semi-arid regions of the world (Johnston *et al*., 2002) that are facing potentially significant climate change (Shaw *et al*., 2005). Lawlor, (1999) stated that the effect of rising atmospheric CO_2 on semi-arid plants will be great because those species showed significantly more increase in yield under CO_2 enrichment. There is therefore a research gap in the literature regarding safflower response to elevated CO_2.

The following sections give more details about the safflower crop.

1.6. Safflower

1.6.1. Safflower biology

Safflower (*Carthamus tinctorius* Linn.) is a member of the family Compositae or Asteraceae same family as sunflower (*Helianthus annus*). It is a branching thistle like herbaceous annual or winter annual plant (Smith, 1996) with the height varying between (0.5 - 1.8 m) (Kaffka and Kearney, 1998).

Seeds need up to 3 weeks to emerge (Herdrich, 2001) and a slow growing rosette stage follows germination. During this stage, near ground level, numerous broad leaves are produced and a strong tap root develops that gives significant drought tolerance. During this rosette stage, safflower seedlings are cold resistant and are even resistant to frost but the crop is a poor competitor with fast growing weeds (Berglund *et al*., 2007). Next, the stem elongates quickly and produces branches, the number of which depends on plant to plant

competition. The duration of this stage is from 4 - 7 weeks, depending on the weather conditions (Dajue and Mundle, 1996) especially temperature (Herdrich, 2001). The stem and branches produce a globular flower capitulum enclosed by clasping bracts which are typically spiny with variation among varieties and individual plants within a variety. Leaf size is also different among varieties and even individual plants (Plate 1.1) and ranges mainly from 2.5 - 5 cm wide and 10 –15 cm long (Dajue and Mundle, 1996). Most leaves are deeply serrate, short and stiff in shape on the lower stem and can be ovate around the inflorescence, most safflower crop types have spineless leaves on the lower stem but upper leaves have degrees of spininess (Smith, 1996). Flowering usually begins in the primary capitulum followed by the secondary capitulum and so forth. Within the capitulum, flowering starts in the outer circle of florets and progresses centripetally over several days up to a week. Early blooms are shades of orange, yellow and red but post-bloom colour is darker and white flowers occasionally occur (Dajue and Mundle, 1996). A safflower flower is composed of petals that attach to a corolla tube at the base of which is an “inferior” ovary. The style and stigma are surrounded by five fused anthers which are longer than the corolla tube (Nimbkar and Singh, 2005). Self-pollination in the tubular florets is normal with generally less than 10% outsourcing. Each capitulum contains 15 - 30 seed (achenes) which develop 4 - 5 week after flowering (Dajue and Mundle, 1996; Oelke et al., 1992). An achene typically consists of 33 - 60% hull and 40 - 67% kernel (Dajue and Mundle, 1996). The typical proximal composition of seed is 32 - 40% oil, 11 - 17% protein and 4 - 7% moisture (Janick and Whipky, 2007). However, the oil content of the seed differed considerably among varieties and across

environmental factors (Coşge *et al*., 2007).Seed weight increases most rapidly during the first 15 days after flowering and reaches a maximum dry weight in about 28 days and the oil amount during this period increases 5 - 10 fold with fatty acid build up beginning about 10 days after flowering (Hill and knowles, 1968). Day length influences the length of the plant growing period with short photoperiods extending development phases. The growing period for autumn sown crops varies from 200 to 230 days (Quiroga *et al*., 2001) In general the safflower requires about 120 - 170 days producing a crop (Kaffka and Kearney, 1998). On average safflower is ready to harvest about 50 - 60 days after the peak of flowering at optimum temperature (Kaffka and Kearney,1998) when most of the later leaves have turned brown and only a very little green remains on the bracts of the latest flowering heads. Seed should rub free of the least mature heads and it is usually harvested using a small grain combine (Berglund *et al*., 2007).

Depending on the types of fatty acids in the oil, two different types of safflower have been recorded and characterized by the fatty acid content; oleic safflower contains high amount about 77% oleic acid and linoleic safflower contains about 77% linoleic fatty (Kaffka and Kearney, 1998).

Table 1.1. Typical fatty acid composition other edible grade specifications of linoleic and oleic safflower types.

Characteristic	***Linoleic safflower***	***Oleic safflower***
Fatty acid		
C16 Palmatic%	5.0	5.0
C18 Stearic %	5.2	2.0
C18: 1 Oleic%	15.0	77.0
C18:2 Linoleic%	77.0	15.0
C18 : 3 Linolenic	<1.0	<1.0
Others:	0.7	1.3
Free fatty acid as (oleic)	0.03	0.03
Ioden value	144.0	92.0
Peroxide value asshipment	0.1	0.1
Refractive Index	1..474	1.690




Plate 1.1. Photographs illustrating safflower plant characteristics and growth stagedevelopments inside -: from left to right; rosette, stem elongation, branching, budding and flowering stages.

1.6.2. Uses of safflower

Safflower is a multipurpose oilseed crop which exhibits potential medical, pharmaceutical and cosmetic importance all over the world (Dajue and Mundle, 1996; Smith, 1996). The following uses have been reported for safflower:

1. Traditionally this crop was grown for its flowers for colouring and flavouring foods. Flowers contain the water soluble yellow dye carthamidin ($C_{16}H_{20}O_{11}$) and a water insoluble red dye carthamin ($C_{21}H_{22}OH.H_2O$). These have been the source of yellow and red dye in the food and industries in Egypt and dye from safflower was used to colour cotton and silk in the 18th century in Italy and France (Dajue and Mundle, 1996). Recently, according to reviews by Emongor, (2010) these yellow and red pigments have been shown to be safe for cosmetic colourings such as face cream, shampoo, perfume or body lotion and hair cream. In Chinese medicine, flower petals have been used as a stimulant for blood circulation and phlegm, healing of fractures, contusions and strain and for various female maladies (Emongor, 2010) and some nutrients have been extracted from florets and have been used in treatments of many illnesses such as menstrual problems, cardiovascular diseases and pain associated with trauma as well as in tonic tea (Singh and Nimbkar, 2006).

2. The seed is also a source of bird food and an oil source (Berglund *et al*., 2007). Also seed has been used in medicine where the seed can be boiled and taken as a remedy for the problem in mensuration to increase blood flow and a mixture of ground safflower seed and mustard oil has been used to reduce rheumatic pain (Emongor, 2010).

3. Oil is used by both food producers and industry (Quiroga *et al*., 2001). Those varieties high in monounsaturated fatty acid (oleic acid) use as heat stable cooking oil for frying food items whilst those high in linoleic oil are valued as a drying agent in paints and varnishes because of non-yellowing characteristics impaired to paint (Berglund 2007). However, Safflower is currently grown mostly for its edible oil, considered as a favourable oil for human consumption due to high quantity (70-75%) of polyunsaturated (Linoleic acid) or mono-unsaturated fatty acid which play an important role in reducing cholesterol level in blood (Nimbkar and Singh, 2005). Safflower oil can also be used in producing Biodiesel fuels in mixtures with other vegetable oils (Demirbaş, 2003).

4. According to Emongor, (2010) a pharmaceutical company called symbiosis, in April 2007 created insulin from genetically modified safflower plant extracts. In India and Afghanistan the tea made from safflower foliage was used to prevent the abortion and fertility in women (Dajue and Mundle, 1996). Male sterility and dead sperm diseases have also been treated successfully using safflower dicotyledons. Dajue and Mundle, 1996). Safflower whole plant can be grazed by livestock or stored as hay or silage, if cut at or just after the bloom stages (Berglund *et al*., 2007) and the nutrition value of silage and hay safflower has been found to be similar or better than oats and alfalfa (Smith, 1996), Sheep and cattle can also graze succulent safflower and stubble fields after harvest (Oelke *et al*., 1992).

5. The meal left after oil extraction is sometimes used as a protein supplement for livestock and poultry feed (Berglund *et al*., 2007) in common with many other oilseed crops such as rapeseed, soy and corn. The meal after oil extraction contains about 20% protein (Oelke *et al*., 1992).

1.6.3. Origin and distribution

Safflower is from the genus of Carthamus L. Which is a member of the Tribe Cynarea, subfamily Tubuliflorea and family Asteraceae The genus Carthamus has members with 20, 22, 24, 44, and 64 chromosomes and include 25 species and sub-specie (Sehgal and Raina, 2011) of which only *Carthamus tinctorius* having 2n = 24 chromosomes is the cultivated type (Singh and Nimbkar, 2006). Safflower is believed to have originated in southern Asia and has been cultivated in India, China, Persia, and Egypt. During the middle Ages it was introduced in Italy, France and Spain and after the discovery of America, introduced to Mexico and Venezuela and Colombia by the Spanish. It was taken to U.S.A in 1925 from the Mediterranean region (Janick and Whipky, 2007) and the weedy progenitors (n = 12) of cultivated safflower such as *C. Flavescens* Spring, *C. Oxyacantha* M.B. and C. *palaestinus* Eig, are widely distributed in these areas (Röbbelen *et al*., 1989).

1.6.4. History and production

Historically, the crop has been restricted to the Middle East part of Asia and Africa but over time it has also been adapted to the semi-arid climatic conditions of the western United States (Dajue and Mundle, 1996) and its production in the Great Plains states began in 1927. Western Nebraska and eastern Colorado had the first commercial production, but now it has become a commercial crop in several Western states and on the Canadian Prairie. Fifty percent of the grown area in the U.S.A is now located in California, but North Dakota and Montana also contribute to the domestic production followed by South Dakota, Idaho, Colorado and Arizona (Armah-Agyeman *et al*., 2002;

Oelke *et al*., 1992). According to Smith and Jimmerson (2005) safflower production in the world increased steadily from the 1990s to 930,000 metric tons in 1997 but after that production decreased to only 604,157 metric tons in 2004 as shown in Table (1.2). In comparison to other oil crops, it has remained as a minor crop around the world. However, recently interest in this crop has been rekindled (Singh and Nimbkar, 2006). It has been estimated that safflower is now being grown in over 60 countries in the world with half of the world's production in India (Singh and Nimbkar, 2006; Singh *et al*., 2001). In Iran the area under cultivation has increased over the last few years and reached 10000 ha during 2008 whereas it was 200 - 300 ha in 1997 (Omidi *et al*., 2009) and it is considered an important dependable oil crop in Iran where the crop is grown in dry areas and also in cropping systems in irrigated areas (Jalali *et al*., 2011). Interest in safflower has been renewed in the last few years in India (Nimbkar, 2002) and in Turkey due to a growth in population and an increasing demand for oil (Istanbulluoglu, 2009a). In North America it has been ranked as one of the most superior oilseed crops (Johnston *et al*., 2002). Moreover, in many of the agricultural areas in the world, this crop is now grown for it is edible oil (McPherson *et al*., 2004). It has been concluded that there are three reasons for renewed safflower production around the world (1) The suitability of safflower for semi-arid region with a shortage rainfall (2) the demand of consumers for healthy oil with lower amounts of saturated fats (3) the industrial and medicinal uses of safflower flowers especially in China (Singh and Nimbkar, 2006).

Table 1. 1. World Safflower production historically. (Smith and Jimmerson, 2005)

Year	*Production in metric tonnes*
1993	815,165
1994	901,443
1995	877,064
1996	903,870
1997	930,091
1998	653,036
1999	869,181
2000	689,556
2001	607,620
2002	601,332
2003	671,485
2004	604,157

1.6.5. Environment requirement

1.6.5.1 Climate

Safflower is a sun loving crop requiring long photoperiods, high temperature and bright sunny days in July and during early September to speed development. The plant requires dry atmospheric conditions and warm temperature during flowering for successful seed set. Safflower needs 2,200 growing degree days to mature (2,200 growing heat units) (Berglund *et al*., 2010) with a 120 day frost free growing season (Herdrich, 2001). However, emerging plants need cool temperature for rosette development and root growth with an average daily temperature of 15 - 20 ^{0}C and then higher temperatures during stem elongation (Dajue and Mundle, 1996).

1.6.5.2. Soil

Deep, fertile, well drained loam soils with good water holding capacity is considered as the best for safflower. But when the rainfall quantity and distribution are adequate the plant can flourish in lower holder water capacity soils (Berglund *et al*., 2010; Berglund *et al*., 2007; Herdrich, 2001; Oelke et al., 1992). It has the same tolerance to salinity as barley (Oelke *et al*., 1992).

1.6.6. Cultural practice

1.6.6.1. Sowing date

In general, in the U.S.A safflower seed is sown in early spring, or whenever the soil temperature is above 40 ^{0}F crop, and needs 8 to 15 days to emerge. In early sowing plants take full advantage of the entire growing season and

delaying sowing to May increases the risk of autumn frost injury and diseases that reduce seed yield and quality (Berglund *et al*., 2010; Berglund *et al*., 2007). In West Asia and North Africa, Iran and Turkey both winter and spring sowings are recommended, but the winter sown crop is significantly higher yielding than the spring sown crop (Abdulhabip *et al*., 2004; Nikabadi *et al*., 2008; Omidi and Sharifmogadas, 2010; Uslu, 2003; Yau, 2007).

1.6.6.2. Irrigation

It is considered an important winter crop in some semiarid regions due to its drought tolerance because of its able to capture the water from depths up to 2.5 - 3 m (Berglund *et al*., 2007). However, fully irrigated winter crops increased seed yield by 4.05 t ha^{-1} and by 3.74 t ha^{-1} for fully spring irrigated crops (Istanbulluogh *et al*., 2009).

1.6.6.3. Fertilization

Safflower is considered an important oil crop particularly under low input conditions (Abbadi *et al*., 2008). The amount of nutrients needed for safflower depends on; yield goal, soil test results and the sequence of the crop in the rotation (Berglund *et al*., 2007) and soil moisture availability and how much irrigation is supplied (Lyon *et al*., 1991). In general, in order to maximize both flower and seed yield in safflower 60N: 30 P_2O_5: 30K_2O is recommended (Nimbkar, 2008). Nitrogen is the most important nutrient for safflower growth and productivity (Lyon *et al*., 1991). Recently, (Dordas and Siouls., 2008 and 2009) demonstrated that nitrogen fertilizer modified physiological parameters,

biomass accumulation and partitioning and as a result the seed yield significantly increased (for more detail see ch 4).

1.6.6.4. Crop rotation

Safflower is the best crop in cropping systems for dry land conditions (Istanbulluoglu, 2009a). The use of cropping systems (i.e. rotation) is an effective way to achieve sustainable agriculture production system especially in dry land areas and improves soil physical properties and also helps to control weeds, pests and diseases (Quiroga *et al*., 2001)

1.6.6.5. Diseases and their control

Leaf spot *Alternaria* (*Alternaria certainty*), has symptoms of large, brown irregular spots on leaves and flower bracts and bacterial blight *(Pseudomonas syringae*) have similar symptoms, together these are the most serious disease under normal rainfall and prolonged periods of high humidity (Berglund *et al*., 2007 Oelket al., 1992) and cause significant damage to photosynthetic tissues and consequently reductions in seed yield have been reported. The fungicide of Quadris (Azoxystrobin) is recommended for foliar application at the first flowering to control or suppress Alternaria leaf spot and bacterial blight is only properly controlled by tolerant variety selection and the use of disease free seed (Berglund *et al*., 2007).

Safflower rust (*Puccinia carthami) is* very common but not a very serious disease, the seed can be treated. The planting of clean, disease free seed, the crop rotation system and field selection are important to control this disease in

the field (Berglund *et al*., 2007). Recently, it has been concluded that safflower is vulnerable to leaf spot disease caused by *Cercospora beticola* (Lartey *et al*., 2005). Safflower, sunflower, lentil and pears are susceptible to white mold (*Sclerotinia sclerotiorum*) of canola and it is best not to follow this crop with safflower in a rotation (Berglund *et al*., 2007).

1.6.6.6*. Insect and other predators and their control*

There are a few insect pests that attack safflower and cause economic damage (Berglund 2007). However, recently the sensitivity of safflower to safflower fly (*Acanthiophilus helianthi*) has been studied in Iran and economic damage recorded under water stress conditions (Hatami *et al*., 2008).

1.7. Aims of the project

The main aims of this study were to investigate the effects on safflower physiology, growth, seed yield and oil content

- Water stress
- Nitrogen fertilizer
- Elevated CO_2
- Elevated CO_2 x nitrogen

The objectives are described later in each of the relevant chapters.

Chapter 2

General Materials and Methods

2.1. Plant material

Safflower seed supplies were not able to be imported from Iraq for this investigation nor were seed companies able to provide reliable certified seed. As a consequence "Richters" Lemon Yellow variety (a non-certified variety) was obtained from Richter Seeds Ltd (herbal seed supplier) and was used throughout the investigations. For all experiments seeds were pre-germinated in an incubator in the dark with a 12 h diurnal temperature fluctuation of 23/12 ^{0}C until radicle emergence (3 days) before sowing in pots.

2.2. Plant container description

Pots of a required dimension were not available commercially and were therefore constructed from 11.4 cm diameter cylindrical polypropylene drainage pipe cut into lengths 30 cm high (Plate 2.1) and used as previously reported (Nasser *et al*., 2008). Each "pot" was placed into a graduated clear plastic beaker to form the base of the pot and so that the level of the drainage water could be monitored when necessary.

Plate 2. 1. Photograph to illustrate the pots and growth media (white colour standard perlite, dark colour are John Innes No.2 (left) and multi-purpose compost (right) used in experiments.

2.3. Growth media

Two types of growth media (Plate 2.1) were used in the different experiments:

2.3.1. John Innes N^{O}. 2 loam based compost was used in the investigation of the effect of drought. However growing plants in this medium in the experiment investigating the effect of nitrogen (see chapter 4) resulted in necrotic plant symptoms which were attributed to an unknown effect of the compost. As a consequence the growing medium was changed to Perlite for the remaining experiments.

2.3.2. Standard grade horticultural perlite (William Sinclair Horticulture Ltd) + hydroponic solution.

2.4. Hydroponic solution

Two types of hydroponic solution were used.

2.4.1. A standard hydroponics growth solution Vita link Max Grow (soft water) supplied by Grow-well Hydroponics Ltd http://www.growwell.co.uk/vita-link-max-hydroponic-nutreint.html (nutrient details given in Appendix 1). This material was supplied in two parts (A & B) and diluted prior to mixing to avoid precipitation of some of the nutrients.

2.4.2. Complete Hoagland's solution minus nitrogen. In order to facilitate the Nitrogen dosing experiment a nitrogen free Hoagland's solution had to be prepared using the following nutrient mix: (Hershey, 1995).

Hoagland solution (per liter of nutrient solution)

10 mL of 0.05 Molar mono calcium phosphate .Ca $(H_2PO_4)_2$
200 mL of 0.01 Molar calcium sulphate dehydrate $CaSO4.2H_2O$
5 mL of 0.5 Molar potassium sulphate K_2SO_4
2 mL of 1 Molar magnesium sulphate $Mgso_4$
1 mL of Micronutrient stock solution (see below)
2.4 mL of iron Chelate stock solution.

Micronutrient stock solution per litre:
2.86 g Boric acid
1.81 g Manganese chloride-4 hydrate
0.22 g Zinc sulfate–7 hydrate
0.08 g Copper sulphate
0.02 g 85% Molybdic acid
Diluted 1: 1000 the micronutrient stock solution to provide the following nutrient:
Boron 0.5 mg L^{-1}
Manganese 0.5 mg L^{-1}
Zinc 0.05 mg L^{-1}
Copper 0.02 mg L^{-1}
Molybdenum 0.01 mg L^{-1}

2.5. Enclosed CO_2 chambers

Controlled CO_2 chambers were constructed from Lexan Ecell D polycarbonate sheets (Gilbert Curry Industrial Plastics Co Ltd) and located in a glass-house. The chambers were 60 cm x 60 cm x 140 cm (width x length x height) (Plate 2.2). Two pipes were connected at the rear side of each chamber, one pipe delivered the air into the chamber, and the other one carried the air out in order to maintain air circulation inside each chamber. For CO_2 elevated chamber, carbon dioxide was supplied using cylinders of compressed CO_2 (BOC gases) coupled to an IRGA Eurotherm^{TM} controller which pulsed CO_2 from the bottled gas to a set point of 850 ppm in investigations of elevated CO_2 (CH 5 & 6). According to the SRES emissions scenarios reported in the Fourth Assessment of the IPCC working group III " Mitigation of Climate Change" in 2007, the concentration of CO_2 projected for year 2050-2080 ranges from 710855 p.p.m. and from 855-1130 ppm for the period 2060-2090. Therefore the range of 850 ppm was chosen to represent the CO_2 enrichment use in this experiment. In investigating the interaction between elevated CO_2 and nitrogen carbon dioxide was also supplied using cylinders of compressed CO_2 (BOC gases) but coupled to an Eco Technics Evolution controller and sensor which replaced the Eurotherm controller.

Exit pipes were reconnected to a single conduit pipe and vented to the outside of the glass-house using a constantly running extractor fan (computer cooling fan) which drew the air out of both elevated and ambient chambers. The conduit pipes were Superflex PU R Anti-abrasive which had an operating temperature

range between -40 ^{0}C and + 90 ^{0}C (Teign Flex, Heathfield Industrial Estate, Newton Abbot, UK).

Holes were cut in the base of each chamber to accommodate 16 pots and to give the chambers sufficient headroom to accommodate the safflower plants through to flower development.

TelairTM monitors were used to measure CO_2, temperature and relative humidity inside the chambers at 6 minute intervals and data logged to Hobo^{TM} data loggers. Data were downloaded from the data loggers every 2 weeks using Boxcar^{TM} software and then the required data exported to a Microsoft Excel file for manipulation. Over the whole growth period for each chamber the weekly average for each measured parameter was calculated. The calculated average data for CO_2 or temperature of all chambers were then compiled into one chart. The relative humidity was monitored only in two chambers (1 elevated CO_2 + 1 ambient CO_2).

Plate 2. 2. Photograph to illustrate the enclosed CO_2 chambers used in experiments.

2.6. Measurements taken

2.6.1. Soil moisture content

The soil water holding capacity was established for the John Innes N^{O}.2, by irrigating two pots until full saturation and then allowing them to drain until drainage flow stopped and the pots weighed. The soil was then allowed to air-dry in the glass-house and the weight measured periodically and Theta Probe^{TM} (Delta T Devices) readings taken. In this way it was possible to determine when soil water content reached 70% of the available water capacity which was taken as a re-watering threshold. Allowing pots to dry until the soil water availability reached 25% of the available water capacity was determined as severe drought. Plants were watered with 200 ml tap water in control (well watered) pots whilst draughted plants were watered with 50 ml tap water to maintain drought but not to kill plants.

2.6.2. Stomatal conductance

Plant stomatal measurements (drought effect) were taken using an AP4 Porometer (Delta-T Devices) (Plate 2.3) .The basis of the method measures the amount of water evaporating from a plant leaf via stomatal pores into the atmosphere to change the relative humidity of a small cavity or cup inside the sensor head and converting this into a reading. This is compared with a calibration plate with holes of defined evaporation characteristics. Readings are expressed in units of seconds per cm^2 of leaf area. A cyclical purging of the head cup using semi-dried air (by passing through a silica gel container) enabled a stable reading to be obtained before recording.

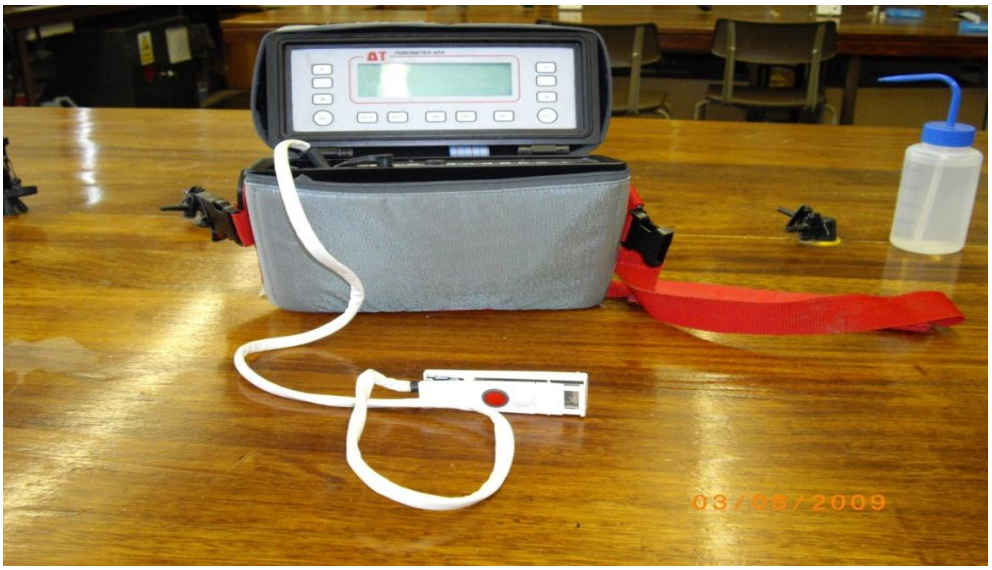

Plate 2. 3. AP4 Porometer (Delta-T Devices.)

2.6.3. Chlorophyll fluorescence measurement

Chlorophyll fluorescence was measured (drought effect) using a plant efficiency analyzer (PEA) (Hansatech^{TM}) (Plate 2.4). The basis of the method is to expose the leaf, after dark adaptation, to the illumination which the sensor unit provides as a pure red light with a peak wave length of 650 nm which is ready absorbed by chloroplasts. During measurement the PEA sensor unit was held over the light exclusion clip and the shutter opened and the ratio of variable fluorescence (Fv) to maximum fluorescence (Fm) was calculated automatically. The light level and recording interval were both selectable from the keypad via the status menu. During a recording the fluorescence received by the sensor unit was digitized in the control box and recorded. The instrument records a number of parameters automatically over a period of a few seconds; $F_{0:}$:is only observed when the first stable electron acceptor of photo system II, called $Q_{A,}$ is fully

oxidized, Fm: is the maximum fluorescence value obtained for the saturating light and the electron acceptor Q_A is fully reduced (maximum excitation), Fv: which is the variable component of fluorescence and is obtained by subtraction from the Fm value and indicates that photochemical quenching at maximum capacity and Tm: is the time at which the maximum florescence occurs.

Fv/Fm (this is a ratio which has been shown to be proportional to the quantum yield of photochemistry and indicates the state of net photosynthesis) was used in this study because is sensitive indication of plant photosynthetic state and plant health.

A- Determination of required dark adaptation time

20 plastic clips were placed on (20 independent) safflower leaves the shatter was closed and the measurement was taken for clips located in sequence from 1 to 40 minutes (approx.). Full light level was used in order to ensure the dark adaptation is enough in all light conditions. Results showed that the Fv/Fm ratio stabilized at 15 minutes showing that 15 minutes provides adequate dark adaptation for safflower.

B- Selecting a saturating light level

20 clips were placed on safflower leaves after 2 minutes the shutter was closed and after 15 minutes of dark adaptation measurements were taken at increasing light level between 10-100%. Results showed that the most stabilized value of Fv/Fm ratio of all leaves used was obtained by 80% level of light.

A measurement was taken in both the morning and afternoon by selecting 3 plants randomly for each treatment in each replicate, the upper expanded leaves of the same age and position were selected then the leaves were dark adapted with plastic leaf clip for 15 minutes before the measurement.

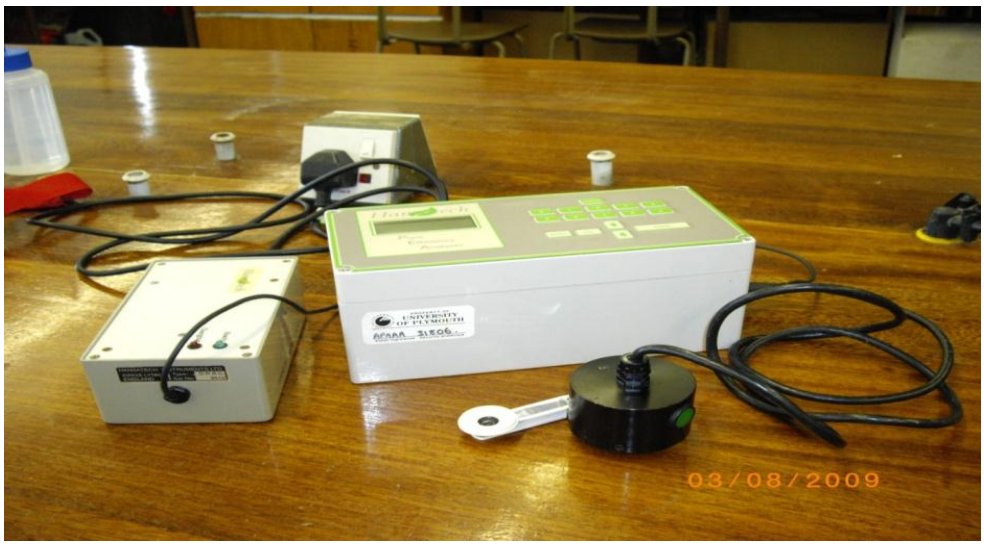

Plate 2. 4. Plant efficiency analyzer (Hansatech) TM

2.6.4. Supplementary radiation measurement

Supplementary radiation to maintain a 12 h. Photoperiod was provided during the winter months in the glass-house using SonT 400W Sodium Vapor lamps. The photosynthetically active radiation (PAR) supplementation was measured on both sunny and cloudy days, using a PAR meter (Skye Instruments Ltd) and ranged between 800 - 1000 µmol m^{-2} s^{-1} at canopy height.

During the winter months when it was dark i.e. at the beginning and the end of the day and when it was cloudy, the photosynthetic active radiation from the supplemental lighting on its own ranged between 180 - 280 µmol m^{-2} s. $^{-1}$

2.6.5. Acidity/ alkalinity of Hoagland's solution (pH) measurement

Measurement of the pH of the Hoagland's solution was taken using a pH meter electrode (Denver Instrument Company, USA) (Plate 2.5). The electrode was calibrated in standard buffer solutions with pH values of 4 and 7 before taking measurements and periodically between measurements. The electrode was immersed in the sample solution and gently stirred until a stable value was recorded. The pH for Hoagland's was between (5.51 - 5.60) and for Hoagland's minus nitrogen between (6.10 to 6.20).

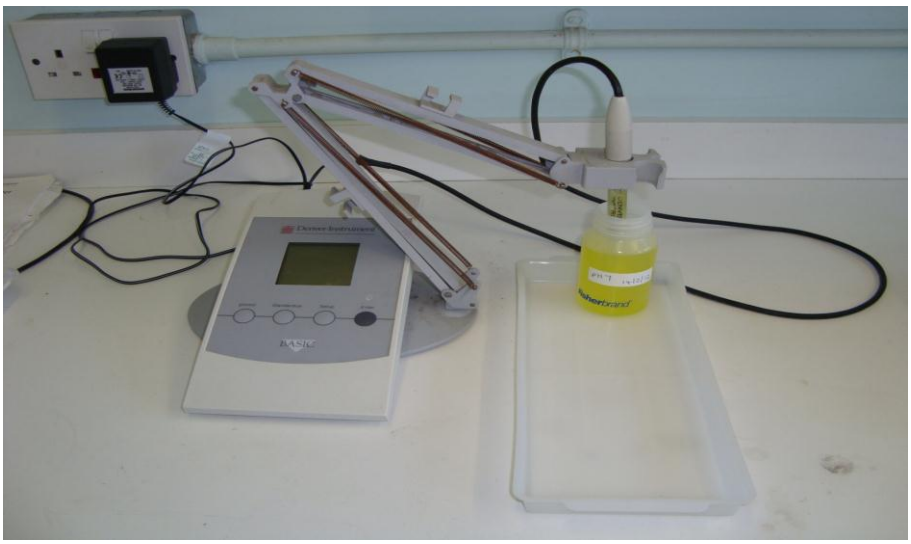

Plate 2. 5. PH meter and electrode (Denver Instrument Company, USA).

2.6.6. Photosynthetic parameters

The photosynthetic activity of the three top expanded leaves for three plants in each treatment within each replicate were measured at anthesis in experiments 2, 3 and 4 (ch 4. 5 & 6) using an LCi Portable Photosynthesis System (ADC BioScientific, Herts. UK) (Plate 2.6). The lace was prepared for use with the

internal battery fully charged and the soda lime was checked to ensure it was in good condition; the plant leaf chamber (PLC) cable was plugged into the lake console connector, the air tube was placed outside of the glass-house and the jaws of the PLC were left open for half an hour before use to stabilize. The flow rate was set at 200 µmol SS^{-1} and stabilization of substomatal CO_2 was used as a guide to settling time for analysis, this was taking about 20 - 40 seconds and the light unit was used at approximately 490 – 520 PAR. The equipment was used with the broad leaf (6 cm^2) chamber with the spectral response from the lamp operated at 11 V DC.

Three parameters are affected by the lamp unit:

- Q_{leaf}

Q_{leaf} effectively provides a measure of the voltage value for Q over the leaf area provided by the chamber in use, and whilst the light level was being adjusted, the Q_{leaf} was seen to vary accordingly on the LCi display until the required level is achieved. The LCi allows the set level for Q_{leaf} to hold for subsequent use in the calculation. The PAR sensor and adaptor are then removed from the chamber and subsequent leaf measurements made with the lamp settings held constant.

Normally Q_{leaf} = Q × Trw, where Trw is the transmission losses through the shield and window of the chamber. Trw = 1, Q_{leaf} = Q. As the PAR sensor in the plane was positioned on the leaf surface, there were no transmissions losses to account for.

- Trw

The Trw average for each type of chamber has been expressed as a fraction of the peak value, for safflower the broad chamber was used and for this chamber, Trw = 0.9.

- H factor

The energy absorbed by the leaf is given by Q × H factor, where the value for H factor quantifies the fraction of visible/infrared energy transmitted/absorbed by the leaf. Since Q was effectively measured at the site of the leaf and most of the infrared energy was removed by the IR absorbing filter built into the lamp unit, H factor is predominantly the conversion from incident quanta between 400 & 700 nm to the radiant energy associated with a tungsten filament. Since this radiant energy is dependent on the filament colour temperature (Tc) as established by the voltage applied, the recommended Hfactors/voltage is 0.160 for lamp voltage 11V and Tc 3000K.

Leaf measurement was made by enclosing the leaf in the leaf chamber. Measurement took up to 4 minutes to readjust to it is new microclimate. During this period CO_2 & H_2O gradually stabilised. After readings were stable a recording was taken.

The system also measured leaf temperature, chamber air temperature, PAR and atmospheric pressure. The PAR at the leaf and the radiant energy balance of the leaf were calculated.

Measured and calculated data were displayed on the LCD on the front panel of the console. By scrolling through the page keys the measured data was

displayed. The data either logged on a PCMCIA type 1 memory card or was sent directly to a dumb terminal via the RS232 serial link connector.

The PC card, which is located in a special holder at the front of the unit, was removed by pressing the eject button and downloaded via a serial link to a PC.

Plate 2. 6. LCi Portable Photosynthesis System (ADC BioScientific Ltd. UK).

2.6.7. Water use efficiency

Instantaneous water use efficiency (WUE) was expressed on an economic yield basis by dividing the biomass production at both anthesis and harvest by the amount of water consumed during the relevant growing period (Conley *et al*., 2001).

2.6.8. Plant morphology, dry weight measurements

The plant height was measured for each plant using a ruler, from the point on the stem at the soil surface to the point of the apical meristem, to the nearest centimeter. Leaf number was counted as all fully expanded leaves on the main stem and branches, and the total leaf area was measured at anthesis using a Delta-T image Analysis System (DIAS^{TM}) (Plate 2.7). The basis of the method is that the device contains a camera which takes images of the leaves and measures their area. After calibration the leaves were flattened and placed under the focal plane of the camera and measurements taken through a computer interface and data stored electronically. LAI was calculated by dividing the total leaf area by the surface area of the pot (Breda, 2003).

Plate 2.7. Plant leaf area meter, Delta-T image Analysis System (DIAS^{TM})

Harvested plants were separated into organs (stem + branch, leaf, capitula) and dried at 80 ^{0}C for 48h in a Gallenkamp drying oven until constant weight and weighed using an Oxford open top-pan balance and an average above ground biomass recorded.

2.6.9. Chlorophyll content measurement

Chlorophyll content of three sub-samples of leaves of three plants selected randomly from each treatment at anthesis was measured. The leaf samples were extracted with acetone 80% (v/v) and the amount of chlorophyll was determined using a spectrophotometer at wavelengths 645 and 663 nm (Porra, 2002)

Chlorophyll extraction

1.1 g of safflower leaves (fresh weight) was placed into a clean mortar

2. 40 mL of 80% (v**/**v) acetone added and the tissue were ground for about 5 minutes using a pestle.

3. The green liquid was carefully transferred to a Buchner funnel containing a pad of whatman No.1 filter paper. After filtering the grinding was repeated with another 30 mL aliquot of 80% acetone. After 3 to 4 minutes this was filtered and added the first extraction.

4. Then the slurry was filtered into the flask containing the other filtrates. The mortar and sides of the funnel were rinsed with 10 mL of 80% acetone to ensure that all the chlorophyll is collected and the filtrated added to flask and

the final volume of the filtered was adjusted to 100 mL by adding additional 80% acetone.

Chlorophyll determination

The amount of chlorophyll was measured using a Heliosepiclon spectrophotometer (Unicam, UK) (Plate 2.8) and was set at wavelength 645 and 663 nm which corresponded to the absorption spectra of Chlorophyll a and b extracted from the leaves.

The optical density (D) of the chlorophyll extracted in a 10 mm cuvette with the spectrophotometer set at 645, 663 and 652 nm was recorded against an 80% acetone solvent blank. Then the amount of chlorophyll present in the extract was calculated on the basis of milligrams of chlorophyll per gramme of leaf tissue according to the following equations:

mg chlorophyll a / g tissue = [12.7 (D 663) – 2.69 (D 645)] × V/ 1000 × W

mg chlorophyll b / g tissue = [22.9 (D 645) – 4.68(D 663)] × V/ 1000 × W

mg total chlorophyll / g tissue = [20.2 (D 645) + 8.2 (D 663)] × V/ 1000 × W

mg total chlorophyll / tissue = D652 × 1000 / 34.5 × v / 1000 x W

D: The optical density reading of the chlorophyll extract at the specific indicated wavelength.

V: the final volume of the 80% acetone – chlorophyll extract

W: the fresh weight in gramme of the tissue extracted.

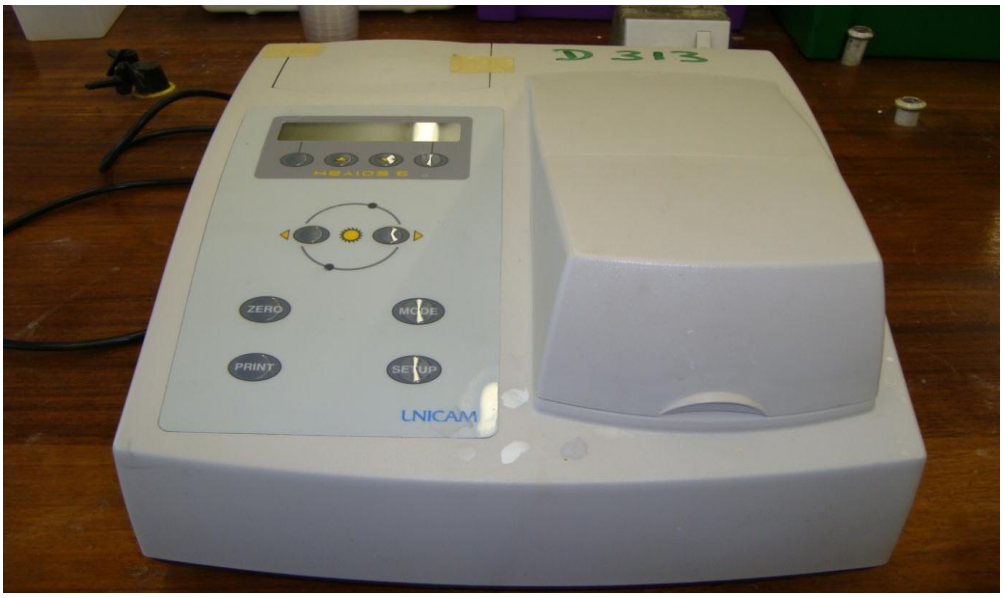

Plate 2.8. Heliosepiclon spectrophotometer (Unicam, UK).

2.6.10. Nitrogen determination of plant parts by Kjeldahl

At anthesis the nitrogen content of different plant organs was determined using Kjeldahl apparatus, Gerhardt, UK Ltd (Bremner, 1996) (Plate 2.9). The basis of the method is the digestion of the sample in strong sulphuric acid in the presence of a catalyst such as sodium sulphate and copper converts the nitrogen compound to ammonium sulphate. The ammonium content in digestion mixture will dissolve and by distillation the amount of ammonia distilled off calculated, and hence the amount of nitrogen determined.

The following steps were followed in conducting the analysis:

1. Digestion: the dried plant materials were weighed and the samples placed into a digestion flask. One sodium sulphate and copper catalyst tablet was added. Then the tubes were put in a digestion block for 3 - 4 h where the tubes

were heated gradually up to 370 ^{0}C and left at this temperature to reflux for 30 minutes and then allowed to cool.

2. Distillation: the digestion solutions were transferred under the fume cupboard to 50 mL volumetric flasks and thoroughly mixed with distilled water to a 50 mL volume. The samples were still warm and were left to cool down for a while, and then the reduced volume was made up again with distilled water to the 50 mL mark and then samples were transferred to 50 mL sealable plastic tubes which were kept in the fridge to be analysed later when the flow injection analyser was available.

3. Titration: Bran and Luebbe Auto analyser 3 (flow injection analyser) was used to analyse the samples for nitrogen concentration. Values were given in mg L $^{-1}$ and nitrogen content as g 100 g $^{-1}$ of the sample dry material calculated as follows:

N content (g100g $^{-1}$) = 50 x N concentration (mg L $^{-1}$) sample dry weight g/1000. The principle of the Auto analyzer operation is that the instrument operates a method known as continuous air segmented flow analysis. An auto sampler is filled with samples, standards and quality controls and the order of analysis is programmed into the computer. A peristaltic pump continuously pumps the entire reagent and the samples from the auto sampler into the chemical manifolds. In the manifolds the samples and reagents are mixed and treated according to the method protocols. On leaving the chemical manifolds the samples are passed through a colorimeter and their absorbance is measured at

a specific wavelength. The computer then compares this to the calibration curve and calculates the concentration of the analyses in the sample.

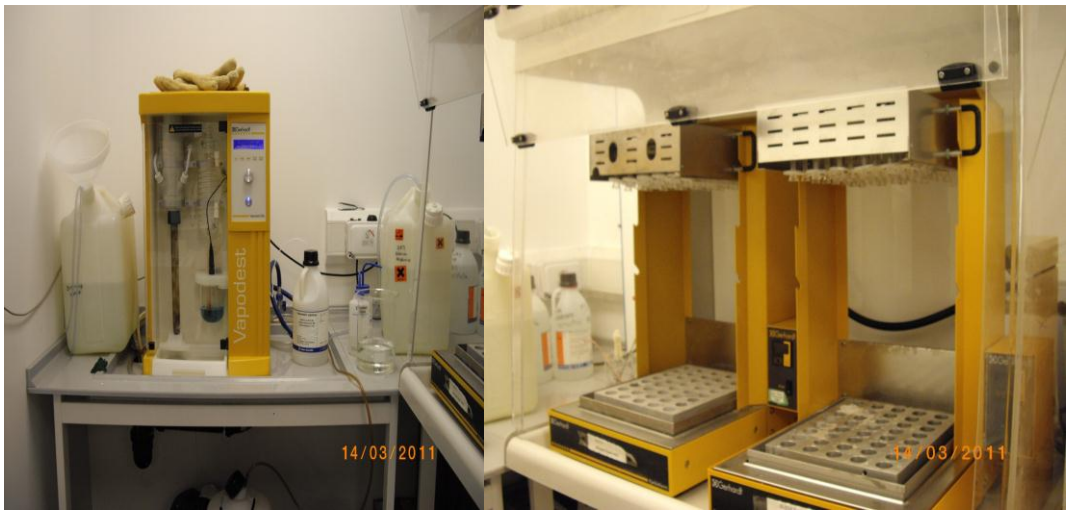

Plate 2.9. Kjeldahl Apparatus (Gerhardt UK Ldt); Distillation unit – vapodestso and Digestion Block – Kjeldatherm.

2.6.11. Seed oil content

Two types of equipment were used in conducting the analysis of seed oil

2.6.11.1. Soxhlet analysis

The oil content was determined following the official AOAC method. The homogenized dry seed was extracted with petroleum ether using a Soxtherm model 41× for 87 min.

The following steps were followed in conducting the analysis:

1. A pinch of anti-bumping granules was placed into each beaker.

2. Samples were weighed into the thimbles and the thimbles were plugged with cotton wool.

3. Thimbles were placed into the unit by fixing the No3 mental adapters to the magnetic ring at the bottom of each condenser, then samples were raised up by pressing the arrow button, (2^{nd} from right on the bottom panel.

4. 140 ml of 40-60 petroleum ether was added to each beaker then the beakers were placed on the Soxtherm.

5. The plastic screen was closed and the machine was run through the program with pre-set boiling and rinsing times.

6. The Soxtherm extraction unit was switched on and the tap water connected to the condensers turned on.

7. Program temperature was set on 150 ^{0}C for petroleum ether and rinsing time determined (Table 2.1).

8. When the run had completed the beakers were transferred into a pre-weighed round bottom flasks and the petroleum ether evaporated using a rotary evaporator and the flasks reweighed and the oil content expressed as a percentage of dry weight (w/w).

Table 2.1. Program details used in the analysis.

Running temperature for Petroleum ether	150 ^{0}C
Temperature limit	200 ^{0}C
Hot extraction	30 minutes
Evaporates A	4X 15 ml
Rinsing time	45 minutes
Evaporation B	3X 15 ml
Evaporation C	Nil
Reduction interval	4 minutes
Pulse	3 seconds

2.6.11.2 Soxhlet extractor

The homogenized fresh extracted with chloroform: methanol (2:1, v/v) using Soxtec system manual 1000 7414, Rev, 3.0. (Stirling University). Total lipids were prepared according to the method of Folch et al., (1957) and non-lipid impurities were removed by washing with 0.88% (w/v) KCl. The weight of lipids was determined gravimetrically after evaporation of solvent and overnight desiccation under vacuum.

The following steps were followed in conducting the analysis:

1. 0.5-3 g of homogenized fresh samples was weighed into an extraction thimble and the weight were record taken to 4 decimal places on LM003. Approximately the same weight of Colette or kieselguhr, added and mixed thoroughly with a spatula and covered with a wad of cotton wool.

2. Extraction cups were weighed (containing 5 -10 glass balls) for each thimble (W1) and recorded to 4 decimal places on a LM003 balance.

3. The Soxtec extraction unit was switched on and the tap water connected to the condensers turned on.

4. Program temperature was set at 160 ^{0}C for chloroform: methanol (2**:**1, v**/**v).

5. The thimbles were placed into the unit by fixing the metal adapters to the magnetic ring at the bottom of each condenser, then samples were raised up by pressing the arrow button, (2nd from the right on the bottom panel).

6. The cups were placed into the holder then half filled with 80 ml of chloroform: methanol (2**:**1, v**/**v) and placed on the metal plate underneath the condensers. The plastic screen was closed, and the machine was run through the program with pre-set boiling and rinsing times.

7. When the run had completed the cups were removed by raising the plastic screen and pressing the arrow button.

8. The cups were placed in the drying oven at 100-105 ^{0}C for between 1- 2h. Then the cops were allowed to cool in a chemical desiccator over the fresh silica gel and reweighed after 1h. The oil content was calculated as % lipid= w2 - w1/ sample weight x 100.

2.6.12. Fatty acid composition

Fatty acid methyl esters (FAME) were prepared by acid-catalyzed transesterification of total lipids according to the method of Christie, (2003). Extraction and purification of FAME were performed as described by Ghioni *et al*., (1996). FAME was separated by gas-liquid chromatography using a Thermo

Fisher Trace GC 2000 (Thermo Fisher, Hemel Hempstead, UK) equipped with a fused silica capillary column (ZBWax 60m x 0.32 x 0.25 mm i.d.; Phenomenex, Macclesfield, UK) with hydrogen as a carrier gas and using on-column injection. The temperature gradient was from 50 to 150 ^{0}C at 40 arc min^{-1} and then to 195 ^{0}C at 1.5 arc min^{-1} and finally to 220 ^{0}C at 2 arc min^{-1}. Individual methyl esters were identified by reference to published data (Ackman, 1980). Data were collected and processed using the Chromcard for Windows (version 2.00) computer package (Thermoquest Italia S.p.A., Milan, Italy). The following steps were followed in accomplishing the analysis:

1 mg of lipid placed into a 15 ml Quick fit test tube and 17:0 free fatty acid standards (Heptadecaenoic acid) added at 10% of the total lipid mass. The organic solvent evaporated off at the nitrogen evaporator.

1. Added 2 ml of the mutilation reagent (1% (v/v) solution of sulfuric acid in methanol.

2. The tubes were flushed with nitrogen and stoppered with a piece of paper to prevent the stopper blowing out when the tub is heated.

3. The tubes incubated overnight (min 16 h, max 18h) at 50 ^{0}C in the hot-block then cooled to room temperature. The tissue or paper removed and 2 ml of 2% $KHCO_{3,}$ added 5 ml of ISO-hexane: diethyl ether (1:1, v/v) + 0.01 % (w/v) BHT.

4. The tubes were gently shaken to mix the content and then centrifuged at 350-400 G for at least for 2 min.

5. The upper organic layer was transferred to another clean test tube using a Pasteur pipette and a further 5 ml is hexane: dithylether (1:1, v/v) added to the original tube mixed and centrifuged as in 8:8 and 8:9 and the upper layer transferred as before. The solvent was evaporated on the nitrogen evaporator.

6. The crude FAME was re-dissolved in 100 µL of ISO-hexane and mixed.

7. Methyl esters were purified by TLC on 20 x 20 cm plate. The samples were loaded on to the TLC plates using a glass Hamilton syringe (rinsed the syringe with ISO-hexane between samples). The plate then chromatographed in ISO-hexane: diethyl ether: acetic acid (90:10:1, v/v) up to 1 - 15 cm from the top of the plate.

8. The plates were then removed from the tank and the solvent allowed evaporating in the fume cupboard.

9. The plates were sprayed with 1% (w/v) iodine in chloroform to visualize the fumes. The outer origins of the plate were masked off with blank glass plates so that only the very outer edge of the bands was exposed. This section was then sprayed lightly with iodine using the atomizer pump. The FAMEs band clearly marked with pencil. The saturated and mono saturated fatty acids are in the upper band and polyunsaturated fatty acids in the lower band. Then the bands scraped from the TLC plate into test tubes using a straight edged scalpel blade.

10. FAMEs were eluted from the silica with 10 mL of iso-hexane: diethyl ether (1:1, v/v) + 0.001% (w/v) BHT. Then mixed and centrifuged as before to sediment the silica. Carefully the solvent was removed to a clean small test tube.

11. The solvent was evaporated on the nitrogen evaporator and the samples transferred to 2 mL glass vials in 0.5-1 mL of iso-hexane, evaporated to dryness and re-dissolved in iso-hexane + 0.01% (v/v) BHT to concentration of 1 mg mL^{-1}. The FAMEs were stored under nitrogen or argon at -18 ^{0}C or less until GLC analysis.

12. After the analysis was completed the results from the GLC were obtained as a weight percent fatty acid composition.

2.7. Statistical analysis

Statistical analyses of data were performed using Minitab v.15 using two-way ANOVA. Significant differences between treatments were determined using least significant differences (L.S.D) at the 0.05 level. Pearson correlations were carried out using SPSS v.19 and significance examined at the 0.05 and 0.01 levels.

For the last experiment (the interaction effect between elevated CO_2 and nitrogen) statistical analysis of data were performed using Minitab v.16 using nested ANOVA (split plot design). Significant differences between treatments were determined using least significances (L.S.D) at the level 0.05.

All graphs were plotted using Microsoft Excel 2010, and the lines fitted with a 2nd order polynomial. All data were tested for normality distribution at $p \leq 0.05$ using Minitab Basic statistics which showed the data were normally distributed and did not require transformation. All data analyses in this thesis were performed on a per plant basis.

Chapter 3

The effect of drought on the physiology, growth, yield and seed oil content of safflowr

3.1. Introduction

The drought is considered to be one of the major environmental stresses for land loss and reduced crop yield, and together with salinity is responsible for a predicted reduction in average yield of up to 50% by the year 2050 as a result of associated climate change events (Wang *et al*., 2003). Therefore, many efforts have been made towards the identification of the choice of crops or varieties suitable for dry land conditions (Öztürk *et al*., 2008) and the interest in safflower has been renewed. As an oilseed crop that is well adapted to arid and semi-arid regions of the world (McPherson *et al*., 2004), safflower is resistance to drought due its deep tap root (Bassiri *et al*., 1977) and coupled with the suitability of its oil for many purposes (Berglund *et al*., 2010) safflower is a candidate species under drought conditions.

In general, the effect of drought stress is usually manifested as a decrease in photosynthesis and growth at the whole plant level (Yordanov *et al*., 2000). Stomatal closure is the one of the first physiological responses to drought which affects the leaf water status and transpiration rate (Kang *et al*., 2002b) with drought reducing stomatal conductance leading to a limitation of CO_2 diffusion into the leaf and thereby into the chloroplast and as a result photosynthetic rate will be reduced (Medrano *et al*., 2002). Non-stomatal limitation of photosynthesis has also been observed such as metabolic impaired adenosine triphosphate (ATP) and decreased ribulose bisphosphate (RuBP), eventually affecting the activity of photosystem under severe drought in C_3 crop species (Flexas *et al*., 2002). However, water stress had no effect on the variation of the maximum fluorescence ratio (Fv/Fm) indicating that drought had no effect on the primary photochemistry of PSII in many C_3 crops including oil seeds such as

sunflower (*Helianthus annus* L.) (Cornic and Fresneau, 2002; Pankovic *et. al*. ; Pastenes *et. al*., 2005; Subrahmanyam *et al*., 2006) and it has been shown that PS II is quite resistant to water stress (Shangguan *et al*., 2000a). The response of plants to drought conditions is determined by the duration of the drought (Bray, 1997), the degree of water stress and the critical stage of growth (Aiken and Lamm, 2006).

In spite of the ability of safflower to survive under dry and hot conditions, and its potential as an oil seed crop for low rainfall areas (Öztürk *et al*., 2008) it is still likely to be affected by drought but such response is not reported widely in the literature. Islam, (2011) has recently reported drought induced physiological modification in five safflower genotypes via reduction in stomatal conductance, relative leaf water content, and osmotic adjustment when water stress was imposed from 80% flowering to maturity (terminal drought) and as a result seed yield seed oil content decreased with significant differences among genotypes. Further, water deficit has been reported to decrease the maximum quantum yield of PSII photochemistry (Fv/Fm) ratio in six safflower genotypes at both heading and pollination stages, with a maximum evaporation rate of 135 mm (among three evaporation rates: 75, 105 and 135 mm) and seed/ head and heads/plant decreased (Miladi and Ehsanzadeh, 2010). Water use efficiency

(WUE) in safflower was increased with increase in the water supply at the vegetative stage in the winter growing season in Eastern India (Kar *et al*., 2007), and (Istanbulluoglu *et al*., 2009) stated that WUE only increased when plants were irrigated at the vegetative stage compared to when they were irrigated only at the seed filling stage in Turkey (winter sown crop).

In addition, Sharrifmoghaddasi and Omidi, (2010) concluded that safflower had a significant net assimilation rate (NAR) and LA when irrigated at all stages of it is grown, including emergence, stem elongation, bud formation, beginning of flowering, 50% flowering, end of flowering and as a result seed and oil yield significantly increased under rain fed conditions. It has been indicated that pre antithesis translocation of dry matter and stored nitrogen is crucial to produce high yields under Mediterranean climatic conditions (Koutroubas *et al*., 2004). However, the stages of flowering and seed filling were considered as the critical stages for irrigation of safflower to produce viable seed and oil yields (Sharrifmoghaddasi and Omidi, 2010). In semi-arid and Mediterranean environments, late sowing of spring safflower led to a lower seed yield due to the later flowering coincides with the terminal drought and extreme heat (Yau, 2007). Moreover, a negative effect of drought on safflower seed yield and seed oil content have been frequently reported under similar conditions with some significant differences in respect to genotypes. The number of capitula per plant and numbers of seed per plant and numbers of seed per capitulum were the important yield component of safflower determining the response to drought (Abel and Driscoll, 1976; Steer and Harrigan, 1986) and these traits decreased under water deficit (Eslam, 2011; Ferasat *et al*., 2008; Miladi and Ehsanzadeh, 2010; Pasban Eslam and Sadeqi, 2008). Also seed and oil yield have been strongly correlated to stem height under drought in safflower (Bagheri, 2011b).

Safflower response to water stress in the field may be different to that under the experimental pot grown conditions and there are no reports in the literature on the effect of water stress on safflower grown in pots. This investigation reports

the results of a detailed study to investigate the effects of drought on the growth, physiology and yield of safflower grown in pots in the glass-house.

3.2. Aim

To evaluate the effect of drought on safflower physiology, growth performance, seed yield and oil content.

3.3. Objectives

3.3.1. Objective 1

Examine the physiological response of safflower to drought via an investigation of stomatal conductance and chlorophyll fluorescence.

3.3.2. Objective 2

Investigate the effect of drought on safflower growth in term of stem height, leaf number and dry matter accumulation in different plant organs.

3.3.3. Objective 3

Assess the seed oil content of safflower with respect the effect of stage of imposing drought.

3.4. Materials and Methods

3.4.1. Experimental design and measurements taken

The experiment was carried out in a semi-controlled glass-house during the year 2009 located at Plymouth University, UK. The experimental design was a randomized block with four replicate comprising of 48 pots filled with John Innes N^{O}.2 loam based compost. The treatments consisted of four watering regimes: 1. Control, where all pots well watered, 2. Mid-season drought which commenced 20 days after planting at the rosette stages, 3. terminal drought which commenced after 60 days from planting during stem elongation and 4. Mid-season + terminal drought from rosette stage through to harvest.

The amounts of water for well watered and drought regimes were established on the basis of the available water capacity of the compost as described in chapter 2. Air temperature was logged using a TinyTag^{TM} data logger. Mean weekly maximum and minimum temperatures during growth period are presented in Figure 3.1.

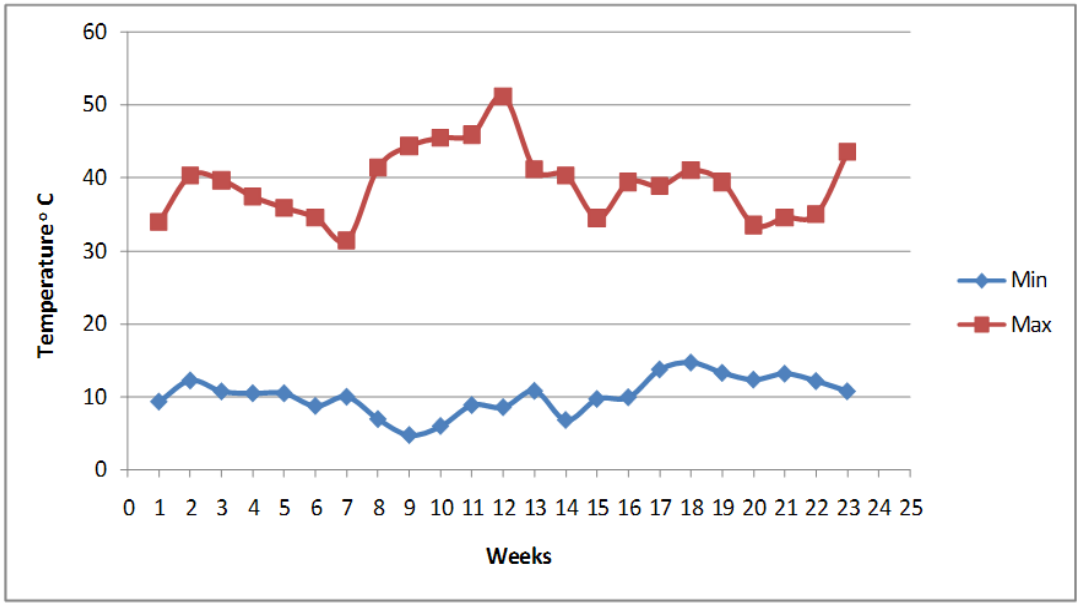

Figure 3.1. Weekly mean maximum and minimum temperatures during the growing period from March to August 2009.

Leaf stomatal conductance was measured (see ch 2) at weekly interval. The best time of day for taking measurements was established by taking hourly readings from 07:00 until 19:00 for the upper expanded leaf for 6 plants in each replicate and each treatment. Results showed that the high transpiration rate occurred in the morning between (09:00 and 11:00) and then declined steadily in the afternoon for both well watered (control) and drought treated (Figure 3.2). Droughted plants tended to have a lower transpiration rate throughout the afternoon period and this typically reflected an imbalance in transpiration losses at the leaves and water uptake from the roots.

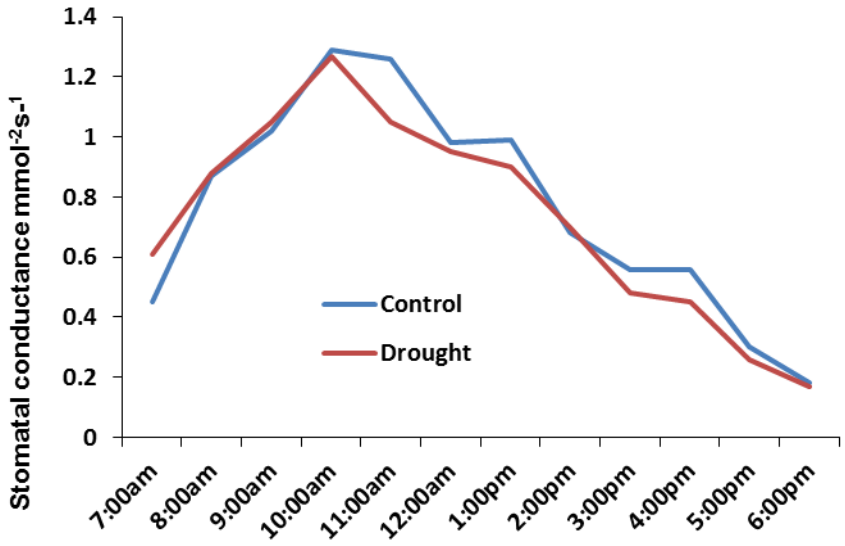

Figure 3. 2. Mean stomatal conductance in the upper leaf of safflower plants through the day.

Given the results presented in Figure 3.2 and in order to make comparisons across treatments it was decided that stomatal conductance would be measured throughout the experiment at two times in the day between 09:00 to 11: 00 in the morning and again in the afternoon from 16:00 to 18:00 on the day after the application of irrigation.

Measurements were taken for three plants for each treatment in each replicate. The upper most fully expanded leaves of the same age were selected. In this way, different leaves were measured each time until branching and then on the same leaves each time. Chlorophyll fluorescence was measured during the growing period at weekly interval (one day after each stomatal measurement) (see ch 2). The days to 50% flowering were recorded. At harvest the plant stem height was determined and number of leaves, branch number, capitula number and seed number counted. Plants organs were dried weighed. The seed were weighed using an Oxford closed top balance and then placed in a -20 ^{0}C freezer for 48 h and then dried by freeze dryer at - 44 ^{0}C for about 1 h to

constant weight. Oven drying of seed was not practiced due to potential volatilisation of oils and lipids. The determined weight loss was taken as moisture. The samples were then ground using a pestle and mortar in preparation for oil analysis (see ch 2). Harvest index was determined by dividing seed yield by total biomass (biological yield).

3.5. Results

3.5.1. Stomatal conductance

During the experiment the stomatal conductance of control plants significantly ($p \leq 0.05$) declined with increasing maturity when measured during the morning at the peak of stomatal conductance (Figure 3.3, Table 3.1 and Table 3.2). Initially there was a small but steady decline in stomatal conductance during the late rosette period but this was followed by a steep decline during stem elongation before returning to a steadier but the lower stomatal conductance until maturity. The reasons for this decline are not entirely clear but the rapid decline occurred at the same time as rapid stem elongation during which times leaves continued to appear. After stem elongation (70 days) measurement of stomatal conductance could be made on the same leaf each time and therefore the later stomatal conductance (70 - 115) days and the subsequent decline is probably due to a leaf age related stomatal closure competency effect.

The imposed drought had the effect of reducing mean stomatal conductance below that of the control as shown in Figure (3.3) and Table (3.1). This is demonstrated more clearly in Figure (3.4) when the control data is subtracted from drought data to give the differences values (“delta conductance”). When drought was applied early (mid-season drought) it caused a big drop in stomatal conductance and was only 50% of the control by day 47 (Figure 3.3) about three weeks after imposing drought. In those pots where the mid-season drought was relieved (at about 60 days) the plants showed some recovery in stomatal conductance until 88 days (3 weeks later) when it was again the same as the control. When drought was applied late (terminal drought) it also reduced

stomatal conductance drastically ($p \leq 0.05$) compared to the control and this reduction continued until maturity (Figure 3.3)

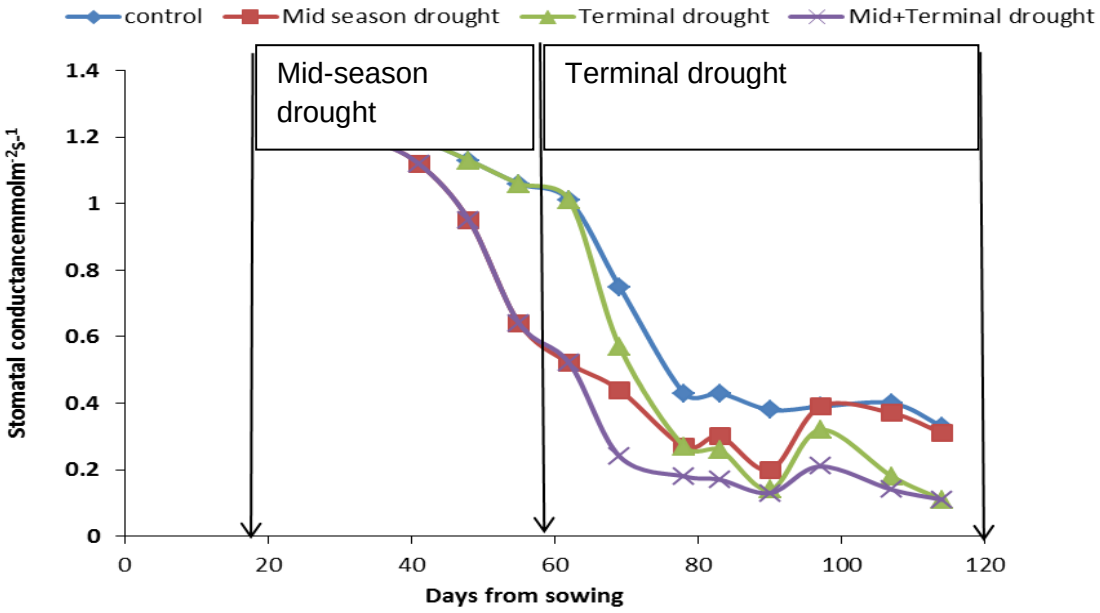

Figure 3.3. Mean of stomatal conductance under different water regimes in the morning during growth period.

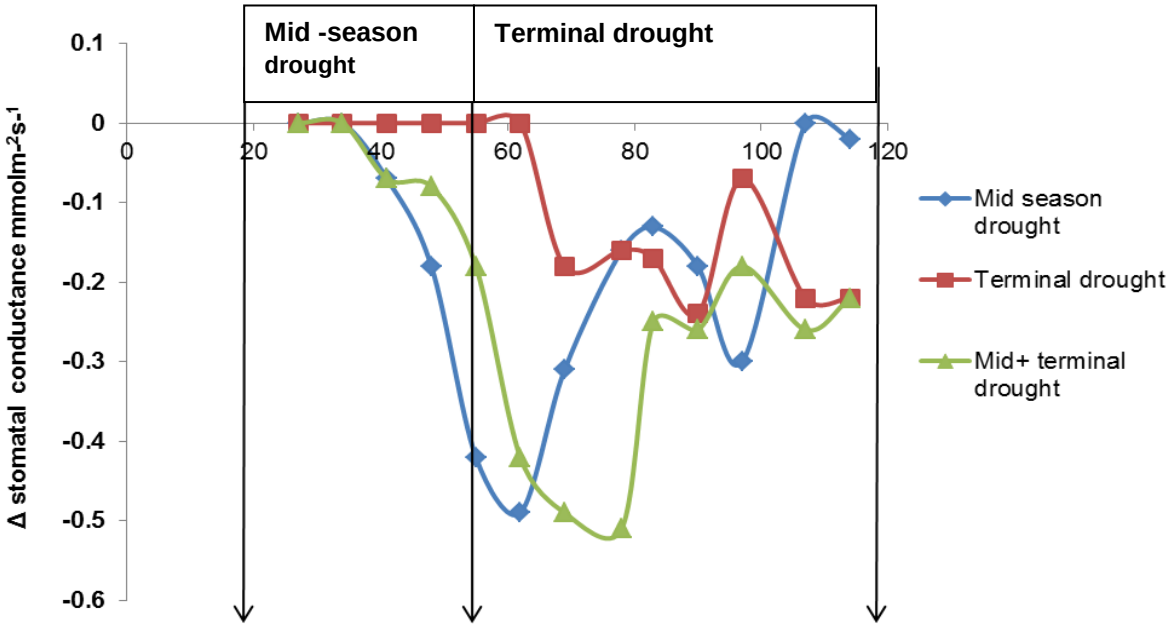

Figure 3.4. Mean of stomatal conductance differences from the control in the morning during growth period.

Finally, when the drought was applied at both times (mid-season + terminal drought) it produced the lowest ($p \leq 0.05$) recorded stomatal conductance of all plants.

Table 3.1. Mean value of stomatal conductance mmol m^{-2} s^{-1}(measured in the morning) under different water regimes during the growth period showing significant differences within a measurement time period (i.e. within a column).

Water regimes	***Days from sowing***												
	27	**34**	**41**	**48**	**55**	**62**	**69**	**78**	**83**	**90**	**97**	**107**	**114**
Control	1.29	1.19	1.88	1.13 [a]	1.06 [a]	1.008 [a]	0.75 [a]	0.43 [a]	0.43 [a]	0.41 [a]	0.26 [a]	0.40 [a]	0.33 [a]
Mid-season drought	1.19	1.19	1.20	0.95 [b]	0.64 [b]	0.52 [b]	0.44 [b]	0.27 [b]	0.30 [b]	0.39 [a]	0.20 [b]	0.32 [b]	0.31 [a]
Terminal drought	1.29	1.19	1.88	1.13 [a]	1.06 [a]	1.008 [a]	0.57 [c]	0.28 [b]	0.26 [b]	0.32 [b]	0.18 [b]	0.18 [c]	0.11 [b]
Mid-season +terminal drought	1.88	1.19	1.12	0.95 [b]	0.64 [b]	0.52 [b]	0.24 [d]	0.18 [b]	0.17 [c]	0.12 [c]	0.13 [c]	0.14 [d]	0.11 [b]
P value	0.581	1.000	0.603	0.000	0.000	0.000	0.000	0.007	0.001	0.000	0.000	0.000	0.000
L.S.D at 0.05	N.S	N.S	N.S	0.022	0.040	0.060	0.13	0.11	0.08	0.06	0.04	0.06	0.059

*Means followed by the same letter within the column are not significantly different at 0.05 levels

Table 3.2. Mean value of stomatal conductance mmol m^{-2} s^{-1} (measured in the morning) under different water regimes during the growing period showing significant differences over time within a treatment (i.e. within a row).

Water regimes	***Days from sowing***														
	27	**34**	**41**	**48**	**55**	**62**	**70**	**77**	**84**	**93**	**100**	**107**	**114**	**P**	**L.s.D at 0.05**
Control	1.29 [a]	1.19 [a]	1.18 [b]	1.13 [b]	1.06 [b]	1.008 [b]	0.75 [b]	0.43 [c]	0.43 [c]	0.43 [c]	0.27 [d]	0.40 [c]	0.33 [d]	0.000	0.12
Mid-season drought	1.19 [a]	1.19 [a]	1.12 [a]	0.95 [a]	0.64 [b]	0.52 [c]	0.43 [d]	0.27 [e]	0.30 [e]	0.30 [e]	0.20 [e]	0.35 [e]	0.31 [e]	0.000	0.12
Terminal drought	1.29 [a]	1.19 [a]	1.19 [a]	1.13 [a]	1.06 [a]	1.008 [a]	0.57 [b]	0.28 [c]	0.26 [c]	0.26 [c]	0.14 [d]	0.18 [d]	0.11 [d]	0.000	0.11
Mid-season +terminal drought	1.19 [a]	1.19 [a]	1.12 [b]	0.95 [b]	0.64 [c]	0.32 [c]	0.18 [d]	0.17 [d]	0.17 [d]	0.17 [d]	0.13 [d]	0.14 [d]	0.11 [d]	0.000	0.10

*Means followed by the same letter within the rows are not significantly different at 0.05 leve

A similar pattern was shown for the stomatal conductance in the afternoon although overall, stomatal conductance at this time was a lot lower than in the morning. For control plants the mean stomatal conductance again significantly declined with increasing maturity (Figure 3.5, Table 3.3 and Table 3.4). There was a steady decline during the early equivalent period for the mid-season drought before it then returned to a slightly reduced level until day 115.

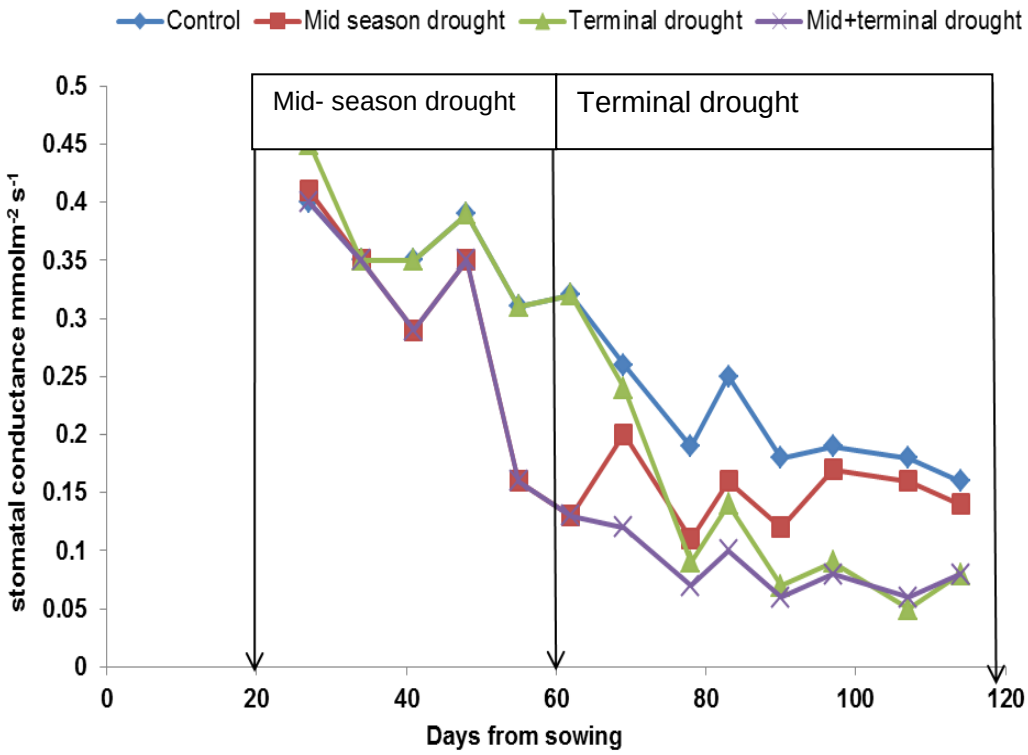

Figure 3.5. The mean of stomatal conductance under different water regimes in the afternoon during growth period.

Drought again had the effect of reducing the mean stomatal conductance below that of control (Figure 3.5. Table 3.3 and Table 3.4). When early (mid-season drought) was applied stomatal conductance was significantly ($p \leq 0.05$) reduced by about 50% at 41 days after sowing. After 63 days the plants recovered after re-watering, but there was a steady fluctuation until 95 days when it was again the same as control.

Stomatal conductance was drastically ($p \leq 0.05$) reduced when late (terminal) drought was applied and this reduction continued in a steady manner until day 115.

The lowest value of stomatal conductance was recorded with the full drought treatment (both mid-season + terminal drought).

Table 3.3. Mean value of the stomatal conductance mmol m^{-2} s^{-1} for each water regimes during growing period in the afternoon showing significant difference within a time period (i.e. within column).

Water regimes	***Days from sowing***												
	27	**34**	**41**	**48**	**55**	**62**	**70**	**77**	**84**	**93**	**100**	**107**	**114**
Control	0.40	0.35	0.35	0.39 [a]	0.31 [a]	0.32 [a]	0.26 [a]	0.25 [a]	0.18 [a]	019 [a]	0.18 [a]	0.18 [a]	0.16 [a]
Mid-season drought	0.41	0.35	0.35	0.35 [b]	0.16 [b]	0.13 [b]	0.20 [b]	0.16 [b]	0.12 [b]	0.17 [a]	0.16 [b]	0.16 [b]	0.14 [b]
Terminal drought	0.45	0.35	0.35	0.39 [a]	0.31 [a]	0.32 [a]	0.24 [b]	0.14 [b]	0.07 [b]	0.09 [b]	0.14 [b]	0.14 [b]	0.083 [b]
Mid-season +terminal drought	0.40	0.35	0.35	0.35 [b]	0.16 [b]	0.13 [b]	0.12 [c]	0.95 [c]	0.06 [b]	0.08 [b]	0.058 [c]	0.058 [c]	0.078 [c]
P value	0.104	0.998	0.998	0.066	0.000	0.000	0.005	0.005	0.038	0.042	0.017	0.007	0.017
L.S.D. at 0.0 5	N.S	N.S	N.S	0.026	0.021	0.069	0.060	0.060	0.092	0.078	0.066	0.13	0.051

*Means followed by the same letter within the column are not significantly different at 0.05 levels.

Table 3. 4. Means value of the stomatal conductance mmol m^{-2} s^{-1} for each water regimes during growing period in the afternoon showing significant differences over time within a treatment (i.e. within a row).

Water regimes	***Days from sowing***														
	27	**34**	**41**	**48**	**55**	**62**	**70**	**77**	**84**	**93**	**100**	**107**	**114**	**P**	**L.S.D**
Control	0.40 ^{a}	0.35 ^{a}	0.35 ^{a}	0.39 ^{a}	0.31 ^{b}	0.32 ^{b}	0.24 ^{b}	0.26 ^{b}	0.25 ^{b}	0.18 ^{c}	0.19 ^{c}	0.18 ^{c}	0.16 ^{c}	0.000	0.073
Mid-season drought	0.41 ^{a}	0.35 ^{a}	0.35 ^{a}	0.39 ^{a}	0.16 ^{c}	0.13 ^{c}	0.20 ^{c}	0.28 ^{b}	0.16 ^{c}	0.12 ^{c}	0.17 ^{c}	0.16 ^{c}	0.14 ^{c}	0.000	0.069
Terminal drought	0.45 ^{a}	0.35 ^{a}	0.35 ^{a}	0.39 ^{a}	0.31 ^{a}	0.32 ^{a}	0.24 ^{b}	0.24 ^{b}	0.14 ^{b}	0.07 ^{c}	0.088 ^{c}	0.14 ^{b}	0.083 ^{c}	0.000	0.072
Mid-season +terminal drought	0.40 ^{a}	0.35 ^{a}	0.35 ^{a}	0.35 ^{a}	0.16 ^{b}	0.13 ^{b}	0.12 ^{b}	0.12 ^{b}	0.095 ^{c}	0.06 ^{c}	0.80 ^{c}	0.06 ^{c}	0.08 ^{c}	0.000	0.053

*Means followed by the same letter within the rows are not significantly different at 0.05 levels.

3.5.2. Chlorophyll fluorescence

The maximum quantum yield of PSII chemistry when measured in the morning for control plants was constant from sowing and from 42 to 65 and 69 days (Figure 3.6, Tables 3.5 and 3.6) with a Fv/Fm ratio value of approximately 0.80 ± 0.03. It then declined substantially at day 65 and day 74 but recovered back to approximately 0.75 for the rest of the experiment. The reasons for this sudden decline are not entirely clear but high temperature inside the glass-house (above 40 ^{0}C) might be responsible for the decline in this period. Exceptionally high temperature themselves may have been responsible for the decline or associated increased stomatal closure and transpiration rate may have accentuated a drought effect.

None of the three water stress treatments had any significant ($p \geq 0.05$) effect on Fv/Fm ratio until 65 days from sowing when there was a small but significant ($p < 0.05$) reduction for terminal and mid-season + terminal drought compared with the control. The pattern of results was similar when measured in the afternoon (Figure 3.7 and Table 3.6) showing the same reduction at 79 and 93 days but in contrast to the morning measurements this recovered and remained low until 107 days. Small but significant reductions associated with terminal and mid-season terminal drought were also evident.

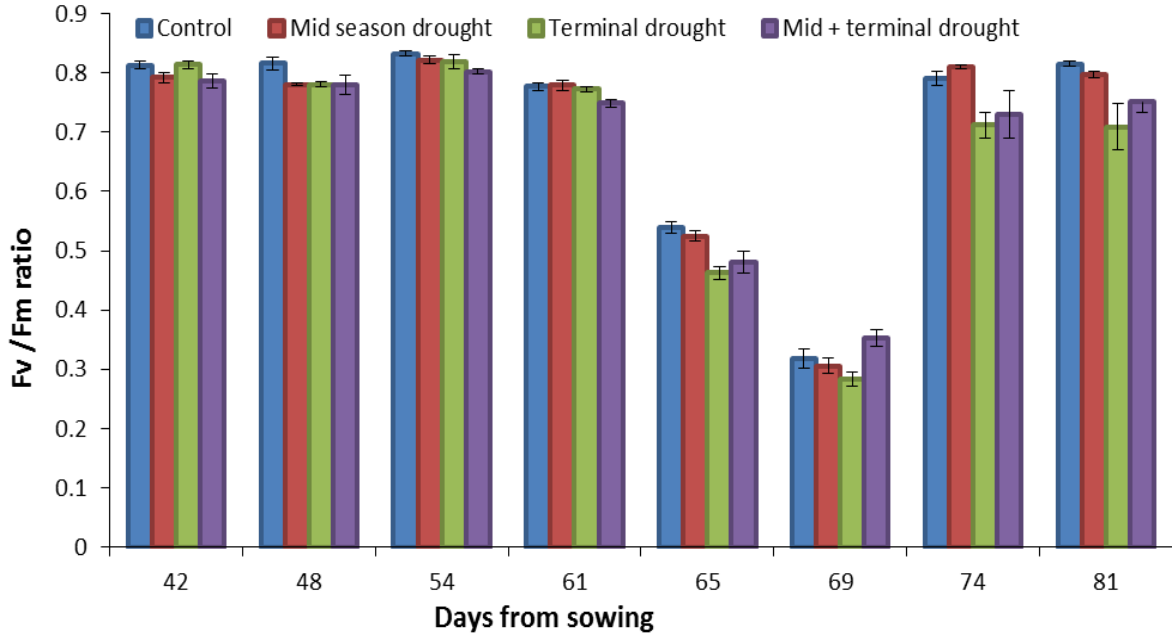

Figure 3.6. Means ratio of variable fluorescence (Fv) to maximum fluorescence (Fm) under different water regimes in the morning during growth period. Vertical bars are standard error of the mean (n= 12) at 0.05 levels.

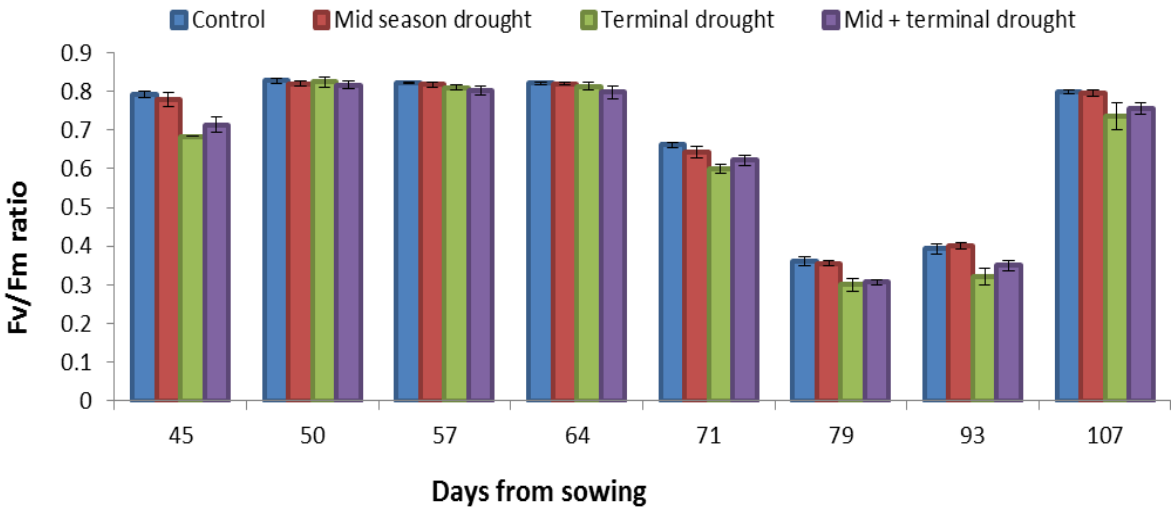

Figure 3.7. Means ratio of variable fluorescence (Fv) to maximum fluorescence (Fm) under different water regimes in the afternoon during growth period. Vertical bars are standard error of the mean (n= 12) at 0.05 levels.

Table 3.5. Mean value of the ratio of variable fluorescence (Fv) to maximum fluorescence (Fm) versus maturity under different water regimes in the morning during growth period.

Water regimes	***Day from sowing***									
	45	***50***	***57***	***64***	***71***	***79***	***93***	***107***	***p***	***L.S.D***
Control	0.81 [a]	0.82 [a]	0.83 [a]	0.78 [b]	0.54 [c]	0.32 [d]	0.79 [a]	0.82 [a]	0.000	0.03
Mid- season drought	0.79 [a]	0.78 [a]	0.82 [a]	0.78 [a]	0.53 [b]	0.31 [c]	0.81 [a]	0.80 [a]	0.000	0.03
Terminal drought	0.81 [a]	0.81 [a]	0.82 [a]	0.77 [a]	0.46 [c]	0.28 [d]	0.71 [b]	0.71 [b]	0.000	0.06
Midseason+ terminal drought	0.79 [a]	0.78 [a]	0.80 [a]	0.75 [a]	0.48 [b]	0.35 [b]	0.73 [a]	0.75 [a]	0.000	0.06

*Means followed by the same letter within the rows are not significantly different at 0.05 levels.

Table 3.6. Means value of the ratio of variable fluorescence (Fv) to maximum fluorescence (Fm) versus the maturity under different water regimes in the afternoon during growing period.

Water regimes	***Day from sowing***									
	45	***50***	***57***	***64***	***71***	***79***	***93***	***107***	***P***	***L.S.D at 0.05***
Control	0.79 [b]	0.83 [a]	0.82 [ab]	0.82 [ab]	0.66 [c]	0.36 [d]	0.39 [d]	0.80 [a]	0.000	0.04
Midseason drought	0.79 [c]	0.83 [a]	0.82 [b]	0.82 [b]	0.64 [d]	0.35 [e]	0.40 [f]	0.80 [c]	0.000	0.01
Terminal drought	0.68 [b]	0.82 [a]	0.81[a]	0.81 [a]	0.60 [c]	0.30 [d]	0.32 [d]	0.73 [b]	0.000	0.08
Mid-season+ terminal drought	0.71 [a]	0.80 [a]	0.80 [a]	0.80 [a]	0.80 [a]	0.62 [b]	0.31 [c]	0.35 [c]	0.000	0.07

*Means followed by the same letter within the rows are not significantly different at 0.05 levels.

3.5.3. Plant development, growth and seed yield at harvest

The time from germination to 50% flowering was 105 days for well watered plant and this was similar to the plants subjected to mid-season drought and terminal drought. However for the mid-season drought time to flowering was reduced to 101 days. Plant morphology in term of plant height, leaf number, branch number and capitula number were significantly affected by drought. Plant stem height was significantly ($p \leq 0.05$) affected by drought but there was no significant effect between mid-season and terminal drought on plant stem height. The smallest plants were observed under mid-season + terminal drought (Figure 3.8), also the extreme water stress (mid-season + terminal) significantly ($p \leq 0.05$) decreased the leaf number as compared with control and other drought treatments (Table 3.7).

Among the water stresses imposed mid-season drought had no significant effect on yield component (branch and capitula number) compared with the control. Well watered plants were superior in producing seed yield and all water stresses significantly reduced seed number compared with the control ($p \leq 0.05$) with reduction under terminal and mid-season + terminal being greater compared with mid-season drought alone.

Table 3.7. Mean of plant development criteria and yield component number/plant, and plant organs dry weight (gpl^{-1}) under different Water regimes at harvest.

Water regimes	*Leaf NO.*	*Branch NO.*	*Capitula NO.*	*Seed NO.*	*Stem and branch dry weight*	*Leaf dry weight*	*Capitula dry weight*	*Fresh seed weight*	*1000 weight*
control	46 [a]	2 [a]	3 [a]	171 [a]	5.40 [a]	3.18 [a]	2.40 [a]	7.00 [a]	41 [a]
Midseason drought	42 [a]	2 [a]	3 [a]	88 [b]	4.05 [b]	2.00 [b]	1.63 [b]	3.60 [b]	41 [a]
Terminal drought	43 [a]	1 [b]	2 [b]	18 **c**	3.98 [c]	2.70 [a]	0.85 [c]	0.66 [c]	35 [b]
Midseason+ terminal drought	37 [b]	1 [b]	1 [c]	13 [c]	2.33 [d]	1.50 [b]	0.95 [c]	0.44 [c]	34 [b]
P value	0.012	0.000	0.002	0.001	0.000	0.007	0.005	0.001	0.000
L.S.D	4.43	0.20	0.85	60	0.91	0.84	0.77	2.44	2.39

*Means followed by the same letter within the column are not significantly different at 0.05 levels

Plant growth, in term total above ground biomass was driven from above ground biomass except seed markedly affected by drought; shoot and branches weighed significantly less ($p \leq 0.05$) under water stress with similar values for terminal and mid-season drought (Table 3.7), whilst mid-season drought had more effect on stems and branches dry weight compared with the control. All water stress regimes significantly ($p \leq 0.05$) reduced leaf weight by same amount compared with the control. For capitula dry weight a significant ($p \leq 0.05$) reduction was recorded only for terminal and mid-season + terminal drought plants compared to the control and mid-season drought.

Seed set in this experiment was a problem and overall low seed set was recorded. However, seed set was in proportion to the treatments. All drought treatments had a significant ($p \leq 0.05$) effect on seed dry weight (Figure 3.10). The greatest reduction was recorded for terminal and mid-season + terminal drought compared with mid-season drought alone. Despite this reduction in seed yield, 1000 seed weight was conserved with only small differences between treatments and only the mid-season + terminal drought had a significantly lower 1000 seed weight compared with the control (Table 3.7). All of the water stress significantly ($p \leq 0.05$) decreased the above ground biomass (Figure 3.9), biological yield (seed dry weight/ above ground dry weight) (Figure 3.11) and consequently decreased harvest index (Figure 3.12) compared with the control.

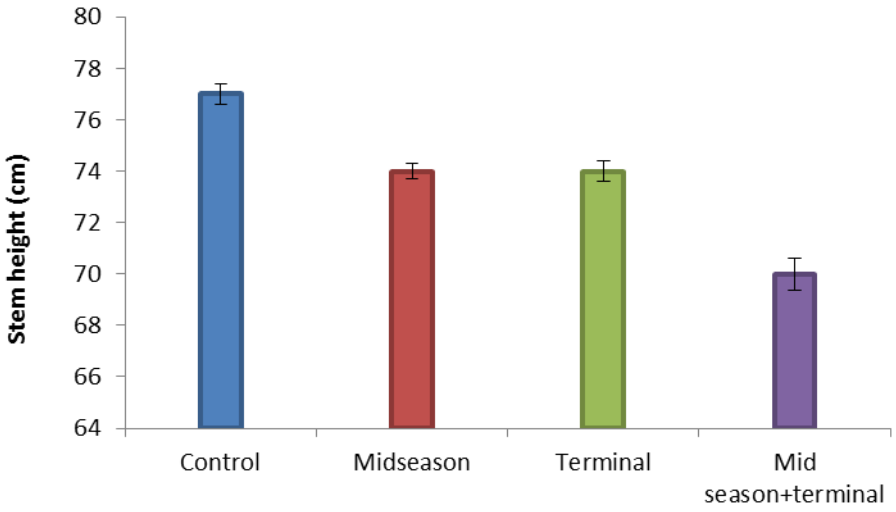

Figure 3.8.Mean of plant height under different water regimes.
Vertical bars are standard error of the mean (n= 12) at 0.05 levels.

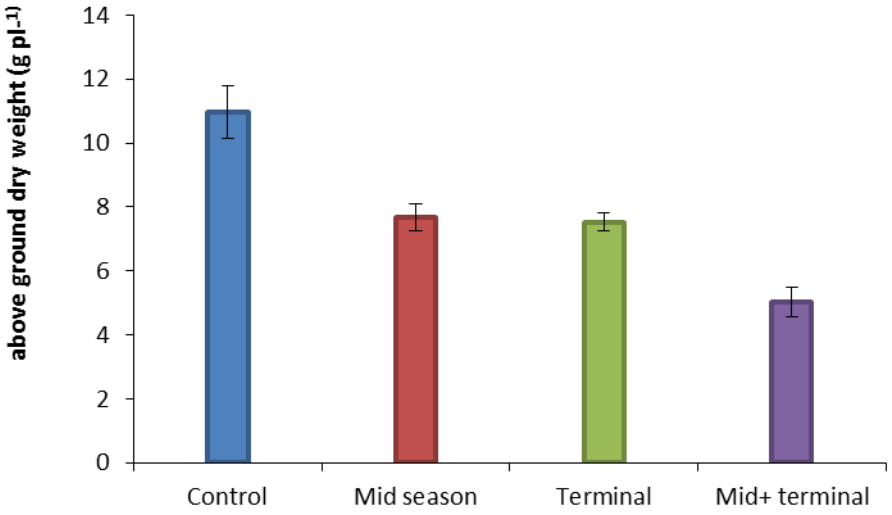

Figure 3. 9. Mean of above ground dry weight under different water regimes.Vertical bars are standard error of the mean (n= 12) at 0.05 levels.

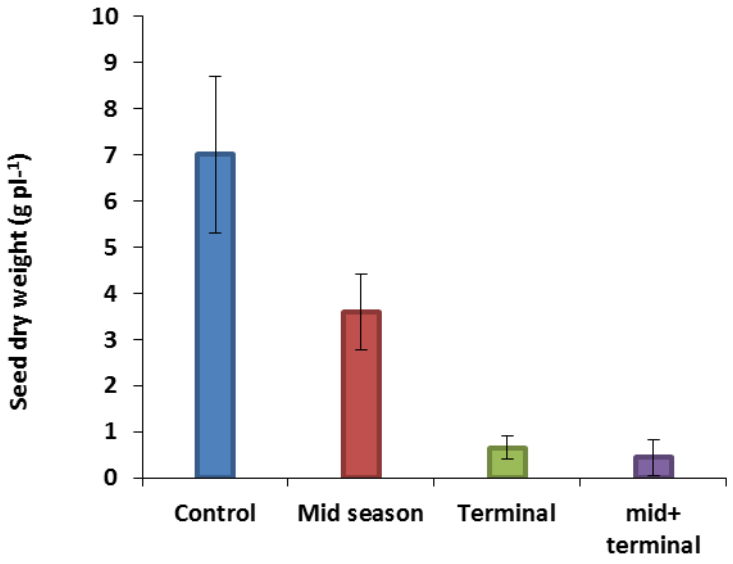

Figure 3.10. Meanof seed dry weight under different water regimes. Vertical bars are standard error of the mean (n = 12) at 0.05 levels.

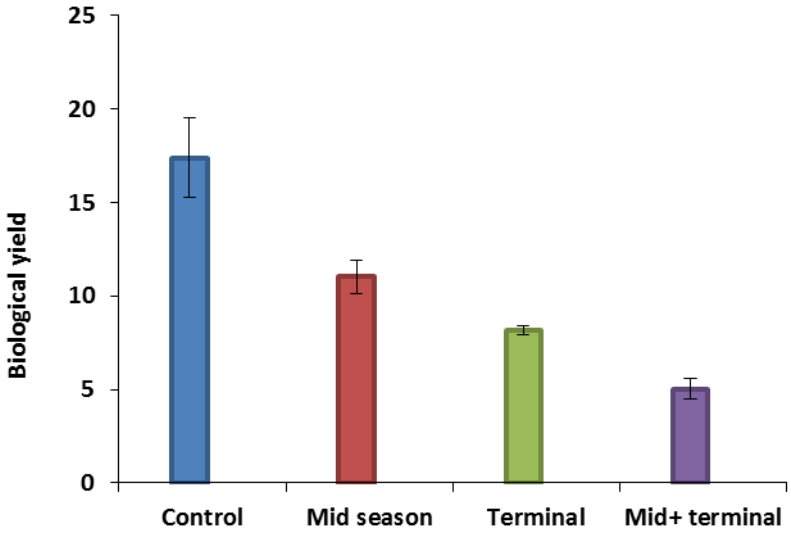

Figure 3.11. Mean of biological yeild under different water regimes. Verticalbars are standard error of the mean (n = 12) at 0.05 levels .

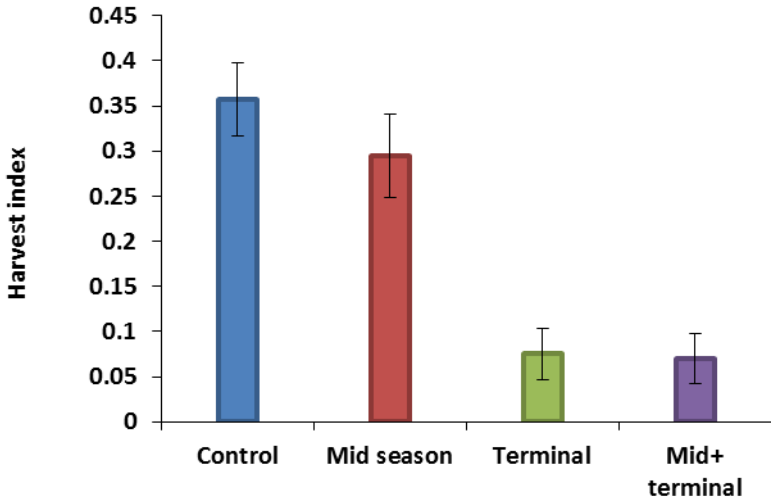

Figure 3.12. Mean of harvest index under different water regimes. Vertical bars are standard error of the mean (n = 12) at 0.05 levels.

3.5.4. Chemical analysis

Due to insufficient quantities of seed obtained at harvest, only seed moisture content and gross oil content were carried out. The average oil concentration of dry seed is shown in Table (3.8) and was significantly decreased ($p \leq 0.05$) by the drought treatments. The highest reduction occurred under terminal drought and with midseason + terminal drought. The seed moisture content was strongly affected by water stresses despite the relatively small effect on 1000 seed weight reported above. Whilst this appeared as an inconsistency it in fact reflects the naturally very low moisture contents of safflower seed and in contrast the high oil and protein content.

Table 3.8. Mean of oil and water of seed content under four watering regimes.

Water regimes	***constituents***	
	% oil	***% moisture***
Control	21 [a]	5.8 [a]
Mid-season drought	20 [a]	2.6 [b]
Terminal drought	18 [b]	0.5 [C]
Midseason + terminal drought	18 [b]	0.4 [C]
P value	0.000	0.000
L.S.D	0.98	0.25

*Means followed by the same letter within a column are not different significantly at 0.05 levels.

3.5.5. Correlations

Pearson correlations (Table 3.9), showed that there is a positive correlation between stem height (H) above ground biomass (AGB), capitula number (NC), seed number(NS), fresh seed weight (FSW) and seed oil content (OC) all per plant, under both well irrigated and droughted treatments. This means that all of these parameters reduced together under drought treatments.

Table 3.9. Pearson correlation among stem height, biomass, fresh seed weight and seed oil content under different water regimes.

	H	***AGB***	***NC***	***NS***
H				
AGB	0.813			
NC	0.879	0.812		
NS	0.836	0.999 **	0.816	
FSW	0.837	0.999 **	0.819	1.000 **
OC	0.793	0.987	0.870	0.980 **

** Correlation is significant at the 0.01 level (2-tailed).

3.6. Discussion

This study confirmed that stomatal conductance increases in safflower in the morning with highest values between 09:00 and 11:00 and thereafter gradually decreases throughout the afternoon (Figure 3.2). This means that the corresponding transpiration rate increases in the morning and thereafter decreases throughout the afternoon. A similar finding was reported for cowpea (*Vigna unguiculata*) (Bates and Hall, 1982). Stomatal conductance decreased in this study with maturity in both normally watered and drought stressed plants and such a result is commonly reported (Field and Mooney, 1983). The water stresses imposed in this experiment reduced stomatal conductance compared to the control and this could be interpreted as decreased relative leaf water content (RWC). This result supports recent work on field grown safflower by Eslam (2011) who indicated that water stress induced decreases in RWC and stomatal conductance and recorded values for stomatal conductance ranging between 0.41 for well watered and 0.23 for drought imposed from 80% flowering to maturity (i.e. terminal drought). In the work reported in this chapter the recorded values for stomatal conductance were about 0.40 to 0.20 for control and drought respectively, from rosette to maturity (midseason + terminal drought) on the same day (see Figure 3.3) and the similarity in data values with those presented by Eslam (2011) lends confidence to the prediction that similar results to those presented here for safflower under semi controlled conditions can also be expected under field conditions. In general, stomatal closure and intercellular CO_2 concentration (*Ci*) is related to soil moisture content in C_3 plant species, and stomatal closure was responsible for a decline in photosynthetic rate under drought (Medrano *et al*., 2002; Tezara *et al*., 1998). Water stress had

no effect on variable to maximum fluorescence ratio (Fv/Fm) indicating that the drought had no effect on primary photochemistry of PSII in many C_3 crops including sunflower (*Helianthus annus* L.) (Cornic and Fresneau, 2002a; Lu and Zhang, 1998; Marco *et al*., 1988; Panković *et al*., 1999; Pastenes *et al*., 2005; Subrahmanyam et al., 2006) showing that PS II is quite resistant to water stress (Shangguan *et al*., 2000a). The same result was reported for safflowers in this study which indicates that the decreases in net assimilation rate are only related to stomatal closure and not to disruption to the biochemistry of photosynthesis. When the temperature became very high inside the glass house (about 40 ^{0}C) (see Figure 3.1) the ratio of Fv/Fm decreased in dark adapted leaves even in well irrigated plants and there was a small but significant reduction in terminal and mid + terminal drought compared with the control (Figure 3.6 and 3.7) and (Table 3.5 and 3.6). This indicated that high temperatures may have been responsible for the decline or associated increased transpiration rate and this may have accentuated the drought effect. Similarly, a combination of high temperature more than (40^{0}C) and water deficit in bean (*Phaseolus vulgaris* L.) decreased the ratio of Fv/ Fm and CO_2 up take but the crop was still able recover from a short term stress (Yordanov *et al*., 1997).

This non-stomatal limitation of photosynthesis has also been observed under severe drought in other C_3 crop species (Flexas *et al*., 2002). Moreover, recently during an experiment on field grown safflower in Isfahan, Iran, using three irrigation treatments across all six safflower genotypes studied, chlorophyll fluorescence Fv/Fm decreased only under the maximum evaporation level (135 mm) when measured at both heading and at pollination stages (Miladi and Ehsanzadeh, 2010).

Plant response to drought conditions is determined by drought duration (Bray, 1997) and the critical stages of plant development at which drought is imposed (Ahmed and Suliman, 2010; Aiken and Lamm, 2006; Sinaki *et al*., 2007). In this study safflower exhibited recovery when water stressed during earlier growth stages (rosette), but when drought was imposed during the elongation stage and/or rosette to maturity (mid + terminal drought) stomatal conductance was significantly reduced (Figure 3.3 and 3.5). Other oil crops such as rapeseed have also shown recovery after relief of early drought (Ahmadi and Bahrani, 2009).

It is frequently reported that a reduction in photosynthesis is mainly due to a reduction in stomatal conductance, and as a consequence this leads to a reduction in the aboveground biomass and seed yield observed (Kang *et al*., 2002b; Mwale *et al*., 2007). This was the case in the current study where safflower significantly accumulated less dry matter under water stresses regimes (Figure 3.9). Thus, water deficit reduced stomatal conductance and as a result the CO_2 uptake and assimilation rate were reduced resulting in biomass reduction. The highest level of reduction recorded was when plants were exposed to the longest duration of drought from the rosette through to maturity compared to either control or other two drought regimes. The least reductions many of which were non-significant in above ground biomass, biological yield and harvest index were produced by plants exposed to mid-season drought (Figure 3.9, 3.11 and 3.12). As a consequence of these biomass changes, seed yield and seed number showed a similar patterns of response (Figure 3.10 and Table 3.7). This is probably because like most seed crops safflower translates a large percentage of its pre-anthesis carbohydrate accumulation to the seed

during late season drought stresses (Koutroubas *et al*., 2004). Grain/seed yield is often determined by total biomass production and the proportion of biomass translocated to grain/seed (Boogaard *et al*., 1996). Other workers have shown that generally, field grown safflower showed a reduction in photosynthesis and other parameters when exposed to drought at different stages of growth (Sharrifmoghaddasi and Omidi, 2010). For example, a study of 12 genotypes of safflower by Sharghi and Bagheri, (2011) concluded that biological yield, seed and oil yield increased under irrigation compared with non-irrigated plants at both rosette and end of elongation stages. In addition, Jalali *et al*., (2011) revealed that a long period of water stress during stem elongation (mid to terminal drought) severely affected safflower growth and yield while a moderate drought at the same stage had no significant effect on these traits. In contrast, the highest average weight of 1000 seeds was recorded for full irrigation and midseason drought while both terminal drought and mid + season drought produced less weight of 1000 kernel weight for field grown safflower (Istanbulluogh *et al*., 2009; Istanbulluoglu, 2009b) whilst here, 1000 seed weights were reasonably well conserved.

Safflower morphology in term of leaf number, stem height and branch number were affected by water stress especially when plants were droughted from the rosette stage right through to maturity. In some safflower cultivars plant morphology was shown to be changed under drought by Bagheri (2011a) but tolerant cultivars showed no change under drought.

For safflower to produce high yields, the number of capitula per plant and numbers of seeds per capitulum are important traits (Abel and Driscoll, 1976; Steer and Harrigan, 1986). Both of these yield components have been shown to

decrease under water stress (Eslam, 2011; Pasban Eslam and Sadeqi, 2008). As observed in the present study the number of capitula per plant and the number of seed per plant significantly reduced under both terminal and mid+ terminal drought compared with the control and the correlation between these parameters (Table 3.9) clearly showed their inter-relationship. This finding was supported by work with field grown safflower at different stages of imposed drought by Mozaffari and Asadi (2006) who observed positive correlation among capitulum diameter and the number of seed per capitulum under drought. Also, Eslam (2011) found that drought during earlier stages of reproductive safflower caused seed and or capitula number reduction. Also in cold dry land grown safflower genotypes in Iran, Alizadeh, (2005) found a positive correlation between seed and oil yield with plant height and capitulum weight. As shown in this study well watered plants produced taller plants with more seed number, inversely under drought the plant height, capitula number and seed number reduced significantly. In addition, high correlation between seed yield and total plant biomass in safflower has been established by Mokhtassi Bidgoli *et al*. (2006) in agreement with the results reported here under both droughted and well watered treatments.

In Iran Kar *et al*., (2007) reported that supplemental irrigation during reproductive phases had a significant effect on increasing seed yield in safflower with some differences between genotypes. Generally, seed yield of safflower from any of the treatments exposed to drought at one or more growth stage was significantly lower than fully irrigated, but highest reduction observed was found to be drought at late vegetative growth stage (Istanbulluoglu *et al*., 2009). In addition, the highest average weight of 1000 seed was found to be in

the ranges of 51 - 55 g and was recorded for the treatments including irrigation application at the late vegetative stage (Istanbulluoglu *et al*., 2009). In contrast, some spring genotypes showed a tolerance to water stress imposed at 80% flowering until maturity in Iran whereas in others water stress at this stage decreased number of seed, seed and oil yield due to limited supply of carbohydrate to the capitula leadings to a reduction in the 1000 seed weight and harvest index (Pasban Eslam and Sadeqi, 2008). In contrast, according to a field study on hybrid and open pollinated safflower by Ozturk *et al*., (2008) non-irrigated plants produced nearly the same seed yield and 1000 weight as irrigated ones but the effect of genotypes and years in both non-irrigated and irrigated treatments for seed production was significant.

Seed yield in the current study was lower than expected for control plants with number per head produced by field growing safflower typically ranged between (15 - 50 achene) (Dajue and Mundle, 1996). The reasons for this are not clear, but one possible explanation is the size of pot in which plants were grown causing restricted root growth. Alternatively, the amount of compost in the pots may not have supplied the plants with adequate mineral nutrition. Another interpretation could be that although the control treatments was thought to be well watered, in reality it was not and even the controls represented a water restricted regimes. Recently it has been shown that safflower produced the greatest head number per plant, seed number per head and consequent seed yield, at 100% water field capacity compared to 75% and 50% field capacity (Ferasat *et al*., 2008; Kazemeini *et al*., 2010). However, seed number was significantly higher in well watered plants compared with the water deficits treatments mostly as a result of the reasons mentioned earlier and also

because drought reduced the ion uptake and nutrient metabolism was affected (Farooq *et al*., 2008). The amount of nutrient taken up by the plant generally depends on the structure and growth rate of the roots, the ion concentration gradient between roots and surrounding soil and soil membrane permeability (Alam and Pessarkli, 1999).

Fai *et al*., (2007) divided development of seed into four different stages: embryo patterning, embryo growth and seed filling and desiccation. Seed storage products such as protein, oil and carbohydrate accumulation start after the completion of embryo growth (Bewley and Black, 1994). The import of sucrose from the vegetative parts of the plant has a fundamental function in the development of seeds as a main source of carbon and energy transportation and as a gene expression regulator (Koch, 2004) and the concentration of starch and soluble sugars increases to their highest values during the first few weeks of seed development (Romano *et al*.,1984). Proteins are mainly produced in the cotyledons throughout the mid to late development stages (Golombek *et al*., 2001) and a marked amount of oil accumulation starts later than protein, but continues even after protein accumulation in cauliflower (*Brassica oleracea* L. var. *botrytis*) (Gorosamy and Thiagarajan , 1998). Many environmental factors affect seed development and composition such as high temperature and soil moisture (Lozovoya *et al*., 2005).

Safflower oil and moisture content were significantly lower ($p < 0.05$) under drought, with the greatest reduction observed during elongation and rosette stages (Table 3.8). The reduction observed was when full drought from rosette to maturity was imposed. Despite this reduction 1000 seed weight was same as well irrigated plants. Unfortunately; due to insufficient seed produced in this

study the protein determination was not performed. Increased seed protein content would be anticipated as typically proteins are accumulated during seed filling prior. As previously found there is a negative correlation between seed oil content and protein under water deficits in safflower (Tuncturk and Çiftçi, 2007).

3.7. Conclusion

It can be concluded from the present study that drought affects safflower physiology, through reduced stomatal conductance and corresponding reduced transpiration rate and intercellular CO_2 concentration leading to a reduction in assimilation and ultimately biomass. The drought did not unduly affect the Fv/Fm ratio and it was stable under all watering regimes, but high temperature decreased the ratio of Fv/ Fm even for well watered plants. This means that the drought did not affect the photosynthetic apparatus unduly.

Safflower physiology and growth recovered after relieving mid-season drought. Seed yield was related to capitula number in both normal and stressed plants. The results indicate that for the maximum economic yield in safflower full irrigation is necessary at growth stages from elongation and branching through to maturity and pre- anthesis biomass allocation and translocation of seed is important. Seed oil content decreased under water stress with the most reduction occurring with water stress at the stem elongation stage and from the rosette stage to maturity.

Chapter 4

The effect of Nitrogen nutrition on safflower physiology, growth and yield

4.1. Introduction

The interdependence of carbon and nitrogen assimilation is well established and the nitrogen budget of the plant is primarily spent in the maintenance of the photosynthetic protein and chlorophyll apparatus although a continuous supply of carbon dioxide and energy is required for nitrogen assimilation and distribution (Foyer *et al*., 2001). With early growth this energy supply comes from seed reserves but as the plant develops it is derived from photosynthesis and therefore forms part of the plant respiratory load associated with growth and development. Adequate nitrogen is required to maintain an optimal photosynthetic rate and consequently crop growth (Gastal and Lemaire, 2002). The importance of nitrogen fertilization to field grown safflower was reported by Zaman and Das, (1990) and physiological responses have been investigated by Dordas and Sioulas (2008) where it was shown that elevated nitrogen levels to (200 kg N ha^{-1}) in the form of ammonium sulfate increased leaf nitrogen concentration and chlorophyll content leading to an increase in photosynthetic rate and stomatal conductance and, consequently, WUE and increased seed yield. During other oilseed crop, sunflower (*Helianthus annus* L.), the photosynthesis rate per unit leaf nitrogen was maintained under nitrogen stress because the initial reduction in photosynthesis with nitrogen deficiency was ameliorated by increased intracellular CO_2.

The total biomass of safflower were taken as an indicator of photosynthetic product translocation and accumulation and Dordas and Sioulas,(2009) concluded that 200 kg N ha^{-1} increased the total above ground biomass at both anthesis and harvest. Also dry matter partitioning to different plant parts, depending on the growing season, was increased.

Leaf photosynthesis is strongly related to LAI, and is directly associated with plant biomass in particular in C_3 plants because the photosynthetic capacity is limited by nitrogen per unit leaf area (Evans, 1989). A multi- year study of field grown safflower at four levels nitrogen fertilizer (0, 40, 80 and 120 kg N ha^{-1} in the form of ammonium nitrate) concluded that plant growth at different stages from stem elongation to maturity including LAI, chlorophyll content, plant organ nitrogen concentration and biomass were not affected by increased nitrogen. However, in other oil crops such as sunflower nitrogen fertilizer enhanced growth and productivity through increasing the LAI and canopy development (Gimenez *et al*., 1994).

Nitrogen levels have been shown to affect safflower crop component and growth performance, Siddique and Oad, considered 120 kg N ha^{-1} as the optimum level for the production of maximum seed yield by significantly producing greater branches, heavier seed weight and more seed number per plant, while more heads per plant and taller plants were recorded in plots treated with 180 kg N $ha.^{-1}$ Also Elfadl *et al*., (2009) reported that 86 kg N ha^{-1} was adequate for maximum safflower seed yield when sown after a crop fertilised with nitrogen fertilizer at the commercial rate. However, neither thousand seed weight nor the number of capitula per plant and consequently seed yield was altered in three varieties of safflower (Gila, KW-74 and Sironaria) at two sites under three rates of nitrogen (0, 40 and 80 kg N ha^{-1}) (Strasil and Vorlicek, 2002).

Safflower seed oil content can be affected by nitrogen fertilizer. In Turkey the recommended N fertilizer rate for safflower seed yield and crude oil was 120 kg N ha^{-1} as ammonium nitrate, and Tuncturk and Yildirim, (2004) concluded that

the oil yield was increased as a result of increasing nitrogen was due to seed yield rather than increased seed oil concentration. In general, the major effect regarding increasing oil yield in safflower in response to nitrogen supply was due to the increased seed (achene) yield whilst the response in terms of oil content was not significant (Abbadi *et al*., 2008).

The fatty acid composition of safflower however has not been studied in relation to nitrogen supply. In other oilseed crops such as oilseed rape, it has been observed that varying levels of nitrogen fertilizer (50, 100, 150 and 200 kg N ha^{-1}) had no significant effects on seed fatty acid composition (Starner *et al*., 1999). Also, neither nitrogen rate nor time of application in two seasons affected seed oil content and its fatty acids (palmitic, stearic, oleic, linoleic and linolenic, arachidic and erucic) composition in rapeseed (Ibrahim *et al*., 1989). In contrast, Zheljazkove *et al*., (2012) found that increasing the nitrogen application from 50 to 150 kg N ha^{-1} increased seed yield and modified the oil content and fatty acid composition in the same species.

It has been reported that safflower seed genotype and sowing date (Gecgel *et al*., 2007) can interact with other environmental factors such as temperature to have an impact on oil content and fatty acid composition rather than nitrogen as in other oil crops (Omidi *et al*., 2009; Zheljazkov *et al*., 2012).

Studying the effects of nitrogen in the field or in potting compost is always complicated by the effect of soil residual nitrogen stores. The use of hydroponic or semi-hydroponic systems however allows nitrogen supply to be controlled precisely. This investigation reports the results of a detailed study to investigate

the effects of nitrogen fertilizer on the growth, physiology and yield of safflower grown in the glass-house using a perlite based hydroponic system.

4.2. Aim

To study the effect of nitrogen nutrition on safflower growth performance, seed yield and quality.

4.3. Objectives

4.3.1. Objective 1

Evaluate the effect of different doses of nitrogen fertilizer on growth parameters (stem height, leaf number, LAI, above ground biomass) and yield components (branch number, capitula number, seed number per plant).

4.3.2. Objective 2

Investigate the physiological parameters (assimilation rate, stomatal conductance, transpiration rate, sub-stomatal conductance and water use efficiency) in response to nitrogen fertilizer.

4.3.3. Objective 3

Investigate the effect of different doses of nitrogen fertilizer on seed quantity and quality by studying the seed oil content and fatty acid composition.

4.4. Materials and Methods

4.4.1. Critique of necrotic plant symptom issues in the glass-house

Four attempts between September 2009 and August 2010 were made to establish this experiment in the glass-house. The following describes the experimental design used, and the plant physiological necrosis issues that affected these plants and the attempts to overcome this problem.

In the first experiment, seeds were transferred (on15th of November 2009) to pots filled with John Innes N^{o}.2 compost (this medium was chosen because it contained low nitrogen reserves). The experimental layout was a randomized block design with four replicate where each replicate comprised 48 pots. It had been planned to apply eight levels of ammonium nitrate nitrogen (0.9, 1.8, 2.7, 3.6, 4.5, 5.4 and 6.3 g per 24 pots) to give the equivalent of (0, 25, 50, 75, 100, 125, 150, and 175 kg N ha^{-1}) in two equal doses. After a month of germination, the first doses of nitrogen levels were supplied, with each weight of ammonium nitrate dissolved in 120 mL distilled water, and using a pipette 5 mL were added to the appropriate pot.

After the first application of nitrogen fertilizer and when the plant had reached a stem elongation stage the plants failed. For most of the plants, the stem died and leaves became yellow and dried up (Plate 4.1 shows these plant symptoms).

Plate 4. 1. Necrotic plant symptom issues in the first nitrogen experiment.

After a further two weeks the experiment was abandoned and was re-established by sowing seeds in the same medium, John Innes N^{o}.2, and in multi-purpose compost. Plants in both compost types again demonstrated the physiological necrosis and die back and the experiment had to be abandoned again.

4.4.2. Diagnosis of the necrosis problem

It was rationalized that the necrosis could be caused either by a pathogen, or a soil/ compost component or seed contaminant or environmental stress. Alternatively the symptoms could be due to a genetic factor of the genotype. The following investigations were carried out in an attempt to diagnose and overcome the problem:

1. Microbiological investigation
2. Elimination of seed–borne contamination sources
3. Investigation of environmental stresses (temperature shock)
4. Investigation of the effect of the genotype and substrate

1. Microbiological investigation

The symptoms were investigated microbiologically using a “Koch’s postulates” approach i.e. isolation and reinfection of an unidentified microorganism. This failed to identify any causative organisms from either the surface of the leaves or from incubation of whole leaves *in vitro* and no hype nor bacterial contaminants could be visualized under the microscope and it was therefore concluded that the symptoms were not associated with an identifiable pathogen. The disorder therefore was either a condition of the seed-lot or associated with the substrate.

2. Elimination of seed–borne contamination

Seed-borne contamination is a common source of the disease carryover on many crops and can be reduced by seed surface sterilization or fungicidal seed dressing. The first experiment was re-established, but the seeds were surface sterilized before germination.

Seeds were immersed in 70% ethanol for two minutes followed by a 5 minute soak in 10% domestic bleach (containing sodium hypochlorite 0.06% active Cl). Seeds were then rinsed six times with sterile water before being placed in the incubator for germination. The germinated seeds were sown on 15th of January 2010 in John Innes N^{o}.2. When the safflower plants reached the stem elongation stage and after the first application of nitrogen the necrosis appeared in the majority of plants and the experiment had to be abandoned again. A few remaining healthy plants were kept from this experiment to examine environmental stress effects.

3. Environmental stress effects

Glasshouses in the UK, particularly small glass-house as used here, are prone to extreme fluctuation in temperature which may go either above or below critical plant temperature thresholds and cause physiological symptoms similar to those shown in Plat 4.1 As nothing is published about safflower thermal tolerances an experiment was conducted in a phytotron to test thermal extremes.

Two pots of healthy plants were tested using a Sanyo M533 incubator under two extreme temperatures (very high, 45 ^{0}C and low to -7 ^{0}C) for 48 h.

Neither temperature shock had any morphological effect on plant health and did not reproduce symptoms in Plate 4.1 showing that safflower appears to be robust in the face of extremes of temperature.

It was concluded that the necrotic symptoms were not caused by extremes of temperature fluctuation. It was also reassuring to establish that safflower appears to be robust in the face of extremes of temperature and this gave reassurance to growing this plant in a semi-controlled glass-house.

4. Investigation of the effect of the genotype and soil type

Four other cultivars of safflower (Sham, Accar 6, Sonl 5 and P125036) were obtained from a Syrian research centre and were used together with the original variety in a substrate experiment to test both the new seeds and some new substrates. The substrates tested were John Innes N^{o}.2, multipurpose compost and perlite standard + hydroponic solution (see ch 2).

The seeds were sown on March 1st 2010. The plants were watered with tap water for John Innes N^{o}.2 and multipurpose compost and the plants grown in perlite were watered with a standard hydroponic solution.

Results showed unequivocally that the necrotic disorder that symptoms appeared in plants of all genotypes growing on both John Innes N^{o}.2 and multipurpose compost (Plate 4.2) whilst the perlite grown plants were completely healthy (Plate 4.3).

Whilst the final problem with the composts was not tracked down for this experiment and the remaining experiments in this thesis Perlite was used as the growth substrate in order to avoid the necrosis problem.

This problem set this project back more than 6 months and demonstrates to future researchers the sensitivity of this species to root conditions associated with growing substrates.

Plate 4. 2. Necrotic plant symptom issues in the glasshouse with John Innes N^{o}.2 and multipurpose compost.

Plate 4.3. Healthy plants at same stage grown in standard perlite.

4.4.3. Experimental design and measurement taken

The nitrogen experiment was finally successfully established during 2010. Germinated seed were planted pots filled with standard grade perlite. The experimental design was a Randomized Block with four replicate with each replicate comprising of 48 pots. Plants were watered with 20 - 100 mL of a standard hydroponic solution Vita Link Max Grow (soft water) A and B every 3 - 5 days for 28 days. Thereafter plants were irrigated using 50 - 200 mL complete Hoagland's solutions minus nitrogen every week. Eight levels of supplementary ammonium nitrogen solution were prepared by dissolving 0, 0.46, 0.91, 1.34, 1.8, 2.28, 2.64, 3.12 g N as ammonium nitrate in 4.8 L of Hoagland's solution and applied to give the equivalent of (0, 25, 50, 75, 100, 125, 150 and 175 kg N ha^{-1}) using 200 mL of the appropriate solution per pot. Nitrogen was applied in 4 doses at monthly intervals. Also 100 - 200 mL of water was applied according to

the demand in between nutrient applications. The given amount of water were recorded every time during the growing period and instantaneous WUE was expressed on an economic yield basis by dividing the biomass production at both anthesis and harvest by the amount of water consumed during the growing period (Conley *et al*., 2001).

Air temperature and humidity were logged using a TinyTag data logger (mean maximum and minimum temperature were 30^{0}C and 60 ^{0}C Figure 4.1). The growing season and the mean maximum and minimum humidity were 100% and 4.6% (Figure 4.2).

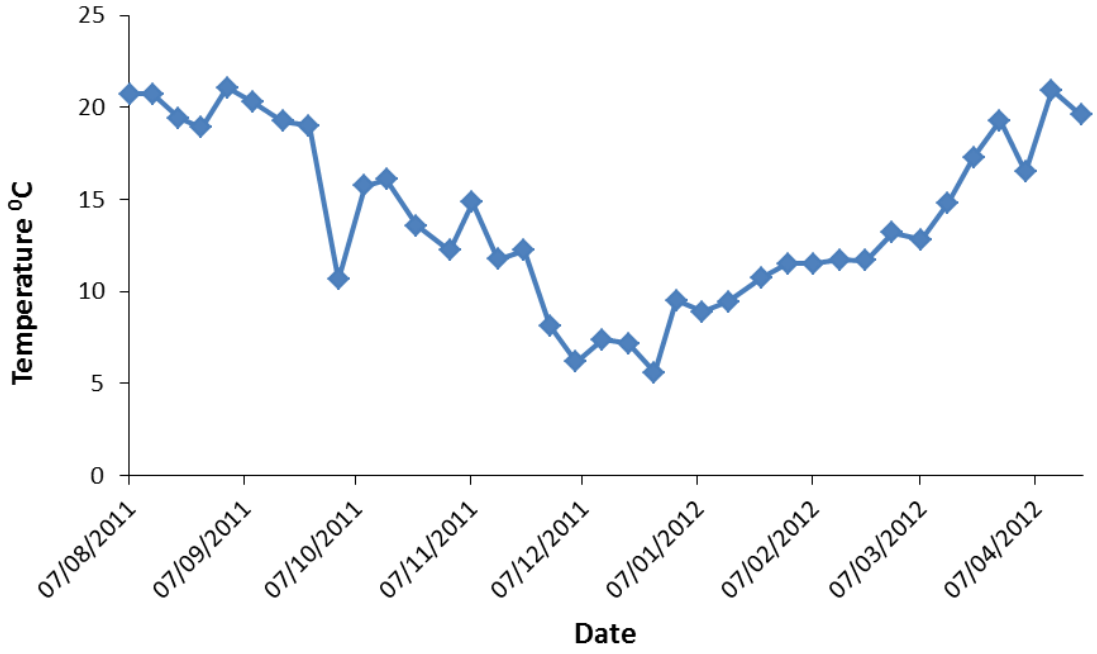

Figure 4. 1.Mean weekly temperature inside glasshouse during the nitrogen experiment.

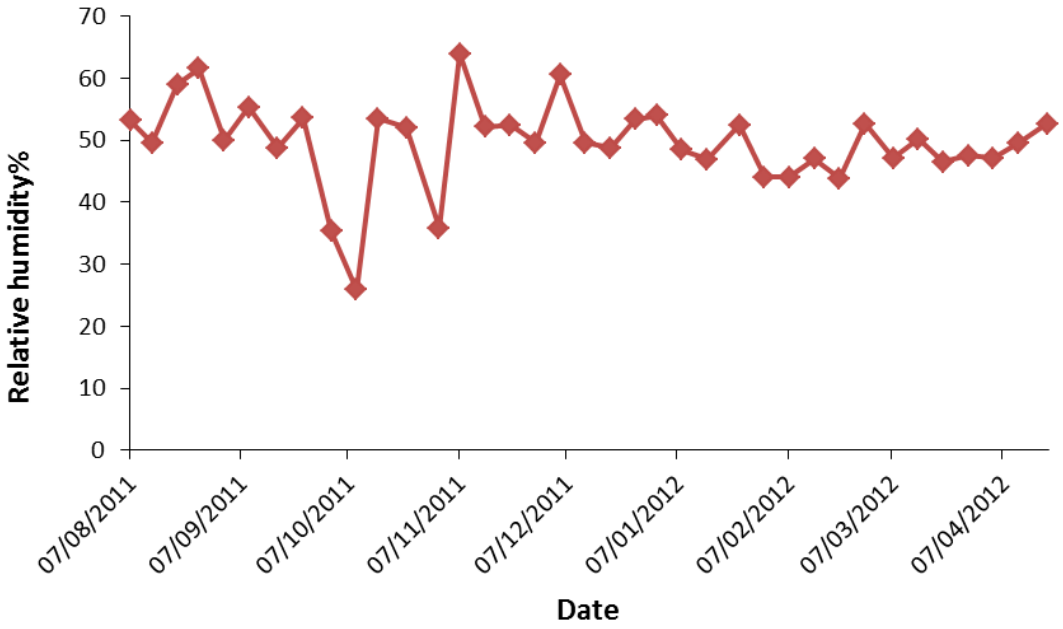

Figure 4. 2. Mean weekly relative humidity inside glasshouse during the nitrogen experiment.

At 50% anthesis the physiological parameters assimilation rate, transpiration rate, stomatal conductance and sub-stomatal CO_2 were measured on the youngest three top expanded leaves of three plants in each replicate for each treatment. After one day half of the plants used for photosynthesis measurement were harvested (24 plants) for measuring stem height, number of leaf, branch number, capitula number, leaf area and LAI, chlorophyll content, plant organs biomass and nitrogen content. Three samples of plant leaves from each replicate and each treatment were used for measuring chlorophyll content. The remaining plants were harvested and plant separated into plant organs (stem + branch, leaf, capitula) and dried then ground and nitrogen concentrations measured. At the end of the experiment the other half of the plants (24 pots) were harvested (15th May 2011) and the average stem height, leaf number, branch number, capitula number, above ground biomass, seed

yield per plant, 1000 seed weight and seed yield per hectare were recorded for each replicate and each treatment. Seeds were sent to Stirling University and seed oil content and fatty acid composition were analysed (see ch 2).

4.5. Results

4.5.1. Physiological parameters

At anthesis, the mean leaf assimilation rate at anthesis increased incrementally with each increase in nitrogen supplied to give a response curve to nitrogen application (Figure 4.3.A). Assimilation rate was significantly increased ($p \leq 0.05$) at 100 kg N ha^{-1}, but increases above this level were not significant. A similar pattern was shown for stomatal conductance at anthesis (Figure 4.3) with a significant increase ($p \leq 0.05$) up to 100 kg N ha^{-1} with no significant increases recorded above 100 kg N ha^{-1}. This led to a corresponding increase in transpiration rate which also showed an incrementally increasing response ($p \leq 0.05$) up to 100 kg N ha^{-1} (Figure 4.3) and the mean transpiration rate was 32% higher compared with the control. While the CO_2 concentration in the sub-stomatal cavity went down slightly with each increase in nitrogen these differences were not statistically significant ($p = 0.539$) (Figure 4.3).

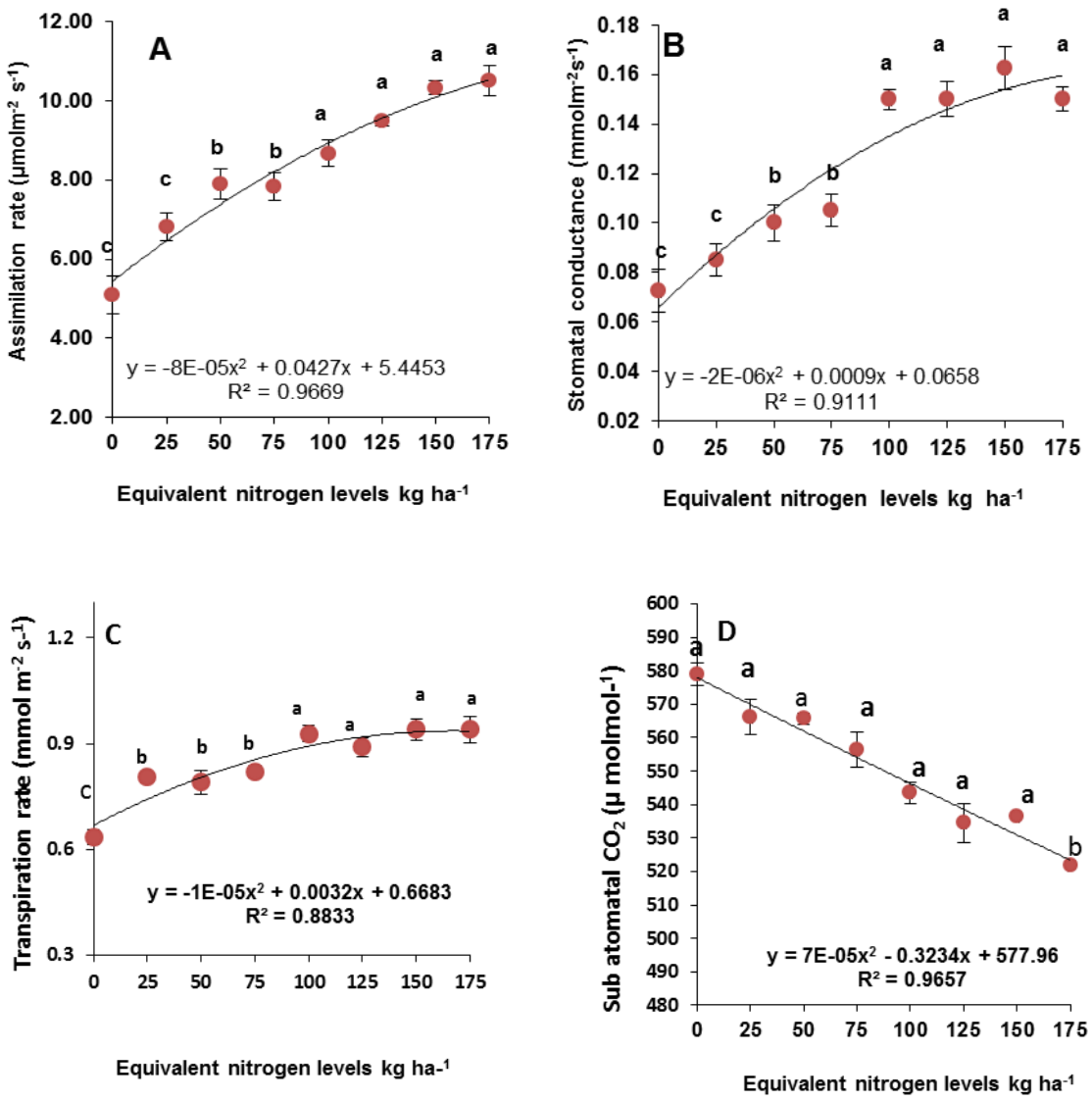

Figure 4. 3. Mean of A. assimilation rate (*A*), B. stomatal conductance (g_s), C. transpiration rate (*E*) and D. sub-stomatal conductance (*Ci*), at 50% anthesis different levels of nitrogen fertilizer. Vertical bars are standard errors of the mean (n= 4) at 0.05 level.

4.5.2. Water use efficiency

WUE from sowing to anthesis and from sowing to harvest was significantly ($p \leq 0.05$) increased with each increase in nitrogen rate compared to the control (Figure 4.4 A and B) . Under the highest nitrogen rate of 175 kg N ha^{-1} WUE increased by 44% at anthesis and by 41% at harvest as compared with the control.

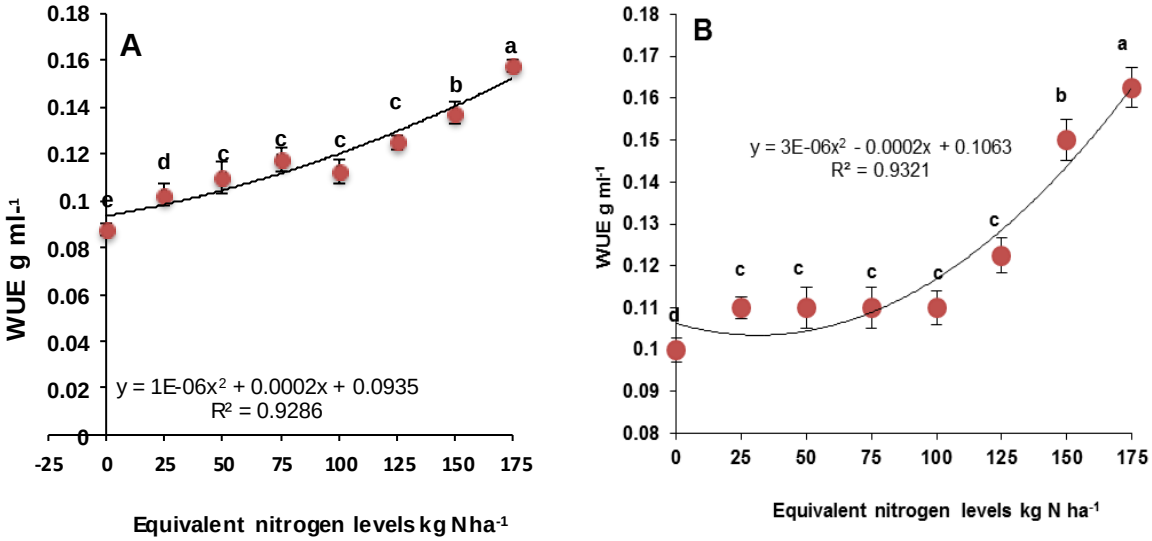

Figure 4. 4. Mean of A. water use efficiency from sowing to 50% anthesis and B. water use efficiency from sowing to harvest at different levels of nitrogen fertilizer. Vertical bars of standard errors of the mean (n= 4) at 0.05 levels.

4.5.3. Plant nitrogen and chlorophyll concentration

At anthesis, the nitrogen content of the plant parts were significantly ($p \leq 0.05$) affected by nitrogen fertilization (Table 4.1) and nitrogen fertilizer affected plant organs differently. Stem and branches responded to each nitrogen level with significant increment in nitrogen content, and the capital responded to most of the range of nitrogen applied. The leaves were less responsive to a highest nitrogen content achieved at 100 kg N ha^{-1}. Chlorophyll a, b and total chlorophyll content at anthesis were significantly ($p \leq 0.05$) affected by nitrogen treatment (Table 4.2). 150 kg N ha^{-1} increased the mean chlorophyll content by 55% compared with the control and no significant increment in chlorophyll a, b and total chlorophyll was observed with nitrogen higher than 150 kg N ha^{-1}.

Table 4.1. Mean of nitrogen concentration (g 100 g^{-1} dry weight) of various plant parts at different nitrogen levels at 50% anthesis.

Equivalent nitrogen treatments kg ha^{-1}	***Stem and branch***	***Leaf***	***Capitula***
0 (control)	0.24 ^{f}	1.28 ^{c}	0.73 ^{f}
25	0.25 ^{e}	1.56 ^{bc}	0.82 ^{e}
50	0.25 ^{e}	1.74 ^{b}	0.89 ^{d}
75	0.25 ^{e}	1.88 ^{b}	0.99 ^{c}
100	0.28 ^{d}	2.14 ^{a}	1.09 ^{b}
125	0.31 ^{b}	2.21 ^{a}	1.08 ^{b}
150	0.30 ^{c}	2.16 ^{a}	1.20 ^{a}
175	0.33 ^{a}	2.34 ^{a}	1.22 ^{a}
P	0.000	0.000	0.000
L.S.D at 0.05	0.03	0.31	0.43

*****Means followed by the same letter within a column are not significantly different at 0.05 levels.

Table 4. 2. Mean values of plant chlorophyll content (mg g^{-1} leaf fresh weight) under different nitrogen fertilizer at 50% anthesis.

Equivalent nitrogen treatments kg ha^{-1}	***Chlorophyll a***	***Chlorophyll b***	***Total chlorophyll***
0 (control)	0.58 **d**	0.40 **c**	0.98 **d**
25	0.85 **c**	0.48 **c**	1.34 **c**
50	0.85 **c**	0.51 **b**	1.40 **c**
75	1.05 **b**	0.58 **b**	1.64 **bc**
100	1 .21 **b**	0.60 **b**	1.83 **b**
125	1.22 **a**	0.58 **b**	1.83 **b**
150	1.42 **a**	0.78 **a**	2.10 **a**
175	1.42 **a**	0.79 **a**	2.20 **a**
P	0.000	0.000	0.000
L.S.D at 0.05	0.25	0.13	0.31

*Means followed by the same letter within a column are not significantly different at 0.05 levels.

4.5.4. Plant morphology, growth and seed yield

At anthesis, plant height was not affected significantly ($p > 0.05$) by nitrogen treatments compared with the control (Table 4.3). Number of leaves branches and capitula per plant significantly ($p < 0.05$) responded to some nitrogen treatments. The greatest mean values were obtained at 125 kg N ha^{-1} with no further response to higher levels of nitrogen, and leaf area index LAI increased incrementally with increasing nitrogen levels reaching a plateau of about LAI 6.27 for the three highest levels of nitrogen (Figure 4.5. A). 125 kg N ha^{-1} increased the LAI by 42 % compared with the control. This value is comparably higher than that recorded in field grown safflower which reached a plateau of LAI about 3.8 (Jalali et al., 2011).

At harvest, the plants showed significant development in response to nitrogen fertilizer. Plant height was significantly ($p > 0.05$) taller when nitrogen increased to more than 75 kg N ha^{-1} compared to the other three lower nitrogen rates. A similar trend was found with the number of leaves, branches and capitula per plant (Table 4.3). The correlation coefficient between seed number and capitula number showed that the seed number and capitula number per plant increased with nitrogen rate to more than 125 kg N ha^{-1} and were highly correlated ($r = 0.946$). Plant growth in term of dry matter accumulation (plant biomass) increased with each increase nitrogen fertilizer. Overall, the above ground dry weight was increased with a 46% and 42% over the control at 175 kg N ha^{-1} at both anthesis and harvest, respectively (Figure 4.5.A and B). Seed yield showed a similar pattern of response towards increased nitrogen levels and seed fresh weight increased by 76% at 175 kg N ha^{-1} compared with the control (Figure 4.5.D).

Table 4. 3. Means values of plant development criteria and yield component with their dry weight (gpl^{-1}), at different levels of nitrogen fertilizer at 50% anthesis.

Parameters	***At anthesis***								***L.S.D at 0.05 levels***
	Equivalent nitrogen levels kg ha^{-1}								
	0	***25***	***50***	***75***	***100***	***125***	***150***	***175***	
Plant height (cm)	93	95	97	93	98	98	102	100	n.s
Leaf number	38 **d**	44 **c**	42 **c**	41 **c**	52 **b**	56 **ab**	62 **a**	54 **b**	8.42
Leaf area	374 **d**	481 **c**	573 **b**	545 **b**	561 **b**	639 **a**	634 **a**	640 **a**	110
Branch number	2 **c**	3 **b**	3 **b**	3. **b**	4 **b**	4. **a**	4. **a**	4 **a**	0.83
Capitula number	3 **b**	4 **b**	4 **b**	3 **b**	3 **b**	4 **a**	4 **a**	4 **a**	0.89
Shoot and branch dry weight	4 **d**	5 **d**	5 **c**	6 **c**	5 **c**	6 **b**	7 **b**	7 **a**	0.79
Leaf dry weight	2 **d**	3 c	3 **b**	3 **b**	3 **b**	4 **b**	4 **b**	4 **a**	0.41
Capitula dry weight	3 **c**	2 c	3 **b**	4 **b**	4 **b**	4 **b**	4 **b**	5 **a**	1.53

*Means followed by the same letter within column are not significantly different at 0.05 levels.

Table 4. 4. Mean values of plant development criteria, yield component with their dry weight (gpl^{-1}), seed number, and 1000 fresh seed weight at different levels of nitrogen fertilizer at harvest.

Parameters	***At harvest***								***L.S.D at 0.05 levels***
	Equivalent nitrogen levels levels kg ha^{-1}								
	0	***25***	***50***	***75***	***100***	***125***	***150***	***175***	
Plant height (cm)	92 ^{a}	93 ^{ab}	94 ^{ab}	98 ^{a}	99 ^{a}	98 ^{a}	100 ^{a}	100 ^{a}	5.13
Leaf number	33 ^{b}	39 ^{b}	41 ^{b}	45 ^{b}	51 ^{a}	54 ^{a}	55 ^{a}	57 ^{a}	6.61
Branch number	2 ^{b}	3 ^{b}	3 ^{b}	3 ^{b}	4 ^{a}	4 ^{a}	4 ^{a}	4 ^{a}	0.75
Capitula number	3 ^{b}	3 ^{b}	4 ^{b}	4 ^{b}	4 ^{a}	5 ^{a}	5 ^{a}	5 ^{a}	0.66
Seed number	6 ^{d}	7 ^{d}	10 ^{c}	9 ^{c}	16 ^{b}	18 ^{b}	23 ^{a}	25 ^{a}	2.66
Shoot and branch dry weight	4 ^{c}	4 ^{c}	4 ^{c}	5 ^{c}	5 ^{ab}	6 ^{a}	5 ^{a}	6 ^{a}	0.82
Leaf dry weight	2 ^{d}	2 ^{d}	2 ^{c}	2 ^{b}	2 ^{b}	3 ^{ab}	3 ^{a}	3 ^{a}	0.73
Capitula dry weight	23 ^{a}	3 ^{b}	4 ^{b}	4 ^{b}	5 ^{a}	5 ^{a}	5 ^{a}	6 ^{a}	0.99
1000 seed weight	33 ^{a}	31 ^{b}	30 ^{b}	29 ^{b}	37 ^{a}	39 ^{a}	36 ^{a}	36 ^{a}	5.96

*Means followed by the same letter within column are not significantly different at 0.0 levels.

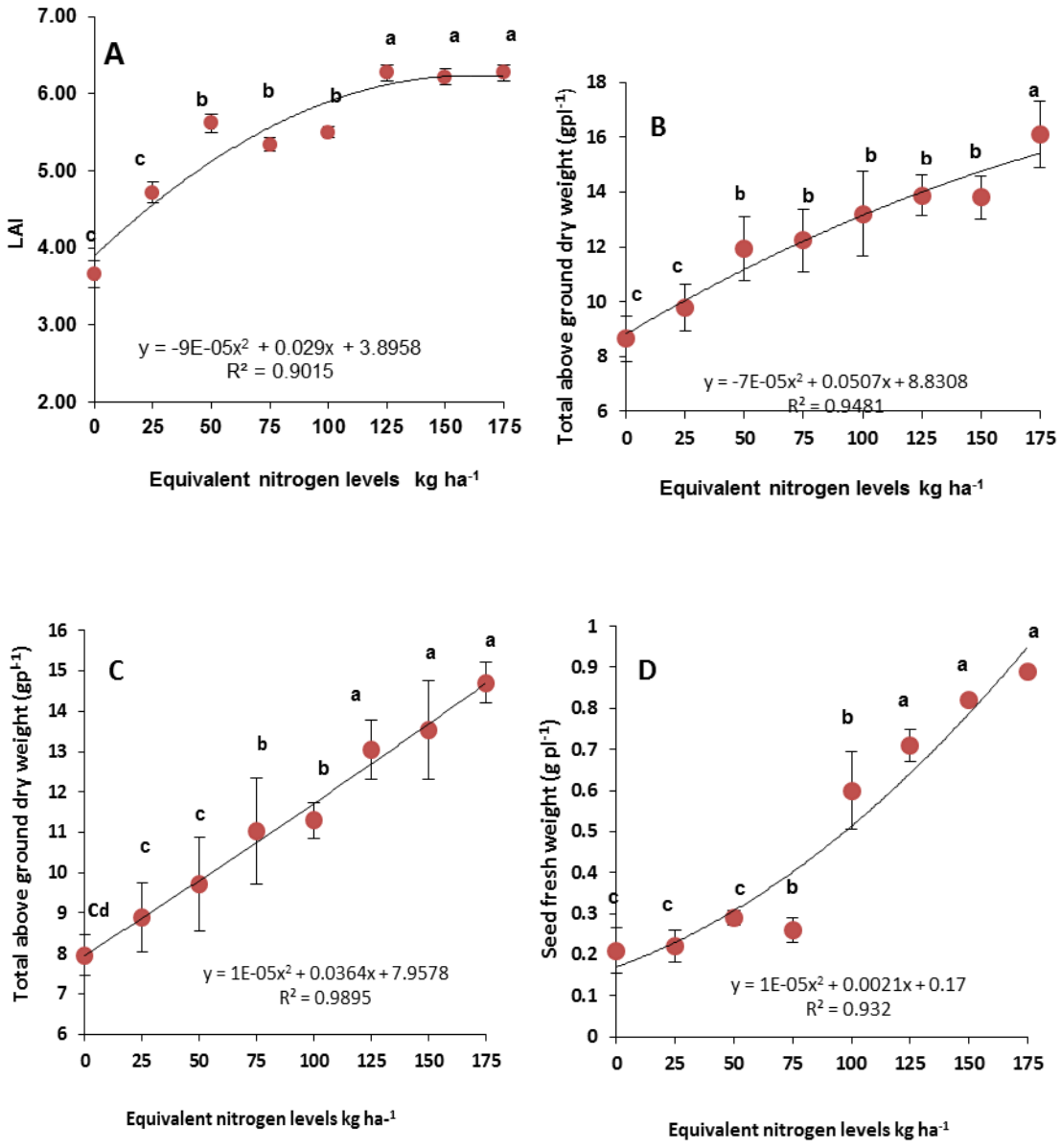

Figure 4. 5. Mean of A. LAI and B. total above ground dry weight at 50% anthesis, C. total above ground and D. seed fresh weight at harvest at different levels of nitrogenfertilizer. Vertical bars are standard errors of the mean (n= 4) at 0.05 levels.

4.5.5. Chemical analysis

Due to an insufficient quantity of seed obtained at harvest, seed oil content and fatty acid analysis were performed on a sample pooled from all replicates (Table 4.5). In comparison with the control the nitrogen fertilization did not affect the seed oil content. However, the total oil yield increased with nitrogen increment above 100 kg N ha^{-1} due to the increase in seed yield per plant.

All treatments resulted in seeds containing slightly lower total oil content relative to the original (parent) seed sown (Table 4.5). None of the fatty acid studies were affected by the application of nitrogen fertilizer. Relative to the original seed the seed from the experimental treatments had some decreased saturated fatty acids (palmitic and stearic) and mono-unsaturated fatty (oleic acid) while inversely, the poly-unsaturated fatty acid (linoleic) levels increased resulting in an overall decrease in the ratio of oleic/linoleic acid.

Table 4.5. The total % of oil and fatty acid composition under different nitrogen and the % oil and fatty acid in original seed (on fresh weight basis).

	Equivalent nitrogen treatments kg ha^{-1}								***Parent***
Seed composition	***0***	***25***	***50***	***75***	***100***	***125***	***150***	***175***	***seed***
Oil	19.5	17.33	19.51	18.75	19.15	18.33	18.40	18.4	21.30
Palmitic C16	6.65	6.61	6.69	6.47	6.44	7.72	6.55	6.63	7.75
Stearic C18	2.23	2.16	2.36	2.29	2.28	2.47	2.32	2.27	2.54
Oleic C18:1n	0.63	0.63	0.63	0.64	0.64	0.71	0.69	0.69	11.65
Linoleic C18: 2n	77.2	77.84	77.03	77.34	77.23	75.80	76.33	77.0	73.63
Linolenic C18: 3n	0.12	0.13	0.12	0.12	0.11	0.11	0.12	0.12	0.12

4.5.6. Correlations

Pearson correlations (Table 4.6) showed that high nitrogen supply significantly increased leaf nitrogen content (LNC) and leaf chlorophyll content (TCC) and was positively related to LAI. This emphasized the importance of nitrogen for leaf growth and for providing the chlorophyll protein complex for photosynthesis, thus increasing the assimilation area and chlorophyll apparatus. Nitrogen input was strongly correlated (at both $p < 0.05$ and 0.01 levels) and to assimilation rate, and was positively correlated to stomatal conductance and transpiration rate and as a consequence WUE was positively correlated to leaf nitrogen content ($p < 0.05$). Ultimately, a strong positive correlation between above ground biomass (AGB) and leaf nitrogen content was (LNC) demonstrated.

Taking all the parameters together, in this study safflower seed yield showed a positive correlation (at both $p < 0.05$ and 0.01 levels) with all of the physiological parameters, WUE, LNC, TCC, LAI and AGB, while seed oil content negatively correlated to seed yield.

Table 4. 6. Pearson correlation among physiological characteristics and above ground biomass at anthesis, above ground biomass at harvest, seed yield and seed oil content at harvest.

	LAI	***A***	***E***	***gs***	***Ci***	***WUE***	***TCC***	***LNC***	***AGB at anthesis***	***ABG at harvest***	***SY***
LAI											
A	0.964 **										
E	0.891 **	0.941 **									
gs	0.867 **	0.933 **	0.925 **								
Ci	0.878 **	0.950 **	0.904 **	0.928 **							
WUE	0.713 **	0.841 **	0.722 **	0.716 **	0.822 **						
TCC	0.899 **	0.979 **	0.953 **	0.936 **	0.967 **	0.848 **					
LNC	0.897 **	0.949 **	0.944 **	0.921 **	0.950 **	0.749 **	0.973 **				
ABG at anthesis	0.929 **	0.960 **	0.878 **	0.891 **	0.961 **	0.801 **	0.954 **	0.972 **			
ABG at harvest	0.898 **	0.964 **	0.871 **	0.911 **	0.978 **	0.872 **	0.970 **	0.929 **	0.963 **		
SY	0.802 **	0.912 **	0.841 **	0.942 **	0.948 **	0.860 **	0.918 **	0.870 **	0.890 **	0.940 **	
OC	- 0.414	-0.454	- 0.5 27	- 0.348	-0.494	-0.483	-0.478	- 0.350	- 0.326	- 0.168	-0.385

*Correlation is significant at the 0.05 level **Correlation is significant at the 0.01 level

4.6. Discussion

This experiment indicated that the safflower leaf assimilation rate at anthesis increased with increasing levels of nitrogen fertilizer. At 100 kg N ha^{-1} the assimilation rate was found to be increased by 42% compared with the control (Figure 4.3), with no significant increment in plant photosynthesis thereafter. This indicates that at 100 kg N ha^{-1} was critical for plants to maximize assimilation rate. Maximum assimilation rate achieved at anthesis has also been previously reported in field grown safflower with a 51% increase in the assimilation rate (Dordas and Sioulas, 2008) and such an increment was about two times higher compared with the control under highest nitrogen level. It can be predicted therefore that similar results to those presented here for safflower under semi-controlled conditions can be expected under field conditions. Such increased assimilation rate is commonly reported in other crops (Cechin and Fumis, 2004; Ciompi *et al*., 1996) and it is considered an essential improvement aspect of the crops physiological response to the removal of a limiting factor. Nitrogen availability typically increases the photosynthetic capacity of C_3 plants because the levels of proteins of the Calvin cycle (including Rubisco accounting for 85 - 90%) and thylakoids are related to increases in leaf nitrogen content (Evans, 1989; Warran *et al*., 2000).

Photosynthetic enhancement could then be interpreted as a result of improvement in the leaf nitrogen status and consequent nitrogen investments in photosynthetic components. This pattern of incremental photosynthetic improvement has been observed in pot grown sorghum at three rates of nitrogen (0% to 20% and 100%) using a Hoagland's solution approach as used

here (Zhao *et al*., 2005). By contrast, nitrogen deficiency is reported to reduce initial activity of Rubisco by 20% and slightly increase starch by about 25% in upper canopy leaves of fully grown sunflower. This eventually led to reduced initial photosynthesis and the intercellular CO_2 increased such that the ratio of assimilation was maintained per unit of leaf nitrogen (Fredeen *et al*., 1991).

It was confirmed also that the photosynthetic rate in both shade and sun plant species was linearly related to leaf nitrogen content (Evans , 1989) and Lawlor *et al.* (1987) detected that the photosynthetic rate can be greater with nitrogen input when measured in warm conditions with an illumination up to 1000 µmol m^{-2} $s.^{-1}$ In contrast, other studies have recorded no effect of nitrogen fertilization on winter wheat leaf assimilation rate (Shangguan, 1997; Shangguan *et al*., 2000b).

Stomatal conductance was increased and led to an increase in transpiration rate at anthesis. Application of the 100 kg N ha^{-1} increased stomatal conductance by 52% and transpiration rate by 32%. Stomatal conductance was up to two times higher under the highest nitrogen level as compared to the lowest at anthesis (Figure 4.3.B). This parameter showed an increase of 27% in other studies (Dordas and Sioulas, 2008). This achievement could be due to the contribution of transpiration rate and stomatal conductance on photosynthetic assimilation rate. There is a clear linear relationship between assimilation rate and transpiration rate as well as between assimilation rate and stomatal conductance (Table 4.6) and this result is strongly supported by findings elsewhere (Broadly, 2000; Cechin and de Fatima Fumis, 2004; Del Pozo *et al*., 2007; Zhao *et al*., 2005).

In contrast, the negative relationship between assimilation rate and transpiration rate and the linear relationship between assimilation rate and stomatal conductance for safflower at antithesis has been reported as a clear effect of nitrogen treatment (Ciompi *et al*., 2008; Dordas and Sioulas, 2008) although no effect of nitrogen treatment has also been reported (Fredeen *et al*., 1991).

A negative correlation between the assimilation rate and sub stomatal concentration of CO_2 was obtained in this study (Table 4.6). This finding was interpreted a result of investments of carbon in photosynthesis with increasing nitrogen supplied which led to a decline in the intercellular CO_2, and this corresponds with that for safflower measured by others (Dordas and Sioulas, 2008).

It was observed that in safflower plants, the highest assimilation rate was in fertilized treatments, related to higher stomatal conductance and transpiration rate leading to the highest water use. On the contrary, this also led to higher production rates and therefore the WUE showed an average increase of about 44% 41% from sowing to anthesis and from sowing to harvest respectively, under the highest nitrogen rate as compared with the control (Figure 4.4.A and B). This can be interpreted as a result of the increase in the above ground dry matter accumulation which has a water cost (Shaw *et el*., 2005) Increased WUE is correlated in consequence of this as reported elsewhere (Dordas and Sioulas, 2008) and previously recorded in other crops and plant species (Fredeen *et al*., 1991). For example, Cechin and Fumis (2004) found that increases in transpiration rate in high nitrogen grown sunflower plants did not result in lower WUS because of a higher rate of photosynthesis.

Safflower responded strongly to increasing nitrogen supply with respect to plant morphology and growth through producing taller plants with more branches and leaves. This result corresponds with Gimenez *et al*., (1994) who found that sunflower growth was enhanced by nitrogen fertilizer through increasing the LAI and canopy development and (Siddiqui and Oad, 2006) reported that increased nitrogen from120 to 180 kg N ha^{-1} produced taller safflower plants. Oad *et al*., (2001) also found a positive response of sunflower stem height to increasing rate of nitrogen fertilizer. Similarity, oilseed rape produced more leaves under 1176 mg N pot^{-1} (as ammonium nitrate) applied at the rosette stage (Svecnjak and Rengel, 2006).

LAI of safflower behaves like many other C_3 plants and strongly responded to nitrogen fertilizer and 125 kg N ha^{-1} increased LAI by 42% as compared with the control (Figure 4.5. A), and was tightly correlated to assimilation rate (Table 4.6). LAI reached a plateau in 6.27 to the highest levels of nitrogen and this was interpreted as plant growth acclimation in term of leaf growth with nitrogen availability. LAI was tightly correlated to assimilation and this was interpreted as improved growth through increasing the cell size as a result of increased photosynthetic rate caused by the doubling of nitrogen as previously established (Lea *et al*., 2001). Similar findings have previously been reported for field grown sunflower by Gimenez *et al*., (1999) and Cechin and Fumis, (2004) and for pot grown sorghum (Zhao *et al*., 2005). It was also reported by Fallovo *et al*., (2009) that LAI was increased by a similar amount with all forms of nitrogen. In contrast, fertilizer from 0 to 120 kgN ha^{-1} did not significantly improve LAI relative to control in field grown safflower and as a result above ground biomass did not change (Yau and Ryan, 2010). Safflower growth in term

of LAI in the current study showed a greater range between 3.66 to 6.27 than that reported in field grown safflower which was recorded as 3.8 under Mediterranean conditions (Iran) (Jalali *et al*., 2011). However, under field conditions in Turkey, LAI was reported to range between 19 to 20 (Yau and Ryan, 2010). This is probably because it was grown in a good soil with a greater root growth which leads to a greater vegetative growth especially when the quantity and distribution of rainfall are good. But in comparison with other crops e.g., durum wheat, the LAI increased by about the same value as produced by safflower in this study and ranged between 1.3 for control and 7 for fertilizing plants (Latiri-Souki *et al*., 1998).

Safflower concentrated more nitrogen in its leaves which led to higher chlorophyll and 150 kg N ha^{-1} increased total chlorophyll content with a 55% increase compared with the control (Table 4.1) and (Table 4.2). Nitrogen fertilizer affected plant organs differently and this may be attributed to the fact that nitrogen tends to be translated from the stem to the leaves and then to the capital in different amounts under different nitrogen regimes applied. Nitrogen supply increases in leaf nitrogen concentration and chlorophyll content was also previously reported for safflower (Dordas and Sioulas, 2008) and sorghum (Zhao *et al*., 2005) and a linear relationship between leaf chlorophyll content and plant nitrogen concentration was reported at anthesis. Consequently, the positive relationship between leaf chlorophyll content and leaf assimilation rate at anthesis was observed (Table 4.6).This is in agreement with Lawlor *et al*., (2001) and other findings revealing that the structures involved in light harvesting in photosynthesis that capture the photon energy are actually a chlorophyll protein complex . Such changes in leaf chlorophyll content in

response to increased nitrogen supply have also been reported in maize (*Zea mays* L. CV. Maya) leaves (Tóth *et al*., 2002). In contrast, none of the four nitrogen levels (0, 40 ammonium sulfate, 80 and 120 kg N ha^{-1} ammonium nitrate) had significant increments of change in safflower organ nitrogen concentration and as a result leaf chlorophyll content as well as biomass was not affected significantly when tested in a field grown multi-year experiments (Yau 2010).

The assimilation rate factors mentioned above led to a higher biomass production both at anthesis and at harvest when nitrogen was increased (Figure 4.5. B and C). This is typically reported elsewhere (Cechin and de Fatima Fumis, 2004;, Huber *et al*., 1989) and Zhao *et al*. (2005) and indicated that total plant dry matter production was strongly affected by nitrogen fertilizer and paralleled shoot/leaf dry matter accumulation. In the current study, safflower plants weighed up two times heavier in comparison to the control when given adequate nitrogen and similar results have been obtained for field grown safflower (Dordas and Sioulas, 2008). Both increasing photosynthesis and biomass led to incremental increase in seed yield. This was interpreted as a result of improvement in leaf nitrogen status by nitrogen fertilization, and clearly demonstrated the role of leaf nitrogen in photosynthesis enhancement and photosynthetic product partitioning. In contrast with this result, Siddiqui and Oad, (2006) stated that for field grown safflower seed yield and yield responded to nitrogen fertilizer with an additional increment in nitrogen rate and beyond a peak of 120 kg N ha^{-1} no significant further increase in response was observed.The increases in seed yield were always associated with an increase in head number (capitula) per plant and thereby seed number (Abbadi *et al*.,

2008). As reported here the seed number positively correlated with capitula number (r = 0.946) and this generally agrees with previous studies (Dordas and Sioulas, 2008; Tuncturk and Yildirim, 2004). In a recent study, safflower seed yield was increased and associated with both head numbers per plant and seed number per head, when fertilized with 100 kg N ha^{-1} applied in three stages, sowing, early elongation and early flowering (Soleimani, 2010). In contrast, in another study the number of heads per plant did not significantly differ from those produced either under control or nitrogen regimes (Elfadl *et al*., 2009).

The seed yield per plant obtained in this study was lower than expected and this is possibly because of high mean relative humidities (about 50%+) associated with an average low temperature (17 ^{0}C) during the flowering period which may have affected head fertility. Safflower is typically grown in arid or semi-arid region of the world (Johnston *et al*., 2002) with a hot and dry condition rather than cool moist climates as found in the UK (Dajue and Mundel, 1996; Knowles, 1976). Also pot size in which plants were grown may have restricted the plant root growth which may affect root nutrient uptake especially during seed formation.

In the current experiment, it was difficult to make a decision about the effect of nitrogen supplied on seed oil composition on a one replicate basis. However, it can be tentatively concluded that the seed oil content and fatty acid composition were not affected by nitrogen fertilizer increment. In both control and nitrogen treatments the seed oil content was less than oil obtained from the original

(parent) seed (Table 4.5) and the oleic linoleic acid ratio decreased in both control and nitrogen treatments relative to the parent seed. This suggests other

environmental factors impact on seed oil content and fatty acid composition. It has been reported that the temperature had little effect on the oil and fatty acid synthesis of the high oleic and linoleic genotypes, but the genotype with intermediate levels of both fatty acids will produce high levels of oleic acid at high temperature and high levels linoleic acid at low temperature (Knowles, 1985). In work on sunflower, Izquierdo *et al*., (2006) reported higher oleic acid concentrated in oil when night temperature increased from 20 to 23 ^{0}C during the seed filling period. For linoleic acid an inverse trend was observed which increased with a minimum night temperature. Temperatures above 23 ^{0}C did not affect fatty acid composition except palmitic acid which was highest at 28 ^{0}C (Samancı and Özkaynak, 2003). Furthermore, Omidi *et al*., (2010) and Zheljazkov *et al*., (2009) have suggested that response of oil content and fatty acid composition of nitrogen is more dependent upon other environmental conditions, genotype and location than nitrogen. This is supported by Gecgel *et al*., (2007) who showed that safflower genotype and sowing date are important factors impacting oil content and fatty acid composition. Nevertheless, nitrogen treatment has been reported to increase both yields and oil levels in irrigated, or higher rainfall areas (Dajue and Mundel, 1996) but many studies have not shown any relationship between nitrogen rates and safflower oil content (Dordas and Sioulas, 2008; Elfadl *et al.,* 2009).

The current study showed a negative correlation between oil content and seed yield. This could be interpreted as a result of increased protein or other seed

contents. Rathke *et al*., (2005) found similar results with no relationship between seed yield and oil content and a negative correlation between oil content and protein in winter oilseed rape and stated that the inverse

relationship under higher nitrogen supply might be due to decreased availability of carbohydrates for oil creation. In contrast, the increased nitrogen application from 50 to 150 kg N ha^{-}1 increased seed yield and modified the oil content and fatty acid composition relative to the control in winter mustard (*Brassica juncea* L.) grown in three different locations in Mississippi (south eastern United States) (Zheljazkov *et al*., 2012).

In addition, the change in fatty acid profile under nitrogen might be dependent upon its time of application For example, Steer and Seiler (1990) found that for sunflower the percentage of palmitic (16: 0) and linoleic (18: 2) acids increased significantly when nitrogen was applied before floret initiation while the stearic (18:0) and oleic (18:1) acids decreased and only stearic acid responded when the nitrogen was applied between floret initiation and anthesis. After anthesis, nitrogen application increased the ratio of oleic/linoleic. By contrast, neither nitrogen rate or time of application in two seasons affected the percentage of rapeseed oil content and its fatty acids (palmitic, stearic, oleic, linoleic and linolenic, arachidic and erucidic) (Ibrahim *et al*., 1989).

4.7. Conclusion

In this study, safflower physiological parameters (assimilation rate, transpiration rate and stomatal conductance) and LAI showed positive and incremental response to nitrogen when measured at anthesis. The combination of increases in both assimilation rate and leaf area contributed to the increase in seed yield. It is noticeable that the seed yield response continued to increase whilst leaf area and assimilation rate slowed down and this indicates that high nitrogen application rate also has an effect on partitioning of plant biomass to the seed component. Water use per g of seed yield increased as nitrogen rate increased indicating that there is a water use cost associated with increasing yield by nitrogen fertilizer but water was used more efficiently as biomass rose in response to increase in nitrogen applied. The seed oil content change and fatty acid profiles did not change dramatically with nitrogen fertilizer increases but there are still uncertainties about the effect of nitrogen fertilizer on seed quality in this study. Further studies need to include an interaction of nitrogen with other factors, especially temperature and also the time of nitrogen application needs to be tested to estimate exactly the effect of nitrogen on safflower seed yield and oil composition.

Chapter 5

The effect of elevated CO_2 on safflower physiology, growth performance and seed oil composition

5.1. Introduction

It is frequently reported (Ainsworth and Long, 2005; Ainsworth and Rogers, 2007; Bowes, 1996; Leakey et al., 2009; Reddy *et al*., 2010; Seneweera and Norton, 2011) that the rate of photosynthesis of C_3 plants grown at elevated CO_2 is higher than in plants grown at ambient CO_2. This is because an increase in the CO_2 availability activates the carboxylation binding site of Rubisco and decreases the oxidation activity, and hence inhibits or reduces photorespiration (Taiz and Zeiger, 2002). In addition, the higher CO_2 reduces the stomatal conductance due to either partial stomatal closure (Ainsworth and Rogers, 2007; Drake and Leadley, 1991; Wheeler *et al*., 1999) or decreases in stomatal density (Shaw *et al*., 2005). Bunce (2004) reported that elevated CO_2 reduces stomatal conductance for many crop plants but the relative reduction is not constant, but is dependent on other factors (light, temperature and humidity) and different species showed different responses with a doubling of CO_2 leading to enhanced WUE (Hsiao and Jackson, 1999a; Hsiao and Jackson, 1999b). As a result of these affect the plant growth is expected to rise in almost all cases but the magnitude of response will differ between functional crops (Ainsworth and Long, 2005; Poorter, 1993).

On the other hand, a reduction in transpiration rate resulting from partial stomatal closure in response to doubling CO_2 can also lead to a rise in leaf temperature and ultimately lead to an increase in transpiration rate which may offset the positive effect of stomatal closure (Allen, 1998; Allen Jr and Prasad, 2004; Kimball *et al*., 2002). Furthermore, stomatal closure reduces the CO_2 diffusion into the leaves which can reduce the CO_2 in the sub-stomatal cavity

and consequently reduce the photosynthetic rate. In this way stomata are said to acclimate to elevated CO_2 (Sage, 1994).

Productivity is strongly related to vegetative growth through LAI increases and is then directly associated with biomass increases. LAI determines the amount of light intercepted and total crop photosynthetic rate (Gastal and Lemaire, 2002). Leaf area and thereby LAI often shows changes with rising CO_2 (Dermody *et al*., 2006; Ewert and Pleijel, 1999; Hirose *et al*., 1997). It is often reported (Campbell *et al*., 2001; Reddy *et al*., 1998; Rodriguez et al., 2001; Manderscheid et al., 2003) that LAI increases under doubled CO_2. As a result photosynthesis of upper canopy leaves increases (Campbell *et al*., 2001; Heinemann *et al*., 2006; Rodriguez *et al*., 2001; Yuelin *et al*., 2005). Contrary to what was observed in many other studies in some cases leaf area and consequently LAI remain unchanged (Hartz‐Rubin and DeLucia, 2001; Sims *et al*., 1999b; Yoon *et al*., 2009) and in other cases elevated CO_2 decreased the leaf area and specific leaf area decreased by 6% in plants exposed to elevated CO_2 (Ainsworth and Long, 2005).

The increases in LAI at the canopy level under elevated CO_2 provide a greater surface for transpiration which increases the water use, in this way the WUE may be offset under CO_2 enrichment (Allen Jr, 1999). But still, the positive effect of rising CO_2 on productivity and dry matter production through increasing photosynthesis, reducing the transpiration rate and WUE improvement related to LAI is frequently pronounced (Carlson and Bazzaz, 1980; Lawlor and Mitchell, 1991; Reddy *et al*., 1995). Concomitantly, as a result of higher biomass allocation towards sinks, grain yield is often reported to increase under elevated CO_2 (Hikosaka *et al*., 2011; Hogy and Fangmeier, 2008; Kimball *et al*., 2001;

Kimball *et al*., 2002; Wu *et al*., 2004). However, under conditions where some other factors are severely limiting the long term elevated CO_2 effect may not be manifested e.g., such as low nitrogen supply (Kanemoto *et al*., 2009; Le Roux *et al*., 2001), and growing plants in pots where plants are limited both in root growth and nutrient availability (Ainsworth *et al*., 2002; Arp, 1991) and low temperature (Long, 1991). In these situations imbalances within the photosynthetic system occur which lead to the accumulation of non-structural carbohydrate which may act as the feedback of the CO_2 stimulation of photosynthesis under CO_2 enrichment. This feedback is known as photosynthetic acclimation (Bowler and Press, 1996; Ghannoum *et al*., 2002). Eventually, with photosynthetic down-regulate a reduction in biomass and seed yield is sometimes observed (Ainsworth *et al*., 2002; Prasad *et al*., 2002). However, more recently, Sinha (2011) indicated that plant organ dry weight was significantly enhanced in three wheat species in spite of photosynthetic down regulation due to higher accumulation of starch and total soluble sugars. In order to sustain the photosynthetic rate under long term exposure to elevated CO_2 and to avoid photosynthetic down-regulation, high sink strength (carbon utilization) is required (Lawlor, 1995; Long *et al*., 2004).

A modification in plant response to expected increases in atmospheric CO_2 and combined temperature change may have thus been underestimated by earlier workers. Cheng *et al*., (2009) found that both whole plant biomass and grain dry weight were reduced under elevated CO_2 in combination with high temperature due to a shortening in crop growth cycle and accelerated development and thereby, the advantages of an increasing CO_2 on assimilation may be offset by supra optimum temperature (Streck, 2005). Therefore, an optimum temperature

is required to maintain the maximum assimilation rate. High temperatures in excess to 36 ^{0}C and low temperature below 18 ^{0}C are identified to diminish carbohydrate translocation through the phloem resulting in down regulation of assimilation in fast growing metabolic sinks (Reddy *et al*., 2010).

Alongside increasing crop growth and yield at elevated CO_2, crop grain/seed quality has been suggested to be altered as a result of reduction in vegetative tissue nitrogen concentration rather than the carbon accumulation during grain filling (Högy and Fangmeier, 2008; Jackson *et al*., 1994; Hogy *et al*., 2011; Lierffering *et al*., 2004) and due to a drop in nitrogen concentration in plant organs protein concentration decreased (Blumenthal *et al*., 1996; Hampton *et al*., 2012; Thompson and Woodward, 1994; Wu *et al*., 2004; Yang *et al*., 2007) but the non-structural carbohydrate (Hogy *et al*., 2009) and lipids increased in cereal crops grain under elevated CO_2 (Sator, 1999; Williams *et al*., 1995). However, the seed oil content was not affected in oilseed rape at elevated CO_2 (Franzaring *et al*., 2008) but Hogy *et al*., (2010) reported that the fatty acid composition changed in the same species at elevated CO_2.

Generally, the beneficial effect of elevated CO_2 has been extensively reported in C_3 crop species for physiology, growth, yield (Kimball *et al*., 2002) but for quality only in some oil crops such as oilseed rape (Högy *et al*., 2010).

To date, there are no reports in the literature concerning the effect of elevated CO_2 on safflower physiology, growth, yield and chemical composition, in spite of the medical, pharmaceutical and economic importance that has been demonstrated for the crop (Berglund *et al*., 2010; Berglund *et al*., 2007; Dajue and Mündel, 1996; Smith, 1996). Safflower is typically grown in the arid or semi-

arid regions of the world (Johnston *et al.,* 2002) that are facing potentially challenging climate change (Shaw *et al*., 2005). Lawlor, (1999) reported that the effect of rising atmospheric CO_2 on semi-arid plants will be amongst the greatest because those species showed significant increase in yield under CO_2 enrichment.

This chapter reports the results of an initial investigation of the effect of CO_2 enrichment on physiology, growth and seed composition of this crop grown in an enclosed chamber using a perlite based hydroponic system.

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5.2. Aim

Study the effect of elevated atmospheric CO_2 on safflower physiology, growth performance and seed yield, seed oil content and fatty acid composition.

5.3. Objectives

5.3.1. Objective 1

Investigate the effect of CO_2 enrichment on some growth parameters (stem height, leaf number, LAI, above ground biomass) and yield components (branch number, capitula number, seed number per plant).

5.3.2. Objective 2

Examine the physiological parameters (assimilation rate, stomatal conductance, transpiration rate, sub-stomatal conductance and water use efficiency) under elevated CO_2.

5.3.3. Objective 3

Investigate the effect of elevated CO_2 on seed quantity and quality by studying the seed oil content and fatty acid composition.

5.4. Materials and Methods

Experimental design and measurements taken

Plants were grown under ambient and elevated CO_2. Pots were watered with 10 - 30 mL of a standard hydroponics growth solution A and B (details given in material and methods) every 3 - 5 day until plants reached the elongation phase. Thereafter the amount of solution added was increased to 50 - 200 mL and supplied every week so as to leave at least 200 mL of drainage fluid in the drainage beaker) until 2 weeks before harvest when the watering was completely stopped. Tap water was supplied in between according to demand and all amounts of water added were recorded for each pot.

Weekly average CO_2 (Figure 5.1), temperature (Figure 5.2) and relative humidity (Figure 5.3) were calculated and recoded. An average of 88.8% RH for ambient and 84.14% for the elevated chamber and an average temperature of 17 ^{0}C during the growth period were recorded. The average CO_2 concentrations in the supplemented chambers were 1074, 1022, 1017 and 997 µmol mol^{-1} in the four chambers respectively with an overall average of 1028 µmol mol^{-1}. These, were higher than originally planned but were still double compared to ambient which was recorded at an overall average of 400 µmol mol^{-1}.

At 50% anthesis (4th June 2011) the physiological parameters assimilation rate, transpiration rate, stomatal conductance and sub stomatal CO_2 were measured on the youngest, three top expanded leaves of all plants (see ch 2). Days from sowing to 50% flowering were recorded.

A day after (32 plants in total) plants were harvested and stem height, number of leaves per plant, branch number, capital number, leaf area and leaf area

index (LAI), chlorophyll content, plant organs biomass and nitrogen content were measured.

The three sub-samples of three youngest expanded leaves of two plants from each chamber were used for measuring chlorophyll and other three sub-samples dried for determination of nitrogen content. Also two of the harvested plants were separated into plant organs (stem + branch, leaf, capitula) and dried and weighed and recorded. Finally, these dried organs were ground and nitrogen concentration measured (see ch 2)

An aphid infestation was diagnosed at the plants at the flowering stage (three days after anthesis) and plants in all chambers were infested but plants in one of the elevated chambers were seriously infested. To manage the problem, the plants were sprayed with recommended chemicals (Garden bug killer containing difenoconazole and thiacloprid).

When most of the leaves had turned brown and only a tone of green remained on the bracts of the latest flowering heads (Berglud *et al*., 2007) the remaining plants were harvested (on 28th July 2011) and the average stem height leaf number, branch number, capitula number, above ground biomass and seed yield per plant for both ambient and elevated CO_2 determined. The percentage total seed oil content was determined on a fresh weight basis, using Soxhlet and percentage of fatty acids determined using GLC (see ch 2).

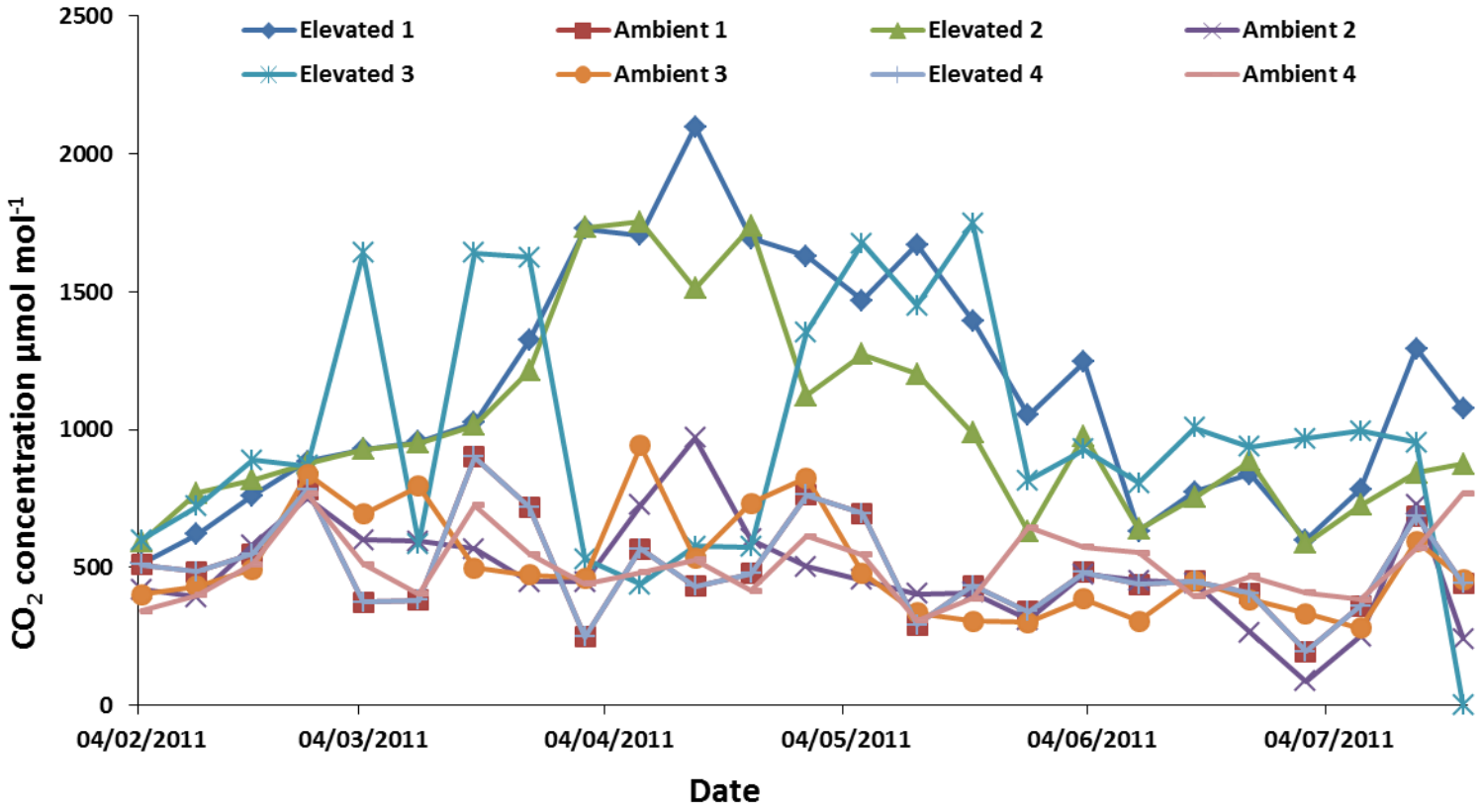

Figure 5. 1. Weekly average CO_2 concentration per chamber over the growth period.

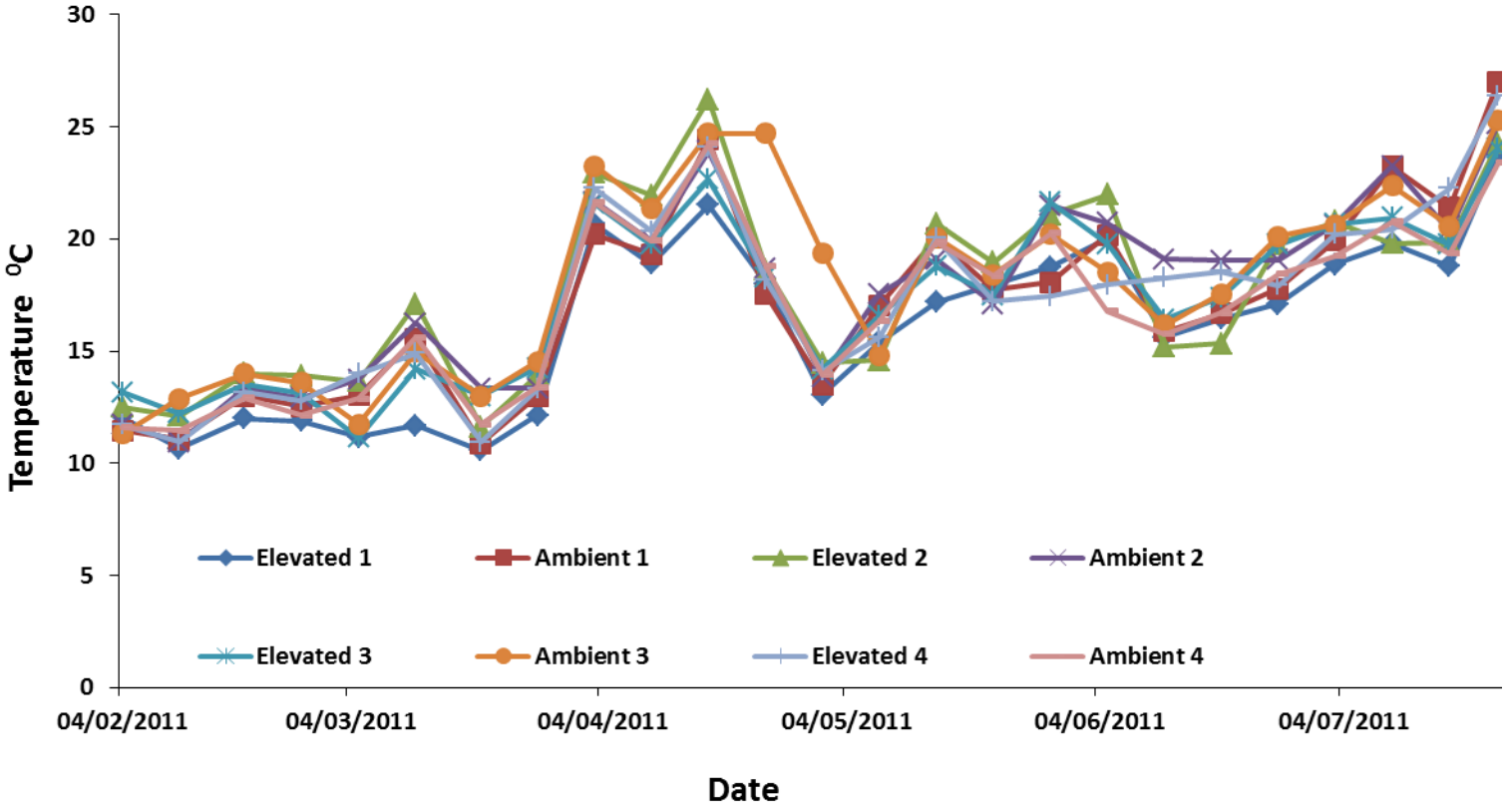

Figure 5. 2.Weekly average temperature per chamber over the growth period.

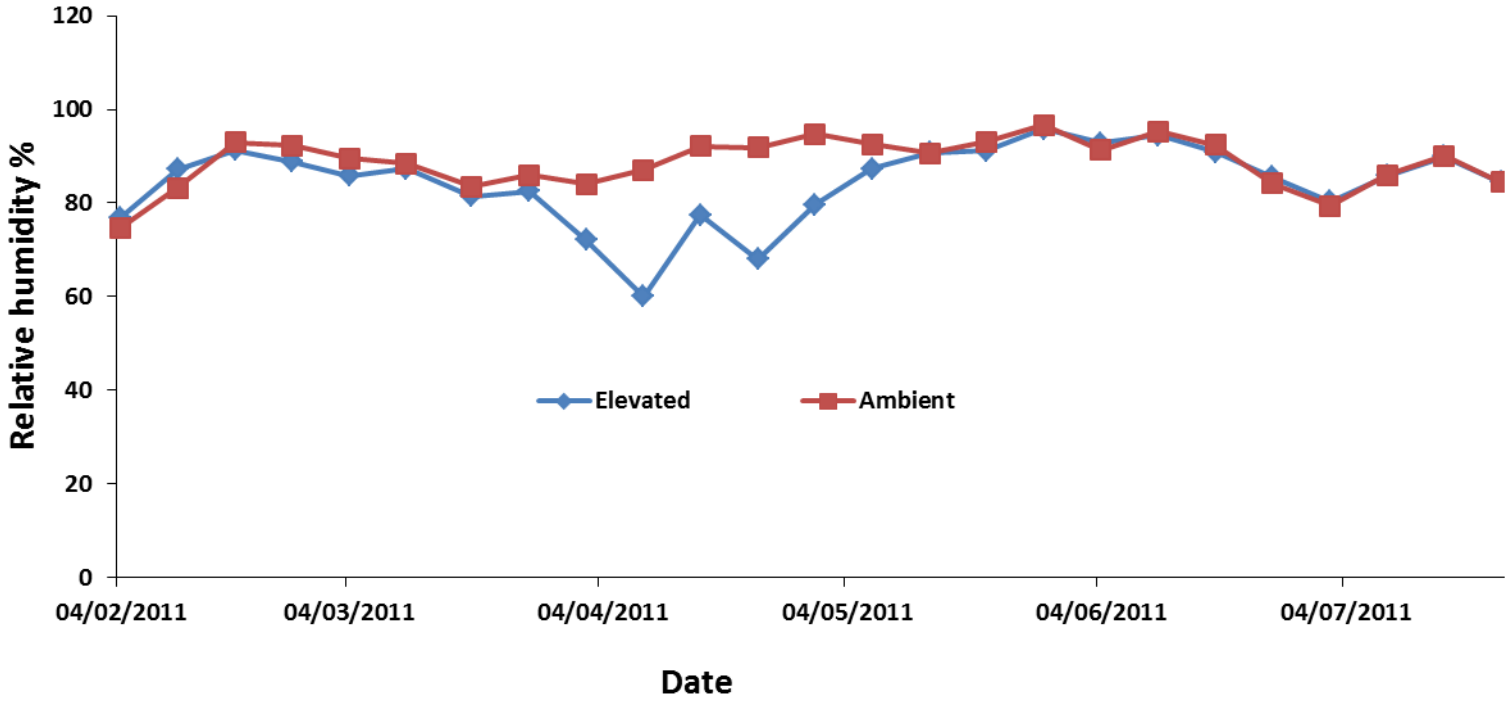

Figure 5. 3. Weekly average relative humidity per chamber over the growth period.

5.5. Results

5.5.1. Physiological parameters

Elevated CO_2 at anthesis significantly ($p < 0.05$) increased mean assimilation rate compared to ambient (Figure 5.4.A) whilst significantly ($p < 0.05$) reducing stomatal conductance (Figure 5.4.B) and thereby the transpiration rate was also significantly ($p < 0.05$) reduced under elevated CO_2 compared to ambient CO_2 (Figure 5.4.C). The CO_2 concentration in the sub-stomatal cavity did not differ between the treatments (Figure 5.4.D).

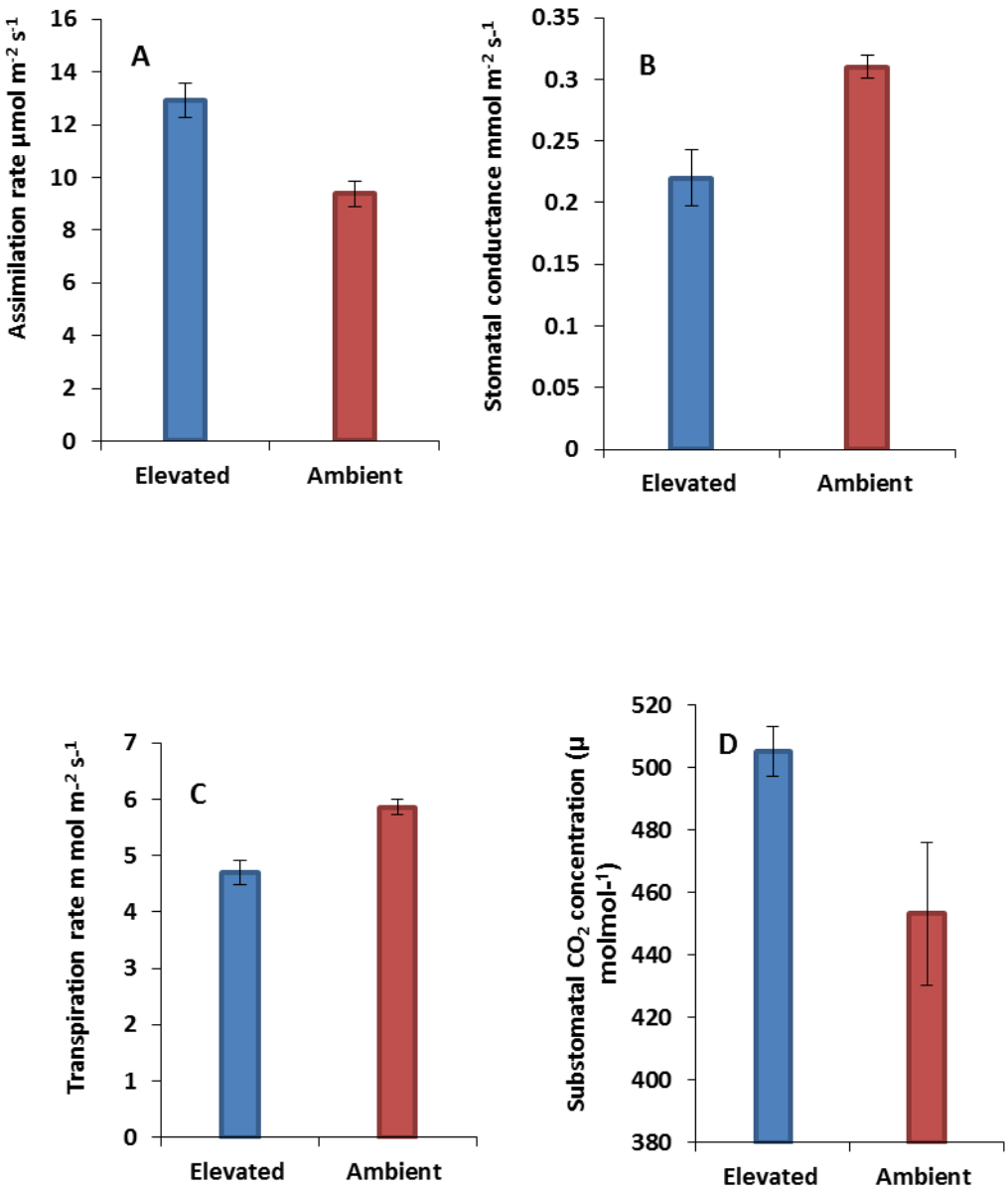

Figure 5.4. Mean of A. assimilation rate (*A*),B. stomatal conductance (g_s), C. transpiration rate (*E*) and D. intercellular CO_2 concentration (Ci) under the elevated CO_2 at 50% anthesis. Vertical bars are the standard errors of the mean (n =16) at 0.0 5 levels.

5.5.2. Plant nitrogen concentration and chlorophyll content

Compared to ambient conditions, plant organs at anthesis significantly concentrated less nitrogen under elevated CO_2 and under both growth conditions the concentration differed in different plant organs. The concentration of nitrogen in the youngest fully expanded leaves, which were used for measuring the assimilation rate at anthesis, was significantly reduced by elevated CO_2 compared with ambient CO_2 (Figure 5.5).

Chlorophyll a, b and total chlorophyll in samples of the same three youngest fully expanded leaves showed that they were not significantly affected by elevated CO_2 treatment (Figure 5.6).

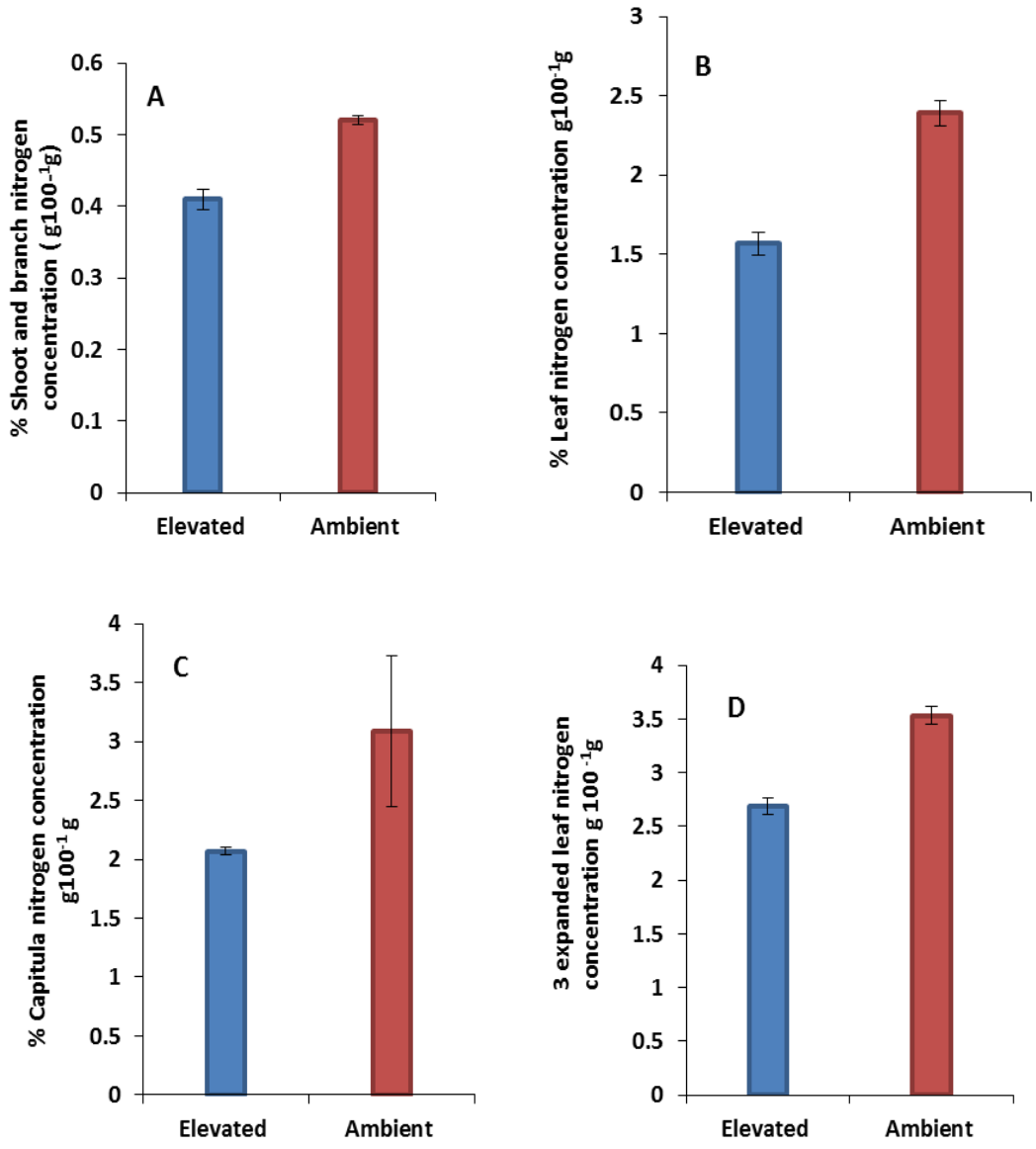

Figure 5.5. Mean of nitrogen concentration ($g100g^{-1}$) in A. shoot and branch, B. total leaves, C..capitula and D. 3 expanded leaves on dry weight basis at 50% anthesis. Vertical bars are standard errors of the mean (n= 3) at 0.05 levels.

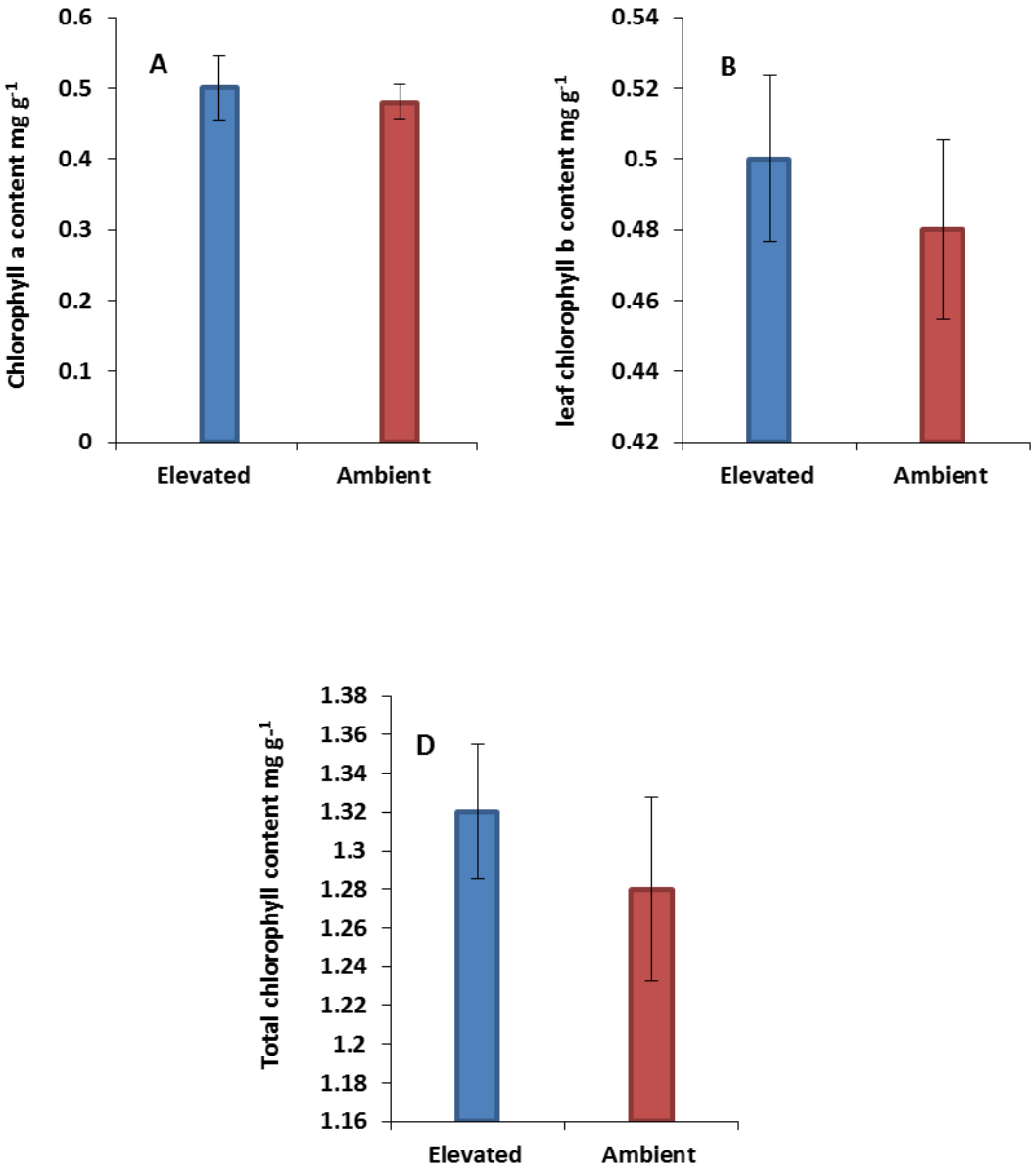

Figure 5.6. Mean of A. 3 expanded leaf chlorophyll a,B. b and C. total chlorophyll content of fresh Weight basis at 50% anthesis under the effect of elevated CO_2.

Vertical bars are standard errors of the mean (n =3) at 0.05 levels.

5.5.3. Plant development, morphology, growth and seed yield

Days to 50% flowering were 78 days for both elevated and ambient CO_2 grown plant. From anthesis to harvest, the plant height did not show any significant effect under elevated CO_2 as compared to ambient CO_2 (Table 5.1). In contrast, the leaf number, and yield components responded significantly to elevated CO_2, with 4%, 50% and 30% increase in the number of leaves, branches and capitula, respectively, when counted at harvest (Table 5.2). Plants grown under elevated CO_2 significantly enhanced leaf area and a consequence leaf area index LAI at anthesis, with a 28% increase as compared to ambient CO_2 grown plants (Figure 5.7.A).

Plants exhibited more growth in term of dry matter accumulation in response to elevated CO_2 (Table 5.1). Elevated in comparison to ambient CO_2 increased total above ground dry weight by 51% and 43% at anthesis and harvest, respectively (Figure 5.8B and C).

Increasing CO_2 had little effect on seed number with only a small increase which was not statistically significant ($p = 0.520$) (Table 5.1). Plants produced an average of 8 seed per capitula which is much less than that reported value for field grown safflower of 15 - 30 seed per capitula (Dajue and Mundle 1996). In general the seed set was lower than expected but sill proportionate to the treatments and fresh seed weight increased by 49% under elevated CO_2 compared to ambient CO_2 (Figure 5.8.D).

Table 5. 1.Means value of plant development criteria and yield component per plantwith their dry weight (gpl^{-1})under the effect of elevated CO_2 at 50% anthesis.

Parameters	***At anthesis***		
	Elevated CO_2	***Ambient CO_2***	***P Value***
Plnat height (cm)	95	91	0.092
Leaf number p	34	30	0.110
Leaf area	37	27	0.000
Branch number	3	1	0.000
Capitla number	3	2	0.000
Shoot and branch dry weight	4	2	0.000
Leaves dry weight	2	1	0.000
Capitula dry weight	3	1	0.000

Table 5. 2. Means value of plant development criteria and yield component per plant with their dry weight (gpl^{-1}) under the effect of elevated CO_2 at harvest.

Parameters	***At harvest***		
	Elevated CO_2	***Ambient CO_2***	***P Value***
Leaf number	31	26	0.000
Branch number	4	2	0.001
Capitula number	3	2	0.006
Shoot and branch dry weight	2	1	0.000
Leaves dry weight	2.07	0.98	0.000
Capitula dry weight	1.61	0.73	0.000
Seed number p	25	17	0.52

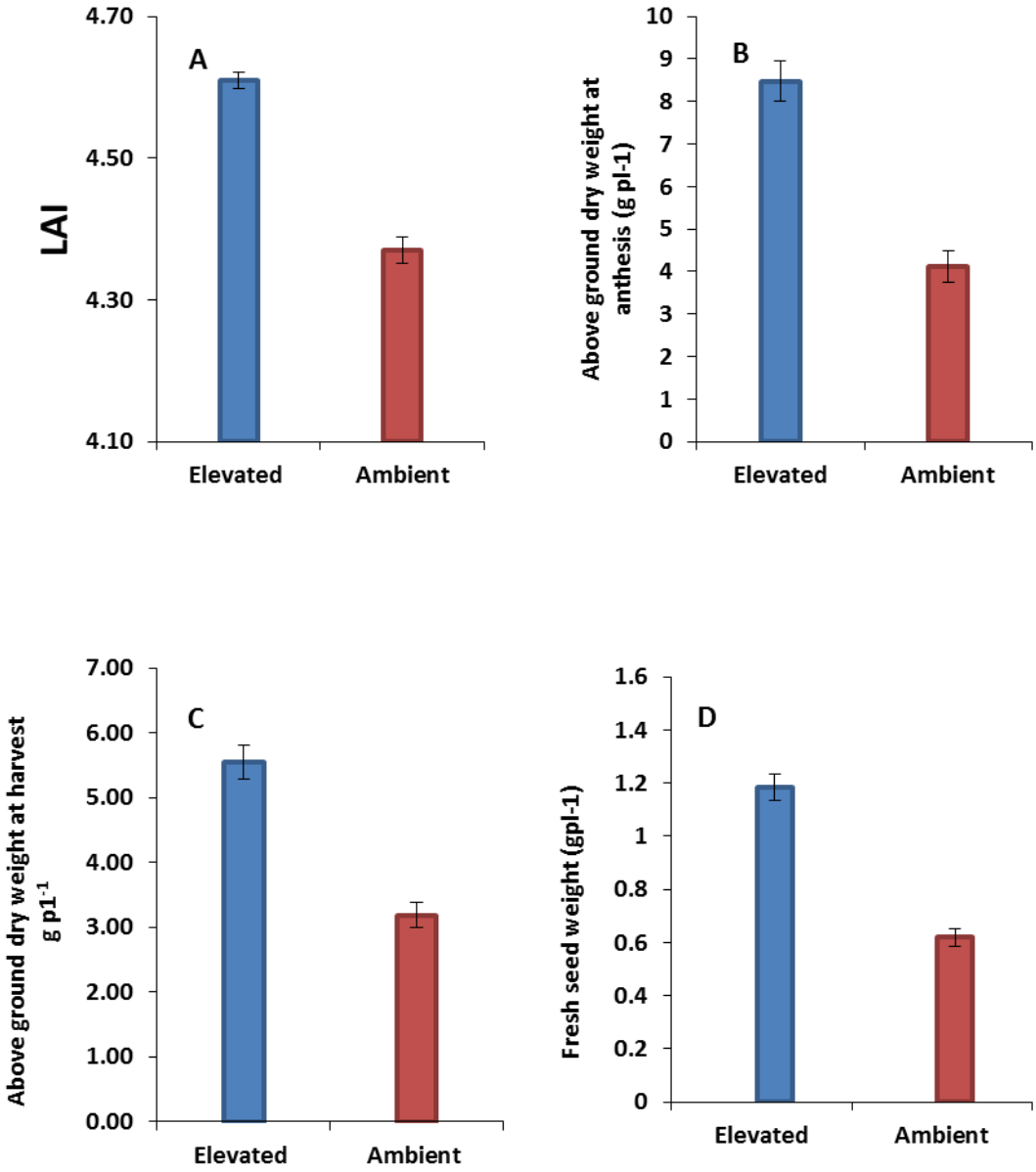

Figure 5.7. Mean of A. LAI and B. above ground dry weight at 50% anthesis, C. above ground dry weight and D. fresh seed weight at harvest under the effect of elevated CO_2. Vertical bars are standard errors of the mean (n= 4) at 0.05 levels.

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5.5.4. Seed chemical analysis

Due to insufficient quantities of seed obtained seed oil content and fatty acid analysis were performed on a sample pooled from all replicates. The elevated CO_2 slightly reduced seed oil content compared to ambient and the seed oil content in both growth conditions was less than the parent seed (Table 5.3).

In comparison with the parent seed the saturated fatty acids (palmitic and stearic) and mono-saturated fatty acid (oleic) were slightly reduced in this study for both CO_2 treatments (Table 5.3), whilst the unsaturated fatty acids (linoleic and linolenic) were increased. In comparison to ambient CO_2 the ratio of unsaturated fatty acids***/***saturated fatty acids slightly increased in oil produced under elevated CO_2.

Table 5. 3. Total % seed oil content and fatty acid composition under the effect of elevated CO_2 compared to ambient CO_2 and original seed.

Parameters	***Parent seed***	***Seeds produced under ambient CO_2***	***Seeds produced under elevated CO_2***
Oil content	21.3	18.44	17.95
Palmitic 16:0	7.75	6.59	6.53
Stearic 18: 0	2.54	2.35	2.19
Oleic 18: 1n	11.65	0.64	0.64
Linoleic 18:2n	73.63	76.96	77.45
Linolenic 18:3n	0.12	0.13	0.14

5.6. Discussion

These results showed that the assimilation rate of safflower increased at elevated CO_2 compared to ambient CO_2.Elevated CO_2 (1000 µmol mol^{-1}) significantly increased the assimilation rate by an average of 27% compared to ambient (400 µmol mol^{-1}) (Figure 5.4.A). This was interpreted to be as a result of increased CO_2 at the carboxylation binding site of Rubisco and hence, inhibited photorespiration under CO_2 enrichment. Such a response has been frequently reported (Andrews *et al*., 1995; Leakey *et al*., 2009) and many workers on several C_3 plants, conducted in different controlled conditions, concluded that the light saturated photosynthetic rates at doubled CO_2 is usually increased to some extent (Ainsworth and Long, 2005; Leakey *et al*., 2009; Reddy *et al*., 2010). Elevated CO_2 in both FACE and enclosed chamber studies has shown a similar direct change in assimilation rate and productivity (Ainsworth and Long, 2005). Similar results have been reported for soybean, dry bean, peanut and cowpea but at the optimum temperature and in the absence of abiotic and biotic stress (pests, diseases and weeds) (Prasad *et al*., 2005). In soybean, it has been reported that elevating CO_2 to 1000 µmol mol^{-1} enhanced photosynthesis more at 22/16 ^{0}C than at 26/20 ^{0}C (SionitI *et al*., 1987) and the optimum temperature is required to maintain this maximum assimilation rate. High temperatures above 36 ^{0}C and temperatures below 18 ^{0}C have been identified to diminish carbohydrate translocation through phloem resulting in down-regulation of assimilation (Reddy *et al*., 2010) because low and high temperature have been shown to cause progressive damage to the protoplasm (Kendall,1952).

In fact, it has been concluded that elevated CO_2 enhances assimilation rate in C_3 plants by an average of 31%, but the magnitude of enhancement, varies among different species and environments (Ainsworth and Rogers, 2007).

In general, modelling studies have reported that assimilation rate and productivity for crops were sustained over time for short term exposure to rising CO_2, but they were down regulated during long term exposure to high CO_2 (Ainsworth *et al*., 2002; Field and Moony, 1986; Rogers *et al*., 1986; Sims et al., 1999; Wheeler *at al*., 1999). Arp (1991) revealed that down-regulation in photosynthetic capacity (acclimation) at long term exposure to high CO_2 was related to the pot size in which plants were grown because the volume of the pots impacted the sink size by restricting root growth and a similar effect might be seen here.

In the current study, elevated CO_2 reduced stomatal conductance at anthesis by 29% and a corresponding decrease in transpiration rate was 18% under CO_2 enrichment treatment compared to ambient (Figure 5.4. B and C). Such results have often been reported (Ainsworth and Long, 2005; Drake and Leadley, 1991; Poorter and Perez-Soba, 2002; Shaw *et al*., 2005; Wheeler *et al*.,1996 a; Wheeler *at al*., 1996b). These reductions may be attributed to WUE improvement and enhanced assimilation rate as reported elsewhere (Drake *et al*., 1997). Elevated CO_2 to 700 µmol mol^{-1} CO_2 increased photosynthesis rate in peanut by an average of 27% across all temperature regimes used (32/22, 36/26, 40/30, and 44/34 ^{0}C) due to reduction in stomatal conductance and leaf transpiration (Gifford *et al*., 2000).

No significant differences were found in sub-stomatal CO_2 concentration between elevated CO_2 and ambient CO_2 at anthesis (Figure 5, 4). This could be explained by more CO_2 investments in photosynthesis on one hand, and stomatal closure reduced transpiration rate at the same time reducing the diffusion of CO_2 in to the leaves at elevated CO_2 on the other. The amount of intracellular CO_2 concentration is frequently reported to be at the same irrespective of the levels of CO_2 (Drake *et al*., 1997; Sage, 1994). Ainsworth and Long (2005) from a meta- analysis review of 12 FACE experiments reported that there was no obvious change in the ratio between atmospheric CO_2 and intercellular CO_2. In contrast, CO_2 enrichment led to an increase in intracellular CO_2 concentration in a total of 17 temperate grasses and herb species leaves (Beerling and Woodward, 1995). The same results were also reported for deciduous forest trees (Korner *et al*., 2005) and the deciduous woody shrub *Lindera bezoin* L. (Cipollini *et al*.,1994).

Plant organ nitrogen content was significantly depressed at high CO_2 compared to ambient (Figure 5.5) leading to an increased ratio of C: N. This trend is consistent with the observation that plants change their nitrogen allocation to optimize energy cost (Cotrufo *et al*., 1998; Jablonski *et al*., 2002; Kimball *et al*., 2002). Moreover, this has been interpreted as an increased ability of plants to use nitrogen more efficacies as a result of the marked increase in photosynthetic rate at doubled CO_2 (Cruz *et al*., 2003) as reported here for safflower. Another explanation is the consequence of a decrease in transpiration rate resulting from reduced stomatal conductance in response to elevated CO_2 which possibly limited plant nitrogen uptake from the root

environment, but this explanation has received little attention in the literature (Kanemoto *et al*., 2009).

Safflower leaves concentrated less nitrogen but this did not lead to big changes in chlorophyll content in elevated CO_2 (Figure 5.6). In previous work on cotton, (Delucia *et al*., 1985) proposed that elevated CO_2 (1000 µmol mol^{-1}) reduced leaf chlorophyll content and the ratio of chlorophyll a/b changed with reductions in transpiration rate and nitrogen content. The reduction in leaf chlorophyll content under elevated CO_2 was due to mild chlorosis in leaves in response to CO_2 increase.

CO_2 enrichment did not enhance the plant height and leaf numbers at anthesis and stem height at harvest, which corresponds to previous finding where no significant effect of two levels of increasing CO_2 (600 and 800 µmol mol^{-1}) in cotton plant height was found. Also the interaction of CO_2 with high temperature (35/25 ^{0}C) did not alter the stem height (Yoon *et al.,* 2009). Seven FACE experiments for 12 species of trees and shrubs showed an increase in stem height, while C_3 crop species showed no change (Ainsworth and Long, 2005). With the growth in doubled CO_2 the leaf number increased by 8%. In contrast, oilseed rape plants grown under elevated CO_2 were markedly taller than those grown under normal CO_2 (Franzaring *et al*., 2008).

Plant yield components in term of leaf, branch and capitula numbers were affected in plants grown under elevated CO_2 (Table 5.1and 5.2) at the same time the dry weight of these reproductive organs increased and this was interpreted as a result of a speed up of plant development during vegetative growth and also photosynthetic product shift to generative growth organs

increased at elevated CO_2 (Franzaring *at al*., 2008). The same result was reported for cotton where the number of bolls per plant increased due to a larger number of secondary branches at elevated CO_2 at optimum temperature for cotton growth (Reddy *et al*., 1998). In previous work on soybean, Rogers *et al*., (1984) suggested that branch growth and development, leaf initiation and leaf expansion are sensitive to carbon availability.

Leaf morphology in term of LAI at anthesis was significantly enhanced by 28% at increase at elevated CO_2 (Figure 5.7.A) probably due to a direct consequence of increased assimilation rate and growth. This agrees with previous findings that the greatest stimulation occurred when the canopy leaf area reached its greatest but the increased LAI did not lead to noticeable changes in canopy light absorption (Manderscheid *et al*., 2003). However, some authors have predicted that LAI would increase in the upper canopy under doubled CO_2 because elevated CO_2 reduces the light compensation point for photosynthesis and thus stimulates leaf production (Campbell *et al*., 2001; Reddy *et al*., 1998; Rodriguez *et al*., 2001) also LAI of peanut (Bannayan *et al*., 2009) and soybean increased under elevated CO_2 (Heinemann *et al*., 2006; Yuelin *et al*., 2005) but in contrast no other significant effect of elevated CO_2 on LAI was found by Sims et al., (1999) and Yoon *et al*., (2009) and elevated CO_2 failed to increase the leaf area and in fact specific leaf area decreased by 6% in plants exposed to elevated CO_2 (Ainsworth and Long, 2005). Ewert (2004) showed in his review the relative importance of LAI for canopy assimilation and biomass accumulation and translocation under varying levels of CO_2 and suggested that under elevated CO_2 and other environmental factors, plant growth and productivity are unlikely to be realized without increasing LAI.

There was a strong response of above ground biomass to elevated CO_2, with an increase of 51% at anthesis and 43% of harvest for plants subjected to high CO_2 compared to ambient CO_2 indicating that the total aboveground biomass correlated with LAI changes and followed a similar pattern (Figure 5.7 B nd C) and this paralleled shoot weight as previously reported (Fangmeier *et al*., 1996; Manderscheid *et al*., 2003; Pinter Jr *et al*., 1996)

The seed yield of a crop is a function of final total biomass production and harvest index (Yang *et al*., 2006a) and the highest seed yield (54%, Figure 5.7 D) recorded here under elevated CO_2 was more a function of the increased biomass rather than any shift in harvest index. The increased biomass at elevated CO_2 was interpreted as a result of photosynthetic capacity increases leading to improved net assimilation rate. Some other studies also show a good correlation between photosynthesis and productivity, e.g., in work on cotton Reddy *et al*., (1995) reported that increasing total biomass was due to higher photosynthesis rates at elevated CO_2. This achievement in above ground biomass and grain weight here was interpreted as a result of increased LAI and assimilation rate and thereby radiation use efficiency Lawlor, (1995) and Manderscheid *et al*., (2003). Yuelin *et al*., (2005) in a study on soybean found that elevated CO_2 from 550 to 750 µmol mol^{-1} increased leaf area index from 4.08 to 7.57% and increased pod number per plant from 6.8 to 11.61%, eventually led to the increase in seed weight per plant from 15.14 to 29.10%.

(Gifford and Evans, 1981) showed that increase in seed yield can come from improved carbon partitioning and storage of photosynthetic assimilates and in agreement with this hypothesis elevated CO_2 enhanced the plant biomass and grain yield in field grown rice (Lieffering *et al*., 2004) and elevated CO_2

increased the grain yield due to increasing LAI and biomass in soybean (Yuelin *et al*., 2005). Furthermore, total plant dry weight increased at two elevated of CO_2 levels (600 and 800 µmol mol^{-1}) and temperature (25/15 and 35/25 ^{0}C) but did not correlate to the change in LAI. Yoon *et al*., (2009) suggested that higher resource use efficiency per unit leaf area rather than enhancing the leaf area for capture of resource.

So as can be seen from this discussion, there are several ways that plant species can respond in its assimilation to increased levels of CO_2 and safflower appears to be in the group which increase both assimilation rate and LAI and thereby light efficiency capture. However, the increased assimilation and LAI did not result in a significant increase in seed number in this study (Table 5.1). Increasing CO_2 had little effect on seed number, the seed number was slightly increased by CO_2 but was not statistically significant ($p = 0.520$). The plants under CO_2 enrichment produced 8 seed per capitula which is less than the recorded value for field grown safflower of 15-30 seed per capitula (Dajue and Munde, 1996). The exact reason for this is not known but it may be explained by the cloudy (short photoperiod) days during bud development and pollination (most of the days were cloudy) in combination with high humidity and low temperature in the enclosed chamber (mean temperature was about 19 ^{0}C and humidity was about 88%) and these could have affected flower fertility especially at the time of pollination. Safflower is typically grown in arid or semi-arid regions of the world with a hot, dry and long day climate (Dajue and Mundle, 1996).The supplemented photoperiod provided here in association with the cloudy days, low temperature and high humidity seems to have been inadequate for safflower flowers to fertilizer adequately. This speculation is

strongly supported by work on soybean where Siontl *et al*. (1987) reported that no soybean seeds were produced at 18/12 ^{0}C across all levels of elevated CO_2 (350, 675 and 1000 µmol mol^{-1}). Similarly, photoperiod of 16 h. and day/night temperatures of 18/28 0C increased the flowering and pod formation in bean (*Vicia faba* L.), conversely, at 5/13 ^{0}C and 10 and 13 h. of photoperiod seed set responded negatively (Ellis *et al*., 1988).

In general, under conditions where some other factors are severely limiting, such as low temperature, the effect of CO_2 elevation might not be noticeable (Long, 1991); Furthermore, Amthor (1998) concluded from his review that yield can be enhanced from CO_2 enrichment to some extent but that other factors have more stimulatory effect on crop seed yield, such as selection of genotypes with increased harvest index and disease resistance, nitrogen fertilizer and chemical weed and pest control. A second explanation for poor seed set may be due insufficient nutrient availability with small pot volumes, for example boron deficiency is known to affect seed set in wheat (Rerkasem *et al*., 1993).

Despite the low seed number there was still a positive effect of elevated CO_2 compared to ambient and this was closely related to the increase in capitula number per plant. From the correlated responses in various crosses Patil *et al*., (1994) found that for yield improvement in safflower the selection for capitula number per plant was effective. In other reports seed number was not affected by elevated CO_2 (Kimball *et al*., 2001; Kimball *et al*., 2002). Whereas, elevated CO_2 (700 µmol mol^{-1}) increased seed number in soybean by an average of 22% due to increased plant biomass (Boote *et al*., 1989).

It was observed that the days from sowing to flowering were not affected by high CO_2, and the same result was found for rapeseed (Franzaring *et al*., 2008) and flowering time has generally been shown to be un affected by elevated CO_2 (Springer and Ward, 2007). Furthermore, Prasad *et al*., (2003) stated that the days from sowing to flowering, pollen viability and seed set are temperature sensitive under elevated CO_2 and ambient to a similar degree. No differences were observed at the start of flowering date at different levels of CO_2 investigated for soybean but the start of flowering showed differences at different temperature regimes (Heinemann *et al*., 2006).

Elevated CO_2 slightly reduced seed oil content compared to ambient, but the seed oil content in both elevated CO_2 and ambient was less than the original (parent) seed oil content recorded as 21%. The net oil yield increased at elevated CO_2 due to increasing seed yield (Figure 5.8) and the same result was reported for rapeseed and indicated that the positive effect of CO_2 enrichment on repressed growth is only moderate after flowering stage (Franzaring *et al*., 2008). In comparison with the parent seed the saturated fatty acids (palmitic and stearic) and mono-saturated fatty acid (oleic) were reduced in this study for both elevated CO_2 and ambient (Table 5.8). In contrast, the unsaturated fatty acids (linoleic and linolenic) were increased. In comparison to ambient the ratio of unsaturated fatty acids***/***saturated fatty acids was slightly increased in oil at elevated CO_2. It is not possible to place much confidence in the suggestion that elevated CO_2 affects the seed oil content, and some changes in fatty acids profile in this study, because it was based on only one replicate. However, it can be speculated that non-structural carbohydrate (carbohydrates that the plant is unable to utilize) and its relative source/sink size has been shifted towards the

seed and reduced the seed nitrogen content and made alterations in seed composition of elevated CO_2. Other environmental factors such as temperature could be important for seed composition (Knowles, 1985).

Unfortunately, due to insufficient seed obtained in this study, the seed nitrogen concentration could not be measured. But a reduction in nitrogen concentration and protein content could be possible at elevated CO_2 due to nitrogen concentration reduction in plant organs at anthesis. As previously suggested the nitrogen uptake was complete at anthesis, and after anthesis was translated from the vegetative pool to the seeds which would lead to a decrease in seed nitrogen content (Fangmeier *et al*., 1999).

In agreement with the current study, recent work on oilseed rape (Högy *et al*., 2010) showed that total oil content of seed was not affected by elevated CO_2 (494 µmol mol^{-1}) but oil yield increased due to increasing seed yield, while fatty acid composition changed only slightly compared to ambient. Also Franzaring *et al*., (2008) found no significant effect of elevated CO_2 (500 µmol mol^{-1}) on oil seed rape seed oil content at compared to ambient. Conversely, elevated CO_2 reduced seed protein content in cereal crops such as rice (Lieffering *et al*., 2004; Yang *et al*., 2006b) and wheat due to a drop in the seed nitrogen concentration and an increase in the non-structural carbohydrate (Högy *et al*., 2009) and lipids in wheat grain (Sator, 1999; Williams *et al*., 1995). Results from these studies suggest that grain quality altered with a rise in CO_2 but for safflower this was not evident.

5.7. Conclusion

This experiment clearly demonstrated that CO_2 levels elevated to approximately twice the levels of ambient, and with an optimal of water and nutrient supply, increased the assimilation rate of safflower at anthesis. This increase can be linked to a reduction in a stomatal conductance and transpiration rate. The above ground biomass markedly increased and was associated with a noticeable increase in LAI and assimilation rate at elevated CO_2 and this increased above ground biomass which was maintained through to harvest. This indicates that elevated CO_2 increased assimilation rate and partitioning of biomass. Seed set was very low at both elevated CO_2 and ambient, and it is suggested that pollination of flowers has failed because the flowering period coincided with short days combined by high humidity and low temperature and the amelioration of these stresses by CO_2 enrichment was not possible. Seed oil content might be reduced slightly by elevated CO_2 and the seed fatty acid profile might also be altered but only in a minor way. Overall, the results reported here suggest that safflower would perform well under conditions of increasing CO_2 levels.

Chapter 6

The interaction effect of elevated CO_2 and varying levels of nitrogen on the physiology, growth development and seed yiel

6.1. Introduction

Elevated CO_2 results in initial stimulation of photosynthesis, but this stimulation is often partially or completely reversed especially under long term exposure (Ainsworth *et al*., 2003; Martínez-Carrasco *et al*., 2005). Sink strength and nitrogen status are the main two factors that have been implicated in the acclimatory response of plants grown at elevated CO_2. Many results from FACE have provided links between nitrogen supply and assimilation acclimation (down-regulation) and concluded that plants grown at low nitrogen supply characteristically accumulate more non-structural carbohydrate and exhibited greater acclimation of Rubisco than those grown at adequate nitrogen supply (Ainsworth *et al*., 2003; Ainsworth and Long, 2005). Therefore, in low nitrogen supply a reduction in photosynthetic capacity is often has been observed at elevated CO_2 (Del Pozo *et al*., 2007; Drake *et al*., 1997; Geiger *et al*., 1999; Harmens *et al*., 2000; Pettersson and McDonald, 1994) and this is because photosynthetic capacity is strongly related to the leaf nitrogen content (Evans, 1989; Sage and Pearcy, 1987) primarily because the enzymes of the Calvin cycle, such as Rubisco, and thylakoids account for most of the plant nitrogen content (Taiz and Zeiger, 2006). Nitrogen is also comparative to leaf chlorophyll content (Evans, 1989). Reduction in nitrogen availability therefore is often associated with a decrease in the amount and activity of Rubisco carboxylation (Ainsworth and Long, 2005; Ainsworth and Rogers, 2007; Long *et al*., 2004; Rogers and Humphries, 2000), a decrease in chlorophyll content and a decline in nitrogen reductase activity (Brooks *et al*., 2000; Geiger *et al*., 1999; Nakano *et al*., 1997). Also gas exchange alteration such as decreased stomatal conductance and transpiration rate can be associated with reduced leaf

nitrogen content under elevated CO_2 (Del Pozo *et al*., 2007; Taub and Wang, 2008). As a consequence of these effects, plant growth response to elevated CO_2 is often decreased under low nitrogen supply (Hocking and Meyer, 1991; Johnson *et al*., 1998). An indirect effect of nitrogen deficiency in photosynthetic acclimation can be a limitation of sink development to utilize any additional of photo assimilation arising from CO_2 fertilization (Rogers *et al*., 1998).

Nitrogen supply not only increases the amount of nitrogen in the whole canopy, but also affects the distribution of nitrogen among the different leaves within the canopy (Dreccer *et al*., 2000). Photosynthetic acclimation resulting from elevated CO_2 could be mitigate by nitrogen nutrition (Del Pozo *et al*., 2007) and the growth under sufficient nitrogen supply and predicted increase in atmospheric carbon dioxide results could optimize the light saturated assimilation rate (Geiger *et al*., 1999; Johnson *et al*., 1995; Radoglou *et al*., 1992; Rogers *et al*., 1996; Sanz-Sáez *et al*., 2010). Low nitrogen supply was not found to aggravate photosynthetic acclimation, but the growth in elevated CO_2 and either high or low nitrogen is dependent on sink***/***source (Farage *et al*., 1998; Jifon and Wolfe, 2002; Pettersson and McDonald, 1994; Stitt and Krapp, 1999). Photosynthetic acclimation may be greater in leaves of plants at higher nitrogen supply than the lower because of higher sink strength of assimilating product. Elevated CO_2 appears to increase nitrogen use efficiency (Hoking and Meyer, 1991; Leaky *et al*., 2009; Zerihun *et al*., 2000) and as a result the negative effect of nitrogen deficiency may be ameliorated by elevated CO_2 (Radoglou *et al*., 1992) and the plant growth, biomass and seed production are sometimes not significantly different between low and high nitrogen treatments at elevated CO_2 (Larigauderie *et al*., 1988).

In general, elevated CO_2 increased rate of growth will typically lead to increased demand for mineral nutrients (Reddy *et al*., 2004) and various research studies have been undertaken to study the interactive effect of elevated CO_2 and nitrogen fertilizer on different crop species, including oil crops such as sunflower (Zerihun *et al*., 2000). Arid or semiarid ecosystems account for about 1/3 of the total land area of the world and are likely to undergo a significant impact of climate change through changes in their biochemistry (Schlesinger *et al*., 1990). Many of these ecosystems are already facing a loss of nutrient due to land use (Evans and Belnap, 1999). In addition, the occasional nature of water availability in these ecosystems has a significant consequence on soil carbon and nitrogen cycling (Austin *et al*., 2004). Safflower is one of the few oil crops which is well adapted to these ecosystems and it shows a good tolerance to dry, cold winters and hot summers (Johnston *et al*., 2002; Quiroga *et al*., 2001). It is of interest to study the interactive effect of elevated CO_2 and nitrogen supply on safflower physiology, growth and seed yield.

6.2. Aim

To assess the interaction effect of elevated CO_2 and nitrogen nutrition on the physiology, growth development and seed yield.

6.3. Objectives

6.3.1. Objective 1

Examine the safflower physiology response to elevated CO_2 under a range of nitrogen supplied, and investigate whether the effect of elevated CO_2 on photosynthesis depends on nitrogen availability.

6.3.2. Objective 2

To determine whether the nitrogen status of different plant organs varies in response to elevated CO_2 under different levels of nitrogen fertilizer.

6.3.3. Objective 3

To investigate the effects of CO_2 enrichment and nitrogen nutrition on canopy development (LAI).

6.3.4. Objective 4

To investigate the interaction effect of CO_2 and nitrogen nutrition on above ground biomass accumulation and biomass partitioning.

6.4. Material and Methods

Experimental design and measurements taken

Plants were grown under ambient and elevated CO_2. The study layout as a split- plot design with CO_2 as the main plot with four chambers supplied with elevated CO_2 and four with ambient air. Nitrogen fertilizer levels were the subplots with four levels of nitrogen (equivalent to 25, 70, 125 and 175 kg N ha^{-1}) chosen because of the results obtained in the nitrogen nutrition experiment (ch 4). Two ambient and two elevated chambers were located on each side of the glass-house and the allocation of elevated or ambient CO_2 was made at random. Each chamber had 16 pots, 4 pots of each nitrogen treatment as shown in Plate (6.1). The germinated seed was sown on 20th October 2011. Pots were watered with 10 - 30 mL of a standard hydroponics growth solution A and B every 3 - 5 days for the first month. Thereafter, plants were irrigated with 50 - 200 mL complete Hoagland's solution minus nitrogen (for detail see chapter 2 and appendix A) every (5-7 days). Nitrogen was applied by supplementing the Hoagland's at watering time. Four levels of ammonium nitrogen solution were prepared by dissolving (0.7, 1.9, 3.14 and 4.22 g ammonium nitrate) (detailed in ch 4).

The weekly average CO_2 (Figure 6.1) temperature (Figure 6.2) and humidity (Figure 6.3) were calculated. An average humidity of 84% for ambient and 89 % for elevated chamber and an average temperature of 14 ^{0}C for both ambient and elevated CO_2 during the growth period were recorded.

The average CO_2 concentrations in the supplemented chambers were 1008.66, 1002.93, 1002.79 and 999.48 µmol mol^{-1} in the four chambers respectively, with

an overall average of 1000 µmol mol-^{1}. These, were higher than original planned set point but were still double compared to ambient which recorded an overall average of 400 µmol mol^{-1}.

At (50%) anthesis on 5th Jun 2012 the physiological parameters: assimilation rate, transpiration rate, stomatal conductance and sub stomatal CO_2 were measured on the youngest, three top expanded leaves of all plants in each nitrogen treatment. One day later the plants in six of the chambers (3 ambient + 3 elevated chambers)were harvested and stem height, number of leaf per plant, branch number, capitula number, leaf area and leaf area index (LAI), chlorophyll content, plant organ biomass and nitrogen content measured. As the number of plants in this experiment for each treatment was limited just 2 sub-samples of three youngest expanded leaves of 4 plants from each nitrogen treatment and each chamber were used for measuring chlorophyll and other 2 sub-samples were dried and from the dried samples 3 subsamples used in nitrogen determination The other 2 remaining chambers were harvested at maturity on 15th July 2012 and the stem height, number of leaf, branch number, capitula number, and number of seed and above ground dry weight were measured (see ch 2 for detail).

The plant nitrogen uptake was calculated the total nitrogen (nitrogen concentration in shoot + branches, total leaf and capitula) multiplied by the total above ground dry weight.

Plate 6. 1 Photograph to illustrate the enclosed chambers used and the coloured labels used to indicate different levels of nitrogen used in experiment.

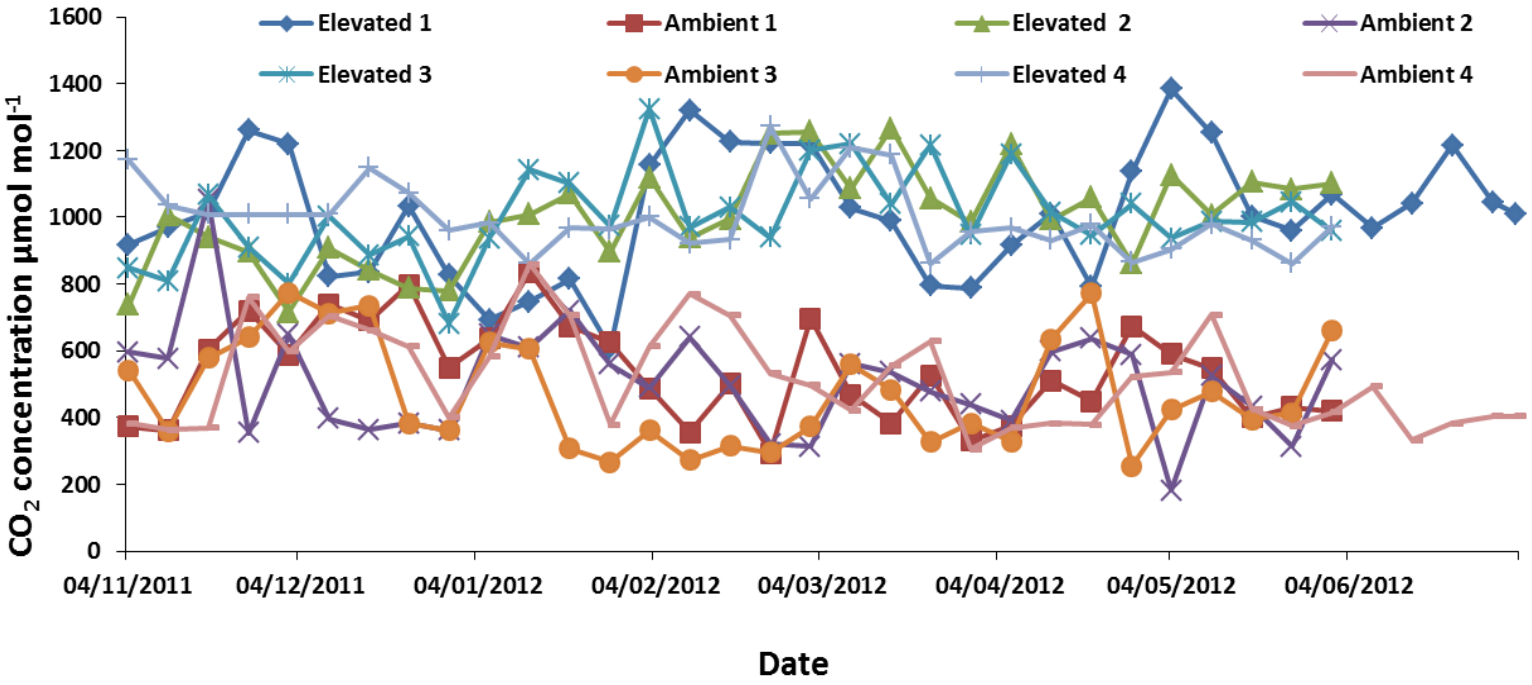

Figure 6. 1. Weekly average CO_2 concentration per chamber over growth period.

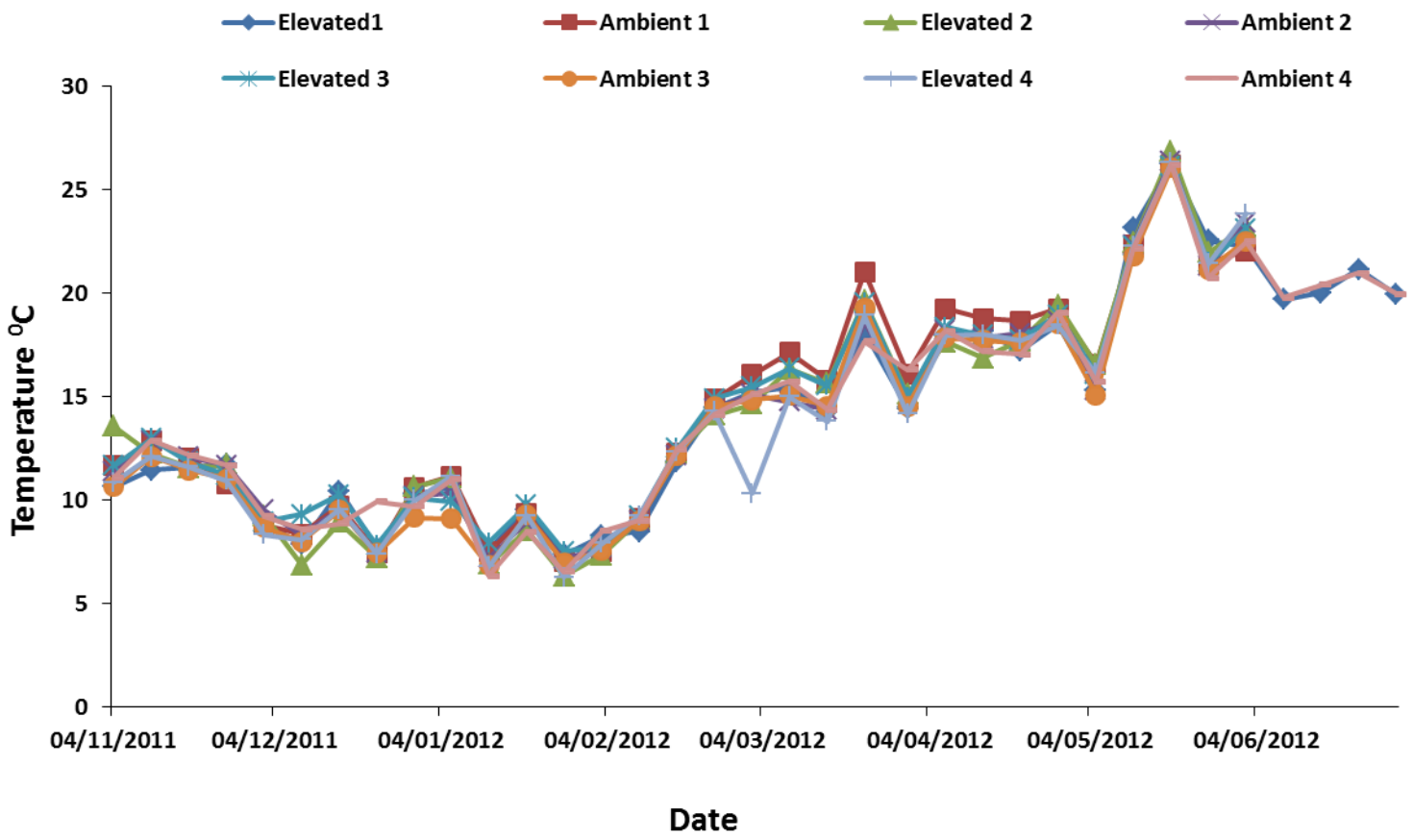

Figure 6. 2.Weekly average temperature per chamber over growth period.

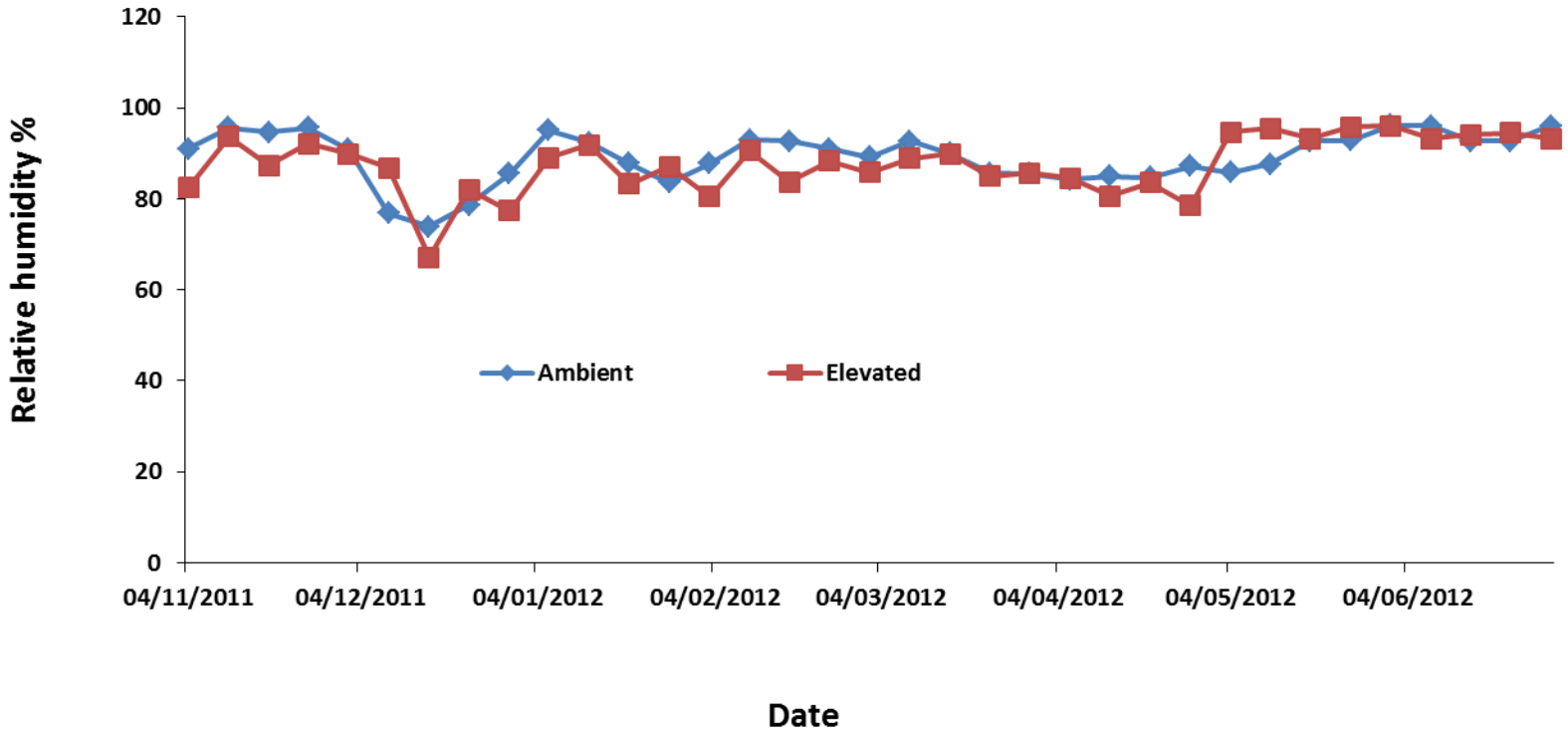

Figure 6. 3. Weekly average relative humidity per chamber over growth period.

6.5. Results

A summary of the results is presented in Table 6.1 and Table 6.2 and each of the results is discussed later in the text**.**

Table 6.1. A summary of the P value and L.S.D. (0.05 level) of main and interaction effects of elevated CO_2 and nitrogen rates on different parameters studied on safflower at 50% anthesis.

Parameters	***P value***			***L.S.D***		
	CO_2	***N***	***CO_2 X N***	***CO_2***	***N***	***CO_2 X N***
Assimilation rate (A)	0.000	0.000	0.968	0.78	1.11	n.s
Intercellular concentration of CO_2 (Ci)	0.000	0.194	0.01	9.66	n.s	n.s
Stomatal conductance (gs)	0.659	0.000	0.529	n.s	0.06	n.s
Transpiration rate (E)	1.000	0.000	0.978	n.s	0.61	n.s
Total leaf nitrogen concentration ($g100g^{-1}$)	0.000	0.000	0.000	0.024	0.034	0.048
Shoot + branch nitrogen concentration ($g100g^{-1}$)	0.000	0.000	0.176	0.018	0.024	n.s
Capitula nitrogen concentration ($g100g^{-1}$)	0.000	0.000	0.006	0.001	0.044	0.062
3 expanded leaves nitrogen concentration ($g100g^{-1}$)	0.000	0.000	0.102	0.072	0.098	n.s
3 expanded leaf chlorophyll a content (mg g fresh weight)	0.559	0.000	0.891	0.020	0.030	n.s
3 expanded leaf chlorophyll b content (mg g fresh weight)	0.280	0.000	0.846	0.026	0.037	n.s
3 expanded leaf total chlorophyll content (mg g fresh weight)	0.420	0.000	0.753	0.075	0.11	0.15
Leaf number	0.000	0.831	0.369	6.1	n.s	n.s
Leaf area index (LAI)	0.000	0.000	0.000	0.04	0.05	0.07
Stem height	0.000	0.016	0.970	2.71	3.83	n.s
Branch number	0.000	0.207	0.217	1.7	n.s	n.s
Capitula number (g pl^{-1})	0.000	0.023	0.666	1.7	n.s	n.s
Leaf dry weight (g pl^{-1})	0.000	0.000	0.048	0.48	0.67	n.s
Shoot+ branch dry weight (g pl^{-1})	0.000	0.000	0.032	0.49	0.69	0.98
Capitula dry weight (gpl^{-1})	0.000	0.000	0.004	0.68	0.96	n.s
Total above ground dry weight (g pl^{-1})	0.000	0.000	0.640	1.42	2.01	n.s

Table 6.2. A summary of the P value and L.S.D. (0.05 level) of main and interaction effects of elevated CO_2 and nitrogen rates on different parameters studied on safflower at harvest.

Parameters	***P value***			***L.S.D***		
	CO_2	***N***	***CO_2 × N***	***CO_2***	***N***	***CO_2 X N***
Stem height (cm)	0.000	0.000	0.026	0.99	1.40	n.s
Leaf number	0.002	0.000	0.327	4.3	6.0	n.s
Branch number	0.000	0.000	0.000	0.9	1.2	1.7
Capitula number	0.000	0.0000	0.011	1.1	1.5	2.1
Seed number	0.000	0.095	0.181	1.2	n.s	n.s
Shoot + branch dry weight (gpl^{-1})	0.000	0.000	0.003	0.46	0.644	0.91
Leaf dry weight (gpl^{-1})	0.000	0.000	0.012	0.35	0.50	n.s
Capitula dry weight (gpl^{-1})	0.000	0.000	0.150	0.093	0.92	n.s
Total above ground dry weight (gpl^{-1})	0.000	0.000	0.001	0.68	0.96	n.s
Seed fresh weight (gpl^{-1})	0.000	0.023	0.590	0.068	0.096	N.S

6.5.1. Physiological parameters

At anthesis, the mean assimilation rate (CO_2 assimilation) increased incrementally with each increase in nitrogen supplied at both elevated CO_2 and ambient CO_2 ($p \leq 0.05$), and was up to 18% higher at elevated CO_2 than ambient CO_2, and the highest value was obtained under the highest nitrogen input (Figure 6.4.A). Plants at the highest nitrogen level had a 37% higher rate of assimilation rate than those grown under the lowest nitrogen level. There was no significant ($p > 0.05$) interaction effect between both CO_2 levels and nitrogen treatments and this reflected in the parallel response curves in Figure (6.4.B). Elevated CO_2 significantly ($p < 0.05$) increased the CO_2 in leaf intercellular spaces and each increment in nitrogen input and was higher by 8% compared with ambient CO_2, and the highest value was elicited at 125 kg N ha. $^{-1}$ Under ambient CO_2 there was no significant effect between nitrogen levels on the intercellular CO_2 concentration (Figure 6.4.C). The plants showed no significant ($p > 0.05$) differences in stomatal conductance in response to CO_2 levels (Figure 6.4.C). Stomatal conductance was significantly ($p < 0.05$) increased with nitrogen and there was a 27% increase at 125 kg N ha^{-1} compared with the lowest level, with no significant interaction effect of CO_2 and nitrogen treatments. Transpiration rate showed a similar pattern of change to stomatal conductance and there were no significant ($p > 0.05$) differences in transpiration rate between CO_2 levels (Figure 6.4.D) but there was a significant ($p < 0.05$) response to nitrogen treatments, with an increase of 28% at 125 kg N ha^{-1} compared with the lowest nitrogen level. There was no significant CO_2 and nitrogen interaction ($p > 0.05$).

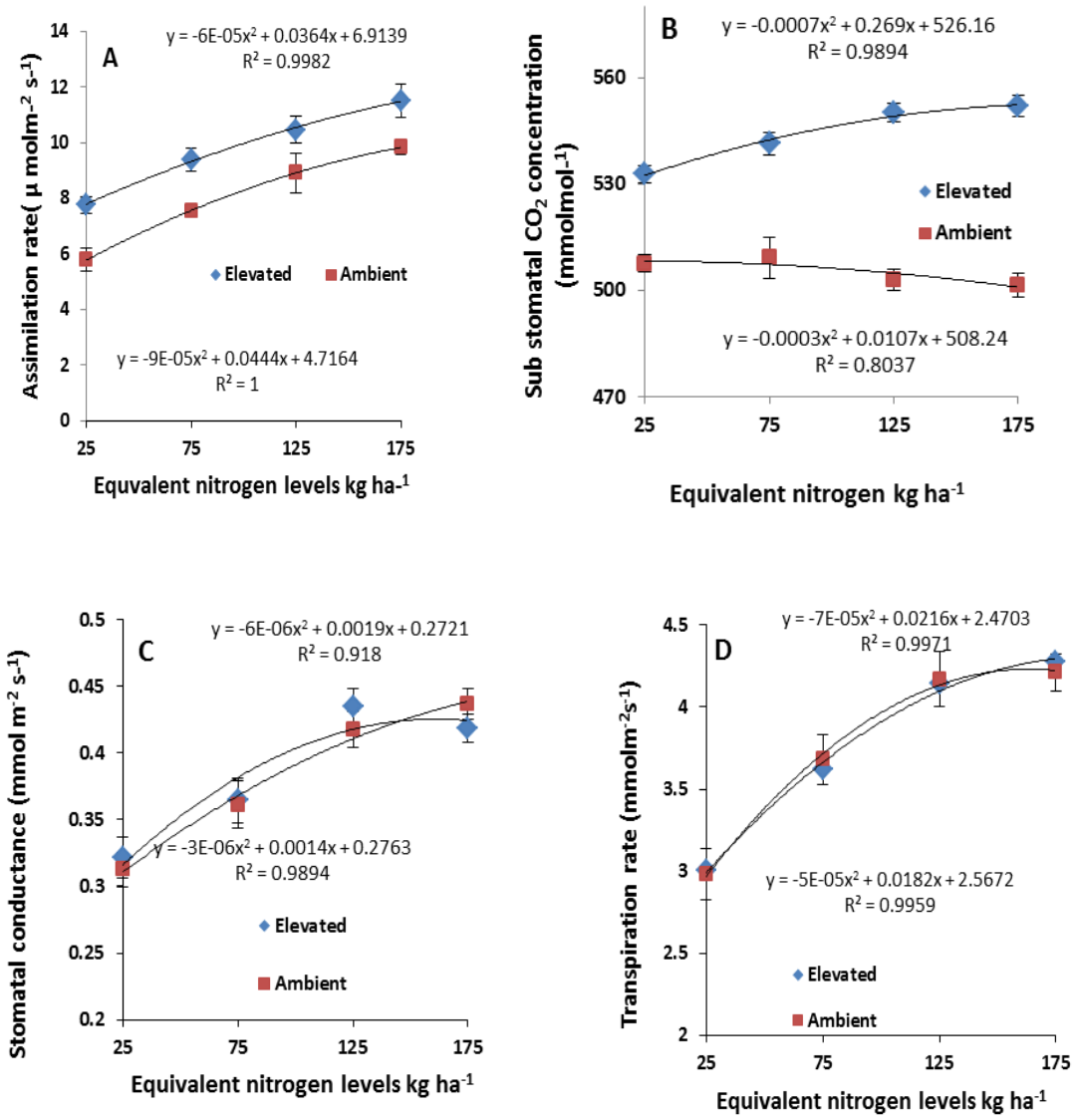

Figure 6.1. Mean of A. Assimilation rate (*A*), B. Intercellular space CO_2 concentration (Ci), C. Stomatalconductance (g_S) and D. Transpiration rate (*E*) at 50% anthesis under elevated CO_2 and different levels of nitrogen fertilizer. Vertical bars are standard errors of the mean (n = 16) at 0.0 5 levels.

6.5.2. Plant nitrogen, chlorophyll content and nitrogen uptake

At anthesis, elevated CO_2 significantly ($p < 0.05$) reduced nitrogen concentration of plant organs compared to ambient CO_2 (Figure 6.5) with the greatest reduction under the lowest nitrogen levels. Plants grown in high CO_2 had a significant reduction in their shoot and branch, leaf, capitula and an expanded leaf nitrogen concentration (7%, 24%, 4% and 8% respectively). There were also reduction in their shoot and branch, total leaf, capitula and expanded leaves nitrogen concentration (27%, 24%, 26% and 30% respectively) compared to ambient CO_2, when comparing the lowest nitrogen input to the highest. In addition, the CO_2 and nitrogen interaction showed significant ($p < 0.05$) effects on 3 expanded leaves and capitula nitrogen concentration, but showed no significant effect on total leaf and shoot and branch nitrogen concentration. Plants under elevated CO_2 and 25 kg N ha^{-1} have taken up 0.30 gpl^{-1} which was same amount taken up by plants under ambient CO_2 and 75 kg N $ha.^{-1}$ Elevated CO_2 significantly ($p < 0.05$) reduced leaf chlorophyll content and the greatest reduction was at the lowest nitrogen input (Figure 6.6), with a 4%, 5% and 8% reduction in chlorophyll a, b and total chlorophyll respectively under the elevated CO_2 compared to ambient CO_2, and a reduction of 24%, 44% and 31% in chlorophyll a, b and total chlorophyll, respectively, at the lowest nitrogen supplied under elevated CO_2 compared with the highest nitrogen supplied. The CO_2, nitrogen interaction showed only significance with total chlorophyll.

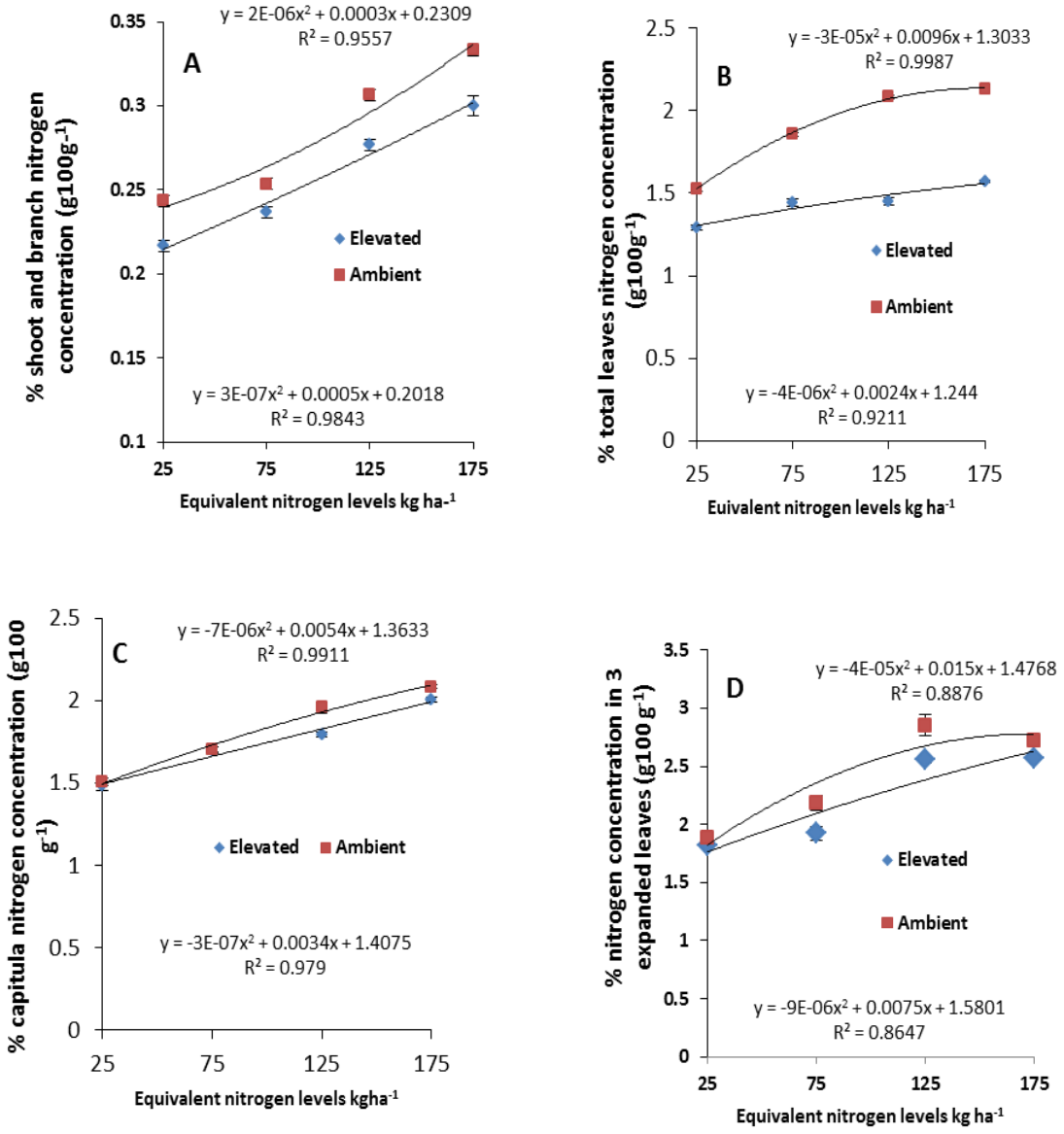

Figure 6.2. Mean of nitrogen concentration in different plant organs on dry weight basis in; A. shoost and branches, B. leaves, C. capitula and in D. three expanded leaf at 50% anthesis under the effect of elevated CO_2 and different levels of nitrogen fertilizer. Vertical bars are standard errors of the mean (n =3) at 0.05 levels.

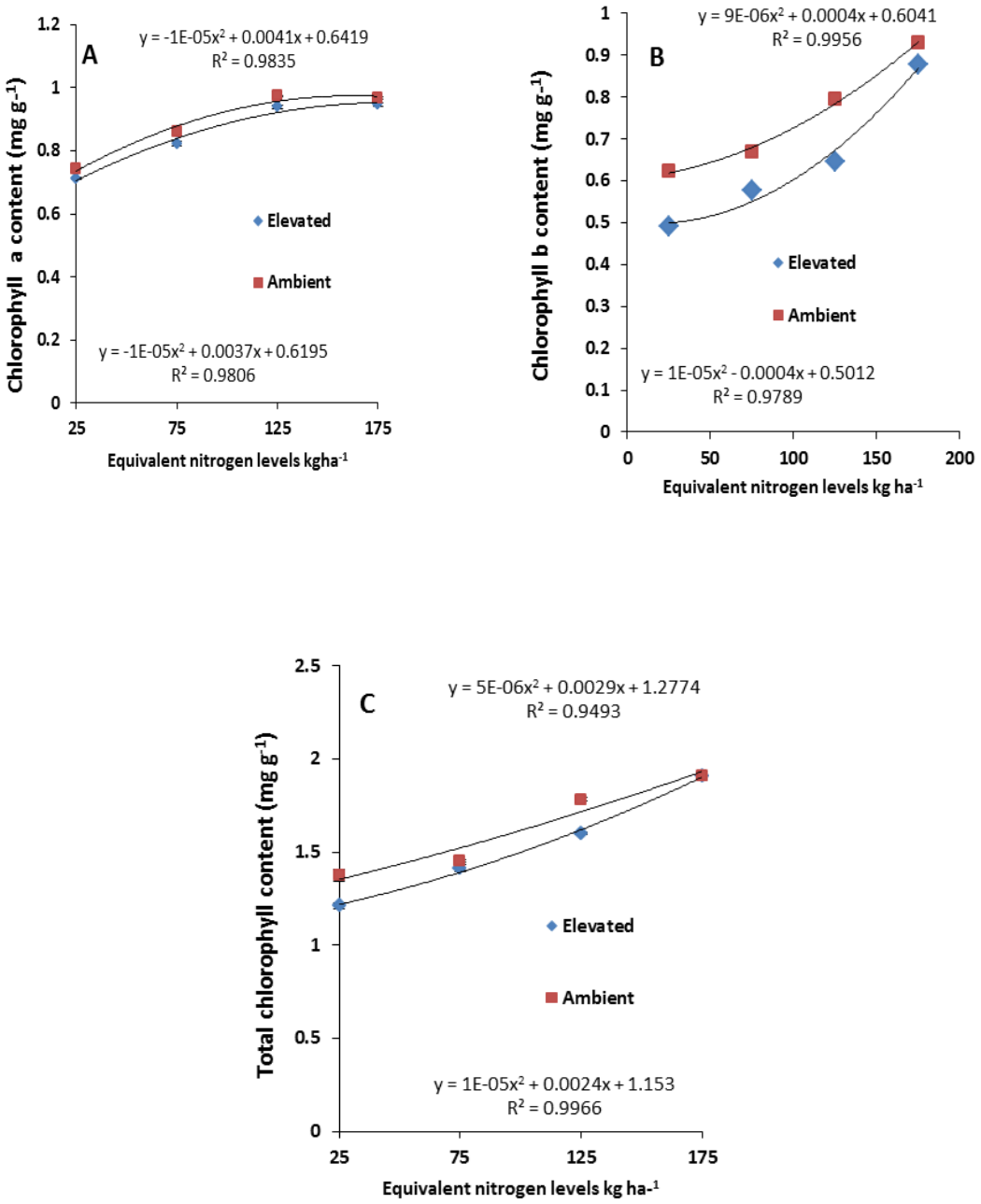

Figure 6.3. Mean of three leaf chlorophyll content on fresh weight basis at 50% anthesis under the effect of elevated CO_2 and different levels of nitrogen fertilizer; A. mean chlorophyll a content, B. mean chlorophyll b content and C. mean total chlorophyll content. Vertical bars are standard errors of the mean (n= 2) at 0.05 levels.

6.5.3. Plant Morphology, growth and seed yield

At both anthesis and harvest, safflower growth in term of stem size and the number of branches, leaves and capitula, as well the leaf area positively responded to elevated CO_2 at all nitrogen levels over the ambient CO_2 (Table 6.3,6.4 and Figure 6.7). Plants grown at elevated CO_2, significantly ($p < 0.05$) produced taller plants with, a 5% increase at anthesis and 4% at harvest over the ambient CO_2 and with an increase of 3% at anthesis and 5% at harvest at 125 kg N ha^{-1} over the 25 kg N ha. $^{-1}$. There was no CO_2 and nitrogen interaction ($p > 0.05$) with respect to plant height. Average leaf area for each plant at anthesis showed significant effects ($p < 0.05$) (Table 6.3) due to elevated CO_2 at all nitrogen treatments. Consequently, LAI significantly increased ($p < 0.05$) at higher CO_2 by 2% over the ambient CO_2 (Figure 6.5) and by 6% at the 125 kg N ha^{-1} compared to the 25 kg N ha^{-1} and LAI ranged between 4.41 to 4.96 under different treatments.

The yield components (branches and capitula number) significantly ($p > 0.05$) increased from anthesis to maturity in response to elevated CO_2 and nitrogen (Table 6.3 and 6.4). At harvest, branch number and capitula number increased by 43 and 50% in plants growth at elevated CO_2 compared to ambient CO_2 respectively, with the highest values in the higher nitrogen input, with an increase of 60% in both branch and capital under 125 kg N ha^{-1} compared to the lowest nitrogen input.

The seed yield in term of seed number obtained in this experiment was again low, but it still significantly ($p > 0.05$) responded to elevated CO_2, with an increase of 49% compared to ambient CO_2, with no significant ($p < 0.05$)

response to nitrogen and the interaction between CO_2 and nitrogen treatments.At both anthesis and harvest, whole plant biomass showed a significant ($p > 0.05$) effect of elevated CO_2 and or nitrogen fertilizer (Figure 6.9 and Figure 10), with no significant interaction of CO_2 and nitrogen ($p < 0.05$). At all nitrogen treatments, plants grown at high elevated CO_2 had up to 34% dry matter at anthesis and 36% at harvest compared to the ambient CO_2, and reached the highest value was under the 125 kg N ha^{-1} nitrogen input, with an increase of 35% at anthesis and 40% at harvest compared with the lowest nitrogen input. The whole plant biomass increment was driven by an increase in shoot and branch dry weight, total leaf dry weight and capitula dry weight, all these parameters were significantly higher ($p > 0.05$) at higher levels of CO_2 compared to ambient CO_2, and they continued accumulating dry matter with each increase in nitrogen levels. Seed yield in term of seed weight, under all nitrogen treatments, was significantly ($p > 0.05$) higher by 47% than ambient CO_2 (Figure 6.11), and the highest value was obtained under the highest level of nitrogen, with a 26% increase compared with other three nitrogen levels, with no significant interaction effect of CO_2 and nitrogen on seed weight.

Table 6.3. Means values of plant development criteria and yield component per plant at 50% anthesis under elevated CO_2 and differenent levels of nitrogen fertilizer.

	At anthesis																
Parameters	**CO_2**		**Nitrogen**				**Elevated CO_2 X Nitrogen**				**Ambient CO_2 X Nitrogen**				**L.S.D at 0.05 level**		
	Elevated CO_2	**Ambient CO_2**	**25**	**75**	**125**	**175**	**25**	**75**	**125**	**175**	**25**	**75**	**125**	**175**	**CO_2**	**N**	**CO_2 N**
Leaf number	97 [a]	90 [b]	93	92	93	95	95	97	98	99	92	88	88	90	**6.1**	**n.s**	**n.s**
Leaf area	495 [a]	483 [b]	461 [d]	486 [c]	498 [b]	510 [a]	472 [c]	489 [b]	501 [b]	517 [a]	449 [c]	482 [c]	495 [b]	503 [a]	**2.8**	**4**	**5.7**
Branch number	6 [a]	2 [b]	4	4	5	5	5	5	7	6	2	3	3	3	**1.7**	**n.s**	**n.s**
Capitula number	5 [a]	3 [b]	4	3	4	5	4	4	7	6	3	3	4	4	**1.7**	**n.s**	**n.s**

***Means following by the same letter within rows are not significantly different at 0.05 levels

Table 6. 4. Mean values of plant development criteria, yield components and seed number per plant at harvest under elevated CO_2 and different levels of nitrogen fertilizer.

	At anthesis																
Parameters	**CO_2**		**Nitrogen**				**Elevated CO_2 X Nitrogen**				**Ambient CO_2 X Nitrogen**				**L.S.D at 0.05 levels**		
	Elevated CO_2	**Ambient CO_2**	**25**	**75**	**125**	**175**	**25**	**75**	**125**	**175**	**25**	**75**	**125**	**175**	**CO_2**	**N**	**CO_2X N**
Leaf number	91 **a**	83 **b**	79 **b**	83 **b**	92 **a**	94 a	83	89	92	99	76	78	92	89	4.3	6.0	n.s
Branch number	7 **a**	3 **b**	3 **c**	4 **b**	7 **a**	7 **a**	4 **b**	5 **b**	10 **b**	10 **a**	3 **b**	4 **c**	4 **b**	5 **a**	0.9	1.2	1.7
Capitula numbr	6 **a**	3 **b**	3 b	3 **b**	6 a	7 **a**	4 **b**	4 **b**	9 **a**	10 **a**	2 **b**	3 **b**	3**b**	5 **a**	1.1	1.5	2.1
Seed number	6 **a**	3	4	5	4	6	6	6	5	8	2	4	4	4	1.2	n.s	n.s

*Means following by the same letter within rows are not significantly different at 0.05 levels.

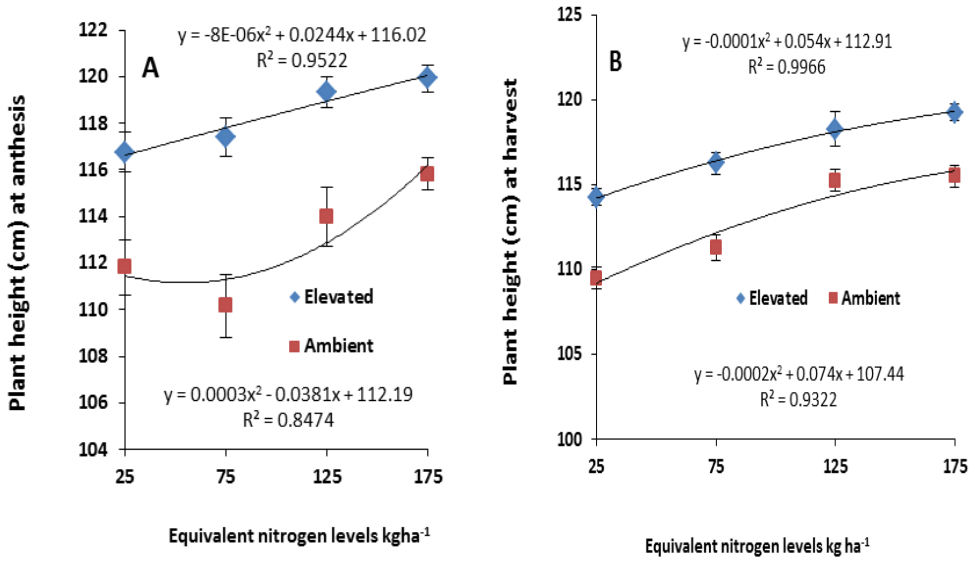

Figure 6.4. Mean of A. plant stem height at 50% anthesis and B.harvest under elevated CO_2 and different levels of nitrogen. Vertical bars are standard errors of the mean (n=12) at 0.05 levels.

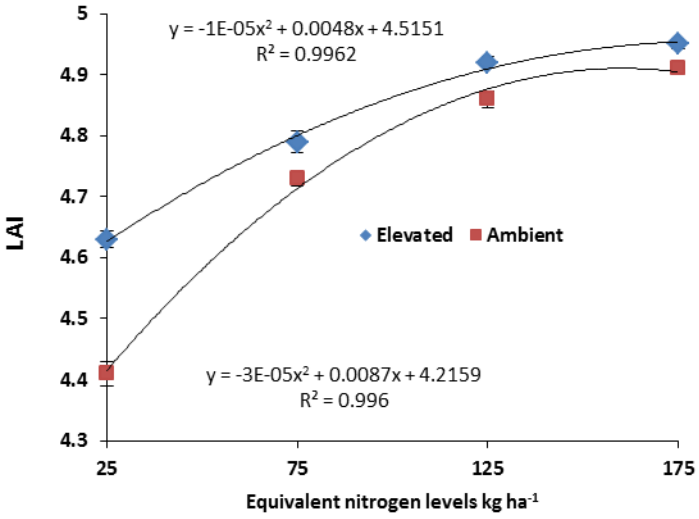

Figure 6.5.Mean of leaf area index (LAI) at elevated CO_2 and different levels ofnitrogen fertilizer at 50% anthesis. Vertical bars are standard errors of the mean (n=12) at 0.05 levels.

Plate 6. 2.Photograph to illustrate the comparison between plant growth (plant height and branch number) in ambient and elevated chambers.

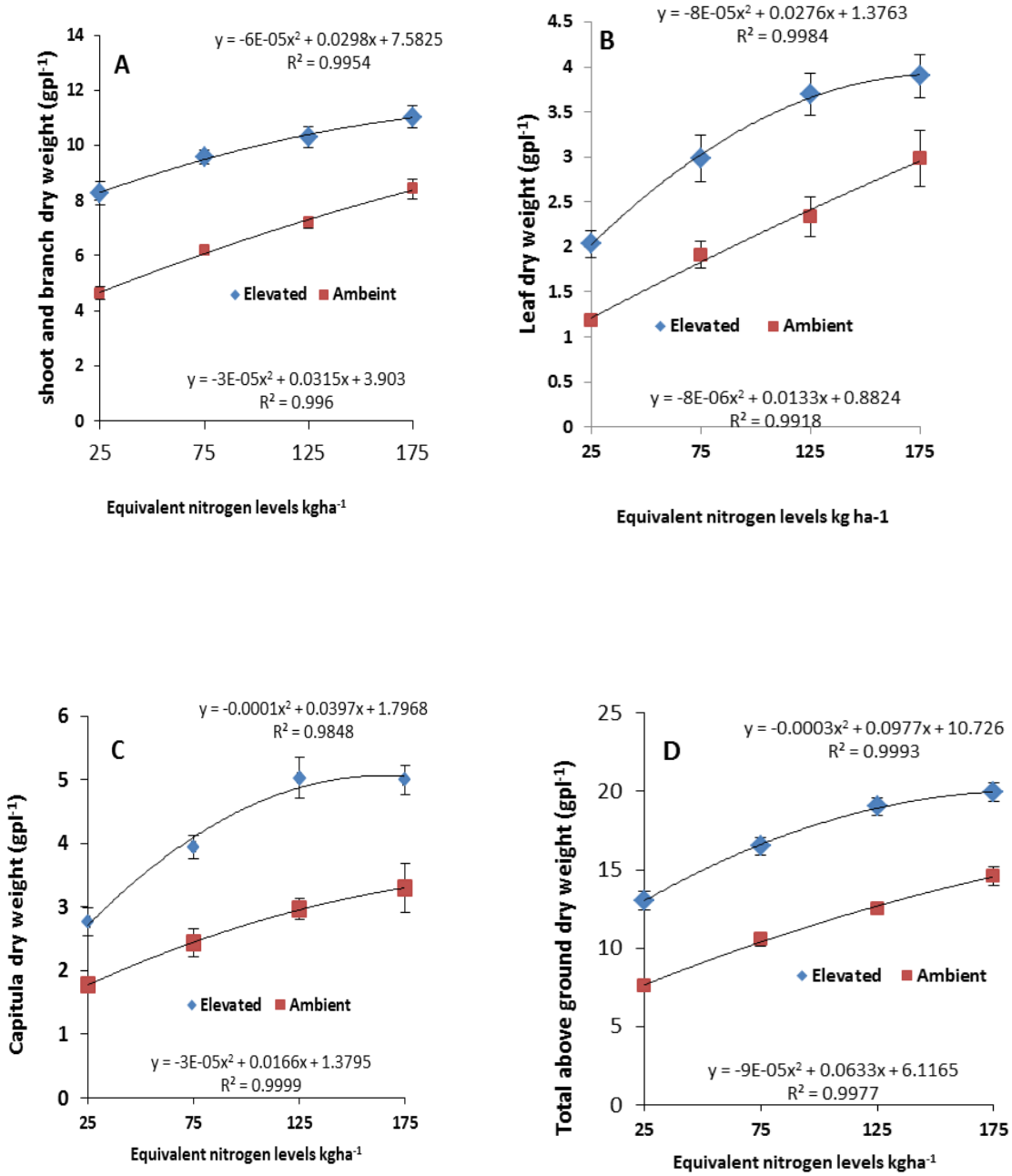

Figure 6.6. Mean of A. shoot and branches,B. leaves,C. capitula and D. total aboveground dry weight at 50% anthesis under elevated CO_2 and different levels of nitrogenfertilizer. Vertical bars are standard errors of the mean (n =12) at 0.0 5 levels.

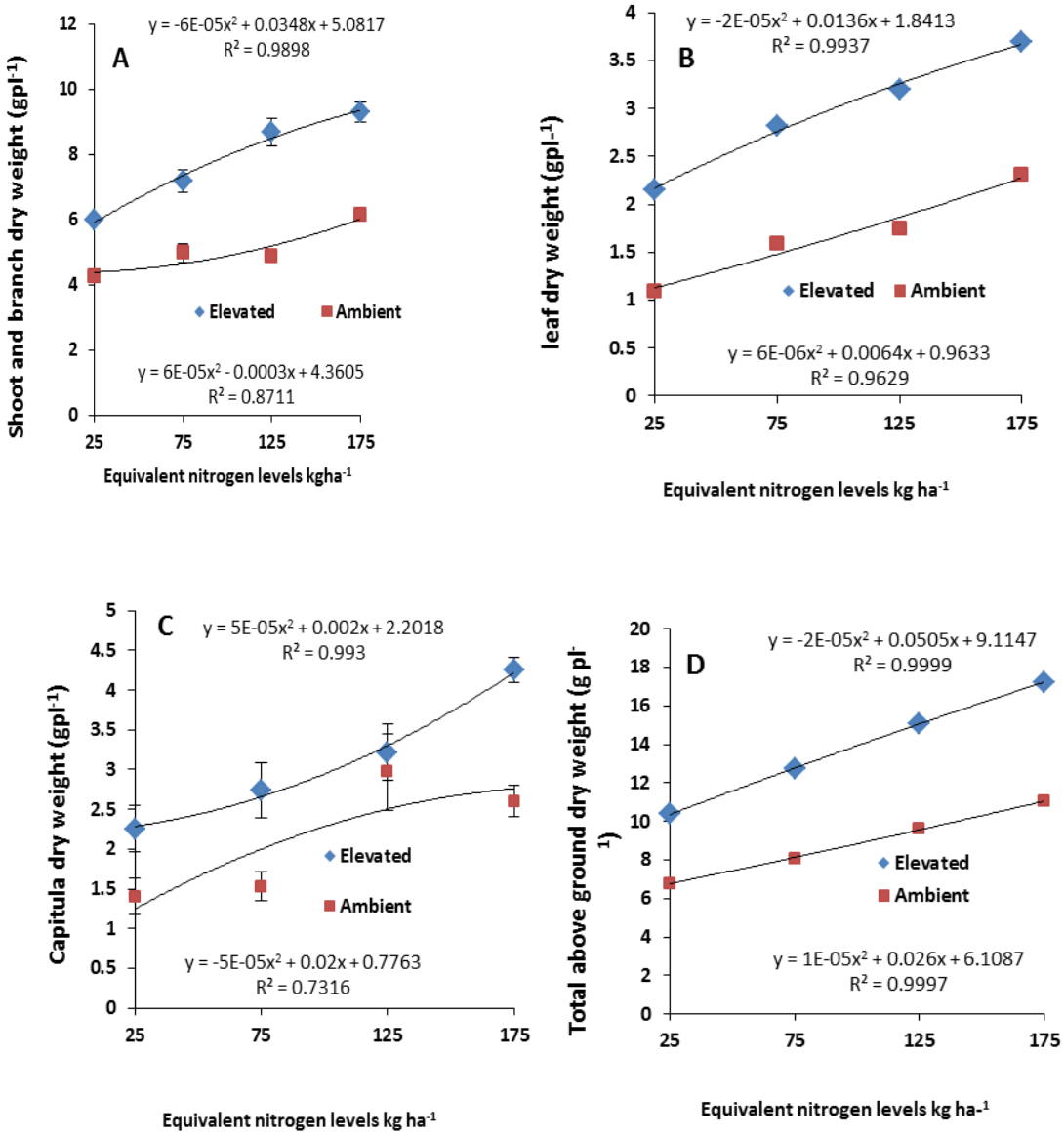

Figure 6.7. Mean of A. shoot and branches, B. leaves, C. capitula and D. above ground dry weight at harvest under elevated CO_2 and different levels of nitrogen fertilizer. Vertical bars are standard errors of the mean (n =4) at 0.0 5 levels.

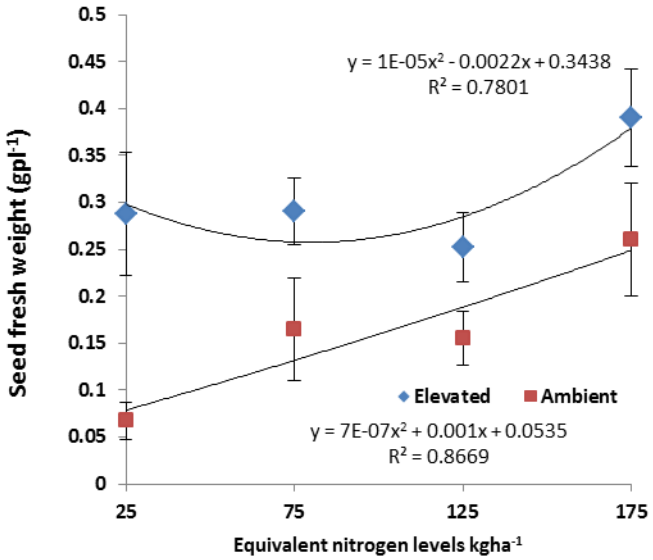

Figure 6.8. Mean of fresh seed weight under elevated CO_2 and different levels of nitrogen. Vertical bars are standard errors of the mean (n=4) at 0.05 levels.

6.6. Discussion

It has been frequently reported that increases in both atmospheric CO_2 concentration and nitrogen supply result in large and sustained increases in light saturated assimilation rate (Geiger *et al*., 1999; Johnson *et al*., 1995; Radoglou *et al*., 1992; Rogers *et al*., 1996b; Sanz-Sáez *et al*., 2010). For example, elevated CO_2 (700 µmol Mol^{-1}) resulted in an increase of 30% and 40% increase in an assimilation rate under high nitrogen and low nitrogen (without nitrogen supplied respectively as compared to ambient CO_2 (350 µmol mol^{-1}) in fully expanded primarily the leaves of *Phaseolus vulgaris* (Radoglou *et al*., 1992). The results obtained at antithesis in this study corroborate these findings at all levels of nitrogen supplied, elevated CO_2 to (1000 µmol mol^{-1}) increased assimilation rate by 18% as compared to ambient CO (400 µmol mol^{-1}) and elevated CO_2 resulted in an increase of 15% in assimilation rate for plants grown with highest nitrogen supply and 26% for plants grown under the lowest nitrogen supply (Figure 6.4.A). This increment was a result of increased intercellular CO_2 concentration at elevated CO_2, and compensated for limited nitrogen in plants grown under elevated CO_2. As a result the stomatal conductance and corresponding transpiration rate did not significantly reduce under the effect of elevated CO_2. At both ambient and elevated CO_2 stomatal conductance and transpiration rate significantly ($p > 0.05$) increased with nitrogen input, with a 26% and 24% at 125 kg N ha^{-1} respectively, compared to the lowest nitrogen levels (Figure 6.4 C and D). These results are consistent with the finding that stomata have been shown to be sensitive to the intercellular CO_2 concentration (Mott, 1988). This can be interpreted as the increases of the carboxylation capacity of Rubisco under elevated CO_2 at high nitrogen supplied

(Ainsworth and Long, 2005; Ainsworth and Rogers, 2007; Jifon and Wolfe, 2002; Long *et al*., 2004). The results presented here coincide with those previously reported by Radoglou *et al*. (1992). So far, the results in this study suggest that the effect of CO_2 and nitrogen on assimilation rate is independent of each other. However, Anten *et al*., (2004) found an interactive effect of nitrogen and CO_2 on canopy Found in gain indicating that photosynthetic rate is the canopy carbon gain, and showed that this interaction resulted from the increase in LAI with increasing nitrogen input, resulting in the interception a higher total quantum yield of light at elevated CO_2.

In fact, the interactive effect between CO_2 and nitrogen on photosynthetic rate and growth could operate through two mechanisms; the nitrogen availability decreases photosynthetic acclimation to elevated CO_2 and this has previously been shown to be more obvious when nitrogen is limited (Stitt and Krapp, 1999), also, long exposure to elevated CO_2 leads to photosynthesis acclimation caused by carbohydrate accumulation, which tends to be more pronounced under lower than higher nitrogen availability (Ainsworth and Rogers, 2007).

In contrast to these studies, the photosynthetic acclimation to elevated CO_2 was more pronounced in high compared to low nitrogen supply because under nitrogen deficiency leaf area, assimilation and sugar levels decreased and allocation of biomass to non-photosynthetic tissue increased (Jifon and Wolfe, 2002). Moreover, Rubisco has not been shown to decrease with nitrogen deficiency under elevated CO_2 (Farage *et al*., 1998; Rogers *et al*., 1998).

In the current study, elevated CO_2, significantly ($p < 0.05$) reduced nitrogen concentration in different plant parts compared to ambient CO_2, and the lowest

concentration was at the lowest level of nitrogen (Figure 6.5). It can be assumed that the reduction in plant tissue nitrogen concentration under elevated CO_2 and at all nitrogen treatments did not lead to photosynthetic acclimation as photosynthetic acclimation is strongly related to a decrease in leaf nitrogen concentration under low nitrogen supplied (Ainsworth and Long, 2005; Sanz-Sáez *et al*., 2010). This can be explained that the photosynthetic rate under elevated CO_2 and 25 kg N ha^{-1} was equivalent to the photosynthetic rate at 125 kg N ha^{-1} and ambient CO_2 and this suggested that the negative effects of low nitrogen can be ameliorated by elevated CO_2 as the amount of nitrogen taken up by the plants under the interaction of elevated CO_2 and 25 kg N ha^{-1} same as the amount taken up by the plants under ambient CO_2 and higher nitrogen rates (0.30 g $Paul^{-1}$). As mentioned earlier, the mean photosynthetic rate (A) for plants grown under the lowest nitrogen level was significantly ($p > 0.05$) higher than for the plants grown under the lowest nitrogen level at ambient CO_2. Therefore, this study lends further support to the hypothesis that elevated CO_2 improves the photosynthetic nitrogen use efficiency (PNUE).

In agreement with this study the PNUE increased by an average of 50% in sunflower under CO_2 enrichment (Zerihun *et al*., 2000). Similar responses were reported here for the leaf chlorophyll content, thus when the nitrogen concentration was reduced in these leaves chlorophyll a, b and total chlorophyll was significantly ($p < 0.05$) reduced at elevated CO_2 with the most reduction under the lowest nitrogen input (Figure 6.6). Reasons for chlorophyll reduction in elevated CO_2 may be related to the leaf nitrogen reduction (Nakano *et al*., 1997), and the decrease in leaf chlorophyll content results in chlorosis as

previously reported (Radoglou and Jarvis, 1992) but no chlorosis was observed here. In a two year study on spring wheat, in one of the years leaf chlorophyll content was not significantly ($p > 0.05$) changed by CO_2, but it was significantly reduced under nitrogen deficiency. While, in the other year, the acclimation of leaf photosynthesis rate and stomatal conductance in elevated CO_2 over the ambient CO_2, was associated with lower total leaf chlorophyll content (Del Pozo *et al*., 2005). There was no significant ($p > 0.05$) interaction effect of elevated CO_2 and nitrogen reported here for leaf nitrogen concentration and all types of chlorophyll content as all increased with each increase in nitrogen levels at both ambient and elevated CO_2. In contrast, Jifon and Wolfe (2002) found that leaf nitrogen and chlorophyll concentration were not significantly affected by elevated CO_2, and the leaf nitrogen concentration was higher under lower nitrogen than high nitrogen, while the chlorophyll content was significantly higher in high nitrogen than low nitrogen plants in all CO_2 levels.

Shoot growth is often used to estimate capacity for the utilization of the photo assimilates and to maintain the photosynthetic capacity under limited nitrogen at CO_2 enrichment (Drake *et al*., 1997). In this experiment, at all nitrogen treatments, shoot growth significantly ($p < 0.05$) responded to elevated CO_2 compared to ambient CO_2 and the plants grown under elevated CO_2 were taller by about 5% than those grown under ambient CO_2 (Figure 6.4.A and B). Similarly, at all nitrogen treatments, LAI positively responded to elevated CO_2 with a 2% increase over the ambient CO_2, The greatest LAI was increased by 6% at 125 kg N ha^{-1} compared to 25 kg N ha^{-1} (Figure 6.8), and these parameters peaked at 125 kg N ha^{-1} at both CO_2 levels. LAI ranged between 4.41 to 4.96.

A proportional increase in leaf area and LAI with nitrogen input is commonly reported (Li *et al*., 2004). Others have also reported that leaf area increased with nitrogen input but at different CO_2 levels were similar indicating that optimum LAI does not increase in elevated CO_2 when nitrogen is low. Two factors may be responsible for this, firstly is that plants grown in elevated CO_2 have higher quantum yield which make its leaves in the canopy more nitrogen limited. The second is a higher dark respiration rate under elevated CO_2 which leads to a higher light compensation point and lowers LAI (Hirose *et al*., 1997). Others, state that LAI increases at elevated CO_2 only when nitrogen take up is high, but not when nitrogen take up is low (kim *et al*., 2001).

In this study increased assimilation rate and leaf area contributed to the increase in above ground biomass (Figure 6.6 and 6.7). The above ground dry weight was increased by 35% and 36% at anthesis and harvest, respectively, under elevated CO_2 and the higher value under the higher nitrogen input, were 35% and 40% at anthesis and harvest, at 125 kg N ha^{-1} as compared with the lowest nitrogen level. The above ground dry weight of plants under elevated CO_2 with the lowest nitrogen rate was 42% higher than the ambient CO_2 and lowest nitrogen rate grown plants. Above ground biomass of all morphological components; shoot, branch, leaf and capitula were significantly ($p < 0.05$) higher at elevated CO_2, and under higher nitrogen levels. This suggests that elevated CO_2 compensates for the negative effect of nitrogen stress. At the same time, nitrogen supply is preventing photosynthesis from CO_2 acclimation as reported by (Kim *et al*., 2003). The short term exposure to elevated (700 µmol mol^{-1}) and ambient CO_2 with alfalfa and three levels of nitrogen fertilizer concluded that plants grown at elevated CO_2 and zero nitrogen significantly

increased photosynthetic activity due to increased nitrogen use efficiency, but there were no significant differences across all CO_2 and nitrogen levels and photosynthetic down regulation had occurred. While, the long term exposure to elevated (700 µmol mol^{-1}) CO_2 markedly enhanced leaf area and plant biomass over the ambient CO_2 when plants were irrigated with nitrogen, photosynthetic activity was maintained (Sanz-Sáez *et al*., 2010). This finding clearly supports the hypothesis that growth in limited nitrogen restricts the development of sink capacity to utilize the photo-assimilate and leads to an accumulation of non-structural carbohydrate which increases the C**:** N ratio, and exacerbates the acclimation of photosynthesis (Ainsworth et al., 2003; Ainsworth and Rogers, 2007). The same was reported for open top grown rice at elevated (700 µmol mol^{-1} CO_2) and three levels of nitrogen (0, 90 and 200 kg N ha^{-1}) (Ziska *et al*., 1996).

In all nitrogen treatments, elevated CO_2 significantly ($p < 0.05$) increased the branch number and corresponding capital number, with the highest values under 125 kg N ha^{-1} compared to the lowest nitrogen input.

In the present study, there was a significant ($p < 0.05$) interaction effect of CO_2 and nitrogen on LAI at anthesis on branch and capitula number when counted at harvest. It can therefore be predicted that the response of LAI, the branch and the capital number of safflower here is dependent on CO_2 concentration, and suggests that 125 kg N ha^{-1} is sufficient for safflower to produce greater LAI, above ground biomass and branch and capital number and depending on LAI leaf and shoot dry matter accumulation. Similarly as reported here for safflower for cotton, significant interaction effects of CO_2 and nitrogen was observed on a branch and boll number, with no significant interaction effect on plant height and

total above biomass (Reddy *et al*., 2004). The explanation for this interactive effect is the increased assimilation rate and growth under elevated CO_2, and the demand for nitrogen input increases and a greater sink capacity can be provided and the photosynthesis rate sustained.

Unfortunately, despite the increased sink capacity in determining yield, and the yield components (branch number, capitula number) in response to elevated CO_2, the seed number obtained was low and it was therefore difficult to indicate which of these factors control the seed set. However seed yield still responded significantly ($p > 0.05$) to elevate CO_2, with an increase of 49% compared to ambient CO_2, with no significant ($p < 0.05$) response to nitrogen and the interaction between CO_2 and nitrogen treatments. As discussed in former chapters, other environmental factors could have an important role in controlling seed set in safflower but they are clearly acting in an even manner independent of the treatments applied in these experiments.

Finally, seed biomass (fresh weight) was a much smaller component of overall biomass than stem, leaves and capitula, so harvest index is rather meaningless and was not calculated. At all nitrogen treatments, seed weight positively ($p < 0.05$) responded to elevated CO_2, with a 47% increase as compared to ambient CO_2, and the heavier seeds obtained under the highest nitrogen rate with a 26% as compared with the three other nitrogen rates, with no significant of CO_2 and nitrogen interaction effect on seed weight (Figure 6.8). At both elevated and ambient CO_2, seed weight increased in a similar pattern. A positive interaction effect of CO_2 and nitrogen supply was shown for rice grain yield, and the increase of grain yield as compared with the final total biomass was greater

at elevated $CO_{2,}$ under the medium and high nitrogen rate, while it was smaller than final total biomass under low nitrogen rate (Kim et al., 2003).

6.7. Conclusion

The results presented here, show that safflower positively responded to elevated CO_2 (from 400 µmol mol^{-1} to 1000 µmol mol^{-1}) and the response was maintained at each increase in nitrogen nutrition input. By and large there were few interactions between CO_2 and nitrogen indicating that they are "simple" limitations to assimilation and production. Since the photosynthetic rate still significantly increased under the lowest nitrogen in response to elevated CO_2 compared to ambient CO_2 it can also be concluded that the negative impact of nitrogen deficiency on photosynthesis can be ameliorated by elevated CO_2. The nitrogen level of 125 kg N ha^{-1} was sufficient for optimum growth and biomass production of safflower in response to elevated CO_2 and this agrees with the nitrogen experiment carried out earlier (ch 4). It can also be concluded that the growth in increased CO_2, need 125 kg N ha^{-1} to avoid photosynthetic down-regulation. It can be concluded that seed yield positively responded to elevated CO_2 and nitrogen fertilizer. For safflower therefore, CO_2 and nitrogen fertilizer can both be regarded as fertilizers with a doubling of CO_2 being equivalent to an increase in 100 kg ha^{-1} of nitrogen at ambient CO_2. This means that the optimum nitrogen level for maximum production is going to be higher as atmospheric CO_2 levels rise.

Chapter 7

General Discussion

Environmental factors, defined as the sum of all external factors and substances affecting the growth, structure, and reproductive capacity of a crop are known as limiting factors (Fageria *et al*., 2010). These limiting factors are water, energy (light), carbon, temperature and nutrients (Chapin *et al*., 1987) and an imbalance of any of these factors will result in stress and ultimately to a restriction of crop growth and yield. The stresses imposed on crops by pollution, drought, salinity, high/low temperature, high/low pH, nutrient deficiency, climatic change or any other stresses affect plant metabolism and modify physiological processes leading to a reduction in plant growth and productivity (Fageria *et al*., 2010). Multiple stresses may be operating at any one time for the crop in the field (Chapin *et al*., 1987). Good farming practice such as fertilizing and irrigation aim to reduce or remove limiting factors and reduce stress to crops. The most certain change in predicted climate change scenarios is in atmospheric carbon dioxide concentrations which are projected to reach 850 µmol mol^{-1} by the year 2090 (IPCC., 2007). This rise is likely to contribute to a rise in the mean global temperature which will in turn alter climatic patterns and rainfall events (Shaw *et al*., 2005). In good growing environments soil nutrient deficits may appear as plant root systems proliferate in response to photosynthetic rate increases (Rogers *et al*., 1999). However, compensation between limiting resources is possible (Fredeen *et al*., 1991). The main target of this study was to investigate whether these some of these stresses (drought, nitrogen deficiency and elevated CO_2) have an impact on safflower growth or whether there can be compensationbetween these stresses.

7.1. Comparison of the results between experiments

Results from this study clearly showed that the physiology of safflower responded positively to the removal of the limiting factors of water, nitrogen and CO_2. As a result LAI and assimilation increased and as a consequence the above ground biomass and seed yield increased. Slight differences between experiments were related to microclimatic conditions for each experiment related predominantly to the glass-house and chambers and the time of year at each experiment was conducted. Plant growth in the chamber (in CO_2 experiments) was in general smaller than in the open glass-house (drought and nitrogen experiments). However, within a small acceptable margin of difference the experiments showed good correlation one with another in terms of productivity and the physiological characters measured. In general, seed set was the main problem with these studies. Plants did not produce as much seed number per head nor per plant as was expected. This could be explained by a number of possibilities including the pot volume in which plants were grown which could have restricted the root growth and lowered the capacity of the roots to absorb the nutrients and water on one hand (in the field the roots can grow to 3 m depth) (Dajue and Mundle, 1996) and create nutrient deficiency. Some trace elements such as boron affect seed set and boron deficiency has been shown to affect seed set in wheat (Rerkasem *et al*., 1993) and it is possible that the Boron levels supplied in the Hoagland's solution were insufficient. The cloudy days and short photoperiods during bud development and pollination, despite best efforts to counteract these with supplementary lighting, in combination with high humidity and temperature in enclosed chambers could also have affected the head fertility. Safflower is typically grown

in arid or semi-arid regions of the world (Johnston *et al*., 2002) with hot, dry and long day climate. (Dajue and Mundle, 1996). This mean that the rosette stage and elongation phase will lengthen under less inductive photoperiods, and exposure to long photoperiod during flowering stage and anthesis will lead to ahigher number of fertile florets, leading to an increased grain number and thereby to a higher yield like other long day crops such as wheat (Gonzalez *et al*., 2003) and it appears that the climate provision in the UK is not conducive to seed set in safflower grown out of season.

A stronger whole plant response was reported from the interaction effect of CO_2 and nitrogen compared to the response from the elevated CO_2 effect alone experiments, where the plants were taller even under ambient CO_2. Number of branches and capitula were greater and plants produced more above ground biomass under the interaction effect of CO_2 and nitrogen rather than CO_2 or nitrogen, or water alone. This is possible because the longest growth period through the winter period of this experiment led to more vegetative growth than plants sown in spring (the elevated CO_2 experiment). A study on two species of safflower, *Carthamus tinctorius* L. and *Carthamus flavescens,* were grown in a greenhouse under high temperature with long photoperiod (20 ^{0}C and 14 h light), high temperature with short photoperiod (20 ^{0}C with 10 h light) and low temperature (14 ^{0}C) regardless of photoperiod and the rosette stage of both species persisted longer under short photoperiod (10 h) than long (14 h) with the greater response for *Carthamus tinctorius* L. and whilst the duration of the rosette was longer under lower temperature with short photoperiod (Zimmerman,1973) and these results support the results reported here.

This in turn led to higher seed yield for each nitrogen treatment under both elevated and ambient CO_2. It can therefore conclude that the highest seed yields were obtained from the interaction of CO_2 and nitrogen compared to the earlier experiments but these were still lower than produced by field grown safflower (Dajue and Mundle, 1996; Oelke *et al*., 1992).

7.2. Comparing results with the literature

Physiologically, safflower showed similar patterns in its physiology as has been reported for several other C_3 plant species (Medlyn and McMurtrie (2005). It showed typical figures that for the increased intercellular CO_2 concentration (400 - 600 µ mol mol $^{-1}$) the transpiration rate was reduced (0.12 – 0.1 mmol mol^{-1}) in the same range of intercellular CO_2 and transpiration rate, photosynthetic rate increased and the recorded value ranged between (10 -12 µmol m^{-2} s^{-1}). Elevated CO_2 to levels of about 1000 µmol mol^{-1} significantly increased assimilation rate by 27% at anthesis compared to ambient CO_2 (400 µmol mol^{-1}) due to increased intercellular CO_2 concentration. This is assumed to lead to an increase in CO_2 at the carboxylation binding site of Rubisco and hence reduced photorespiration. In addition, stomatal conductance reduced by an average of 29% and a corresponding decrease in transpiration rate occurred by an average rate of 18% attributed to the increased photosynthetic rate. Such a result has also been reported for other species (Ainsworth and Long, 2005; Ainsworth and Rogers, 2007; Bowes, 2004). However the magnitude of enhancement varies among different species and environments (Ainsworth and Rogers, 2007) especially among the oil crops that have been previously studied such as sunflower (Cheng *et al*., 2000) and peanut at optimum temperatures (Vu, 2005) showed a similar pattern of change and same pattern showed by safflower in this experiment. Several studies on different crops showed that assimilation rate significantly increased with increasing CO_2 to some extent but with time this was down regulated, a phenomenon referred as photosynthetic acclimation (Ainsworth *et al*., 2002). The most common explanation for this phenomenon under elevated CO_2 was the concomitant accumulation of

nonstructural carbohydrate in the leaf due to a small sink size that did not have the strength to take up all the carbohydrate produced resulting in a feedback down regulation. This has often been attributed to the size of pots in which plants were grown and the volume of pots restricting root growth and decreasing the nutrient availability (Arp, 1991; Sims *et al*., 1999a). Such down-regulation however was not apparent in the experiments on safflower reported here.

Safflower growth in the terms of leaf area and LAI positively responded to CO_2 enrichment. At anthesis LAI showed an increase by an average of 28% of elevated CO_2 compared to ambient as a direct consequence of increased assimilation rate and growth. Thus LAI attributed to an increase in net crop assimilation rate in this study. This extent of increase is often reported and it is concluded that the greatest crop photosynthesis occurred when the canopy leaf area reached its greatest (Manderscheid *et al*., 2003). Recently, the LAI of peanut (Bannayan *et al*., 2009) and soybean (Heinemann *et al*., 2006; Yuelin *et al*., 2005) were also reported to have shown increased LAI at elevated CO_2 and as a result the plant photosynthetic rate and above ground biomass increased. In contrast with some other studies there was no significant response of LAI to elevated CO_2 (Sims *et al*., 1999a; Yuan *et al*., 2009) and elevated CO_2 actually decreased the specific leaf area (Ainsworth and Long, 2005). Furthermore some authors have concluded that increased LAI decreased the crop photosynthesis rate because they have predicted that elevated CO_2 reduced the light compensation point for photosynthesis and thus stimulated leaf production which put a greater respiratory load on the crop (Campbell *et al*., 2001; Rodriguez *et al*., 2001).

In the current study, elevated CO_2 increased LAI and at the same time reduced transpiration rate and the water use efficiency (WUE) was improved and such results have also been reported elsewhere (Ainsworth and Long, 2005; Bowes, 2004). However, others have also reported that leaf area increases in response to elevated CO_2 increase the surface area for transpiration with the other words the increased leaf area led to increase the photosynthesis rate but at the expense of water used (Allen, 1999). Since WUE is a product of the use of water per unit of dry matter produced, efficiencies can be lower in crops with bigger biomass, as was the case here, but still use more water than a smaller biomass crop. Stimulation of a crop canopy can therefore have a water use cost despite being more efficient.

The assimilation rate factors mentioned above and increased LAI normally contribute to higher dry matter accumulated and as a consequence seed yield is usually increased and this response is widely documented (Lawlor, 1995; Long et al., 2006; Manderscheid *et al*., 2003; Yang *et al*., 2006a; Yuelin *et al*., 2005). However, contrasting results have also been reported when other limiting factors were not optimized e.g. high temperature (Cheng *et al*., 2009; Heinemann *et al*., 2006) or because of photosynthetic acclimation (Ainsworth *et al*., 2002; Arp, 1991).

Safflower yield components (number of branches per plant and number of capitula per plant) significantly increased at elevated CO_2 lying the foundation for higher seed yield but in these experiments the seed set was poor because the flowering and pollination of flower discussed above. It is concluded that the fertilization effect caused by elevated CO_2 did not have any effect on the pollination problem. In a similar manner low temperature was found to modulate

the positive effect of elevated CO_2 on soybean during seed set (SionitI *et al*., 1987) so this is not a unique observation. Seed number increases in response to elevated CO_2 is commonly found (Jablonski *et al*., 2002; Kimball *et al*., 2002; Wu *et al*., 2004) and whilst seed set was low in the safflower experiments the relative differences between treatments were as predicted and proportional to the treatments applied.

Elevated CO_2 slightly reduced seed oil content in safflower, and slightly changed fatty acid composition compared to ambient. However it is not possible to place much confidence in these observations because the full analysis was based on a pooled sample due to seed sample size. A reduction in seed nitrogen concentration And protein content could be possible at elevated CO_2 due to nitrogen concentration reduction in plant organs at anthesis result of changes in nitrogen use efficiency. It is usually reported that the rate of growth per unit of nitrogen in the plant increases in response to elevated CO_2 (McKee and Woodward, 1994; Rogers and Dahlman, 1993). In fact, some recent studies have indicated that there are no significant effects of elevated CO_2 on seed oil content and only slight changes in fatty acid profiles (Franzaring *et al*., 2008; Högy *et al*., 2010) and a considerable number of studies suggest that the grain quality in cereal altered with a rise in CO_2 due to a drop in the seed nitrogen concentration (Högy *et al*., 2009; Lieffering *et al*., 2004; Yang *et al*., 2006b). Other workers have reported an increase in grain lipid as an indicator of altering quality in response to elevated CO_2 (Sator, 1999; Williams *et al*., 1995). Thus, whilst the safflower seed quality data are on a pooled sample and not subject to replication, the profiles observed are in line with the, rather limited, published literature and this gives credence to the results.

It is well established that nitrogen is one of the most important mineral nutrients for crop growth and yield and crops need nitrogen in larger quantities compared with other mineral nutrients (Forde *et al*., 1999). There is an interdependent relationship between carbon and nitrogen assimilation. The nitrogen budget of the plant is spent in the maintenance of the photosynthetic protein and chlorophyll binding apparatus and in converse a continuous supply of energy from carbon dioxide fixation is required for nitrogen assimilation and distribution (Foyer *et al*., 2001). In maintaining the balance of carbon and nitrogen, a number of enzymes play major roles and one of these is the glutamate dehydrogenase (GDH) shunt to return the carbon in amino acids back into the carbon metabolism reactions and the tri-carboxylic acid cycle (Miflin and Habash, 2002). Nitrate reductase (NiR) and major foliar glutamate synthase activity use ferredoxin as a reductant of carbon to assimilate nitrate into the amino acids glutamine, glutamate, asparagine and asparate. In illuminated leaves 80% of the reductant necessary is generated directly by the photosynthetic electron transport chain and from the respiratory oxidation of fixed carbon (Foyer *et al*., 2001)

To put it succinctly, increased nitrogen availability increased leaf Rubisco and chlorophyll content (Lawlor *et al*., 2001) which led to an increase in leaf area through increasing the number of cells and their size (Lea *et al*., 2001) and as a result the LAI increased which increased the amount of light intercepted and the radiation use efficiency increased and photosynthesis efficiency was maintained (Gastal and Lemair, 2002). Consequently in a majority of C_3 crop species the growth and productivity are significantly enhanced by nitrogen (Lawlor *et al*., 2001) and this includes oil crops. For example in sunflower, Cechin and Fumis,

(2004) reported that high nitrogen supply led to a significant increase in shoot and leaf dry matter accumulation in greenhouse grown plants resultant from increased leaf nitrogen content, photosynthesis rate and water use efficiency. Also in the field, three rates of nitrogen (0, 100 and 200 kg N ha^{-1}) on the two hybrids of safflower (CW9048 and CW9050) was tested by Dordas and Sioulas (2008) and the results showed that leaf nitrogen content, photosynthesis rate, chlorophyll content and water use efficiency positively related to leaf nitrogen content and significantly increased at 200 kg N ha^{-1}. As a result, plant biomass and yield component (number of branch and number capitula per plant) seed yield significantly increased, while seed oil content was not affected. Dordas and Sioulas (2009) concluded that 200 kg N ha^{-1} increased the total above ground biomass at both anthesis and harvest. Also dry matter partitioning into different parts, depending on the growing season, was increased. These findings were strongly confirmed by the results obtained in this study, under semi-controlled glass-house conditions where safflower seed yield in term of seed number and individual seed weight significant increased with each increase in nitrogen level. These increments were the results of the increased photosynthesis rate, stomatal conductance and consequence transpiration rate which were all positively related to leaf nitrogen content and leaf chlorophyll content. Consequently water use efficiency and above ground biomass also increased continually with increased nitrogen level, but seed oil content and fatty acid composition were not affected by nitrogen input. It is suggested that other environmental factors affect the seed oil content and fatty acid composition more and recently Gecgel *et al*., (2007) reported that the genotype and sowing date are the most important factors that control seed oil content and

fatty acid composition in safflower. These results confirm other studies which did not show any relationship between nitrogen rates and safflower seed oil content (Dordas and Sioulas, 2008; Elfadl *et al*., 2009). Also in other oil seed crops such as oilseed rape an inverse relationship between seed oil content and high nitrogen supplied has been found and suggests that high nitrogen might decrease availability of carbohydrates for oil creation (Rathke *et al*., 2005).

In spite of the amelioration of a negative impact of nitrogen deficiency on crop growth by elevated CO_2(Larigauderie *et al*., 1988; Radoglou *et al*., 1992) long term growth at elevated CO_2 leads to photosynthetic elongation in several species caused by carbohydrate accumulation which tends to be more pronounced under lower than higher nitrogen availability (Ainsworth and Rogers, 2007). Elevated CO_2 increases the photosynthetic rate initially at both low and high nitrogen and it enhances the root and shoots growth and biomass allocation under higher nitrogen and creates strong sinks. Ultimately, photosynthesis is maintained better under higher nitrogen (Ainsworth and Rogers, 2007). In turn, increases in both atmospheric CO_2 concentration and nitrogen supply result in large and sustained increases in the assimilation rate (Geiger *et al*., 1999; Rogers *et al*., 1996a; Rogers *et al*., 1996b; Sanz-Sáez *et al*., 2010).

As reported here for safflower at anthesis, the assimilation rate increased incrementally significantly with each increase in nitrogen supplied at both elevated CO_2 and ambient CO_2 and suggested that effects of CO_2 and nitrogen on assimilation rate are independent of each other. At all nitrogen treatments assimilation rate was up to 18% higher at elevated CO_2 than ambient CO_2, and the highest value was obtained under the highest nitrogen. The photosynthetic

rate under elevated CO_2 and 25 kg N ha^{-1} was equivalent to the photosynthetic rate at 125 kg N ha^{-1} and ambient CO_2 and this suggested that the negative effects of low nitrogen can be ameliorated by elevated CO_2 as the amount of nitrogen taken up by the plants under the interaction of elevated CO_2 and 25 kg N ha^{-1} same as the amount taken up by the plants under ambient CO_2 and higher nitrogen rates (0.30 g Pl^{-1}). As a result plant growth in term of leaves number, leaf area, LAI, plant height, branches number, capitula number above ground dry weight were significantly higher under the elevated CO_2 at 25 kg N ha^{-1}compared to ambient CO_2 and 125 kg N ha^{-1}. Also at anthesis plant growth in term of plant height, leaf number, branch number and capitula number significantly responded to increased nitrogen levels at both elevated and ambient CO_2 but at all nitrogen treatments, they were significantly higher at elevated CO_2 compared to ambient CO_2 the higher nitrogen input. There was only a small interaction effect between elevated CO_2 and nitrogen on the LAI at anthesis, the number of branches, capitula and seeds per plant and total above ground biomass at harvest. It was clearly indicated that the highest nitrogen availability provided the biggest size of the sink that utilized the additional photoassimilate at elevated CO_2. Plants at 175 kg N ha^{-1} produced significantly the highest seed yield at elevated CO_2 compared to other three nitrogen levels. This finding strongly supports the hypothesis that nutrient availability led to a larger sink to utilize additional photoassimilate under elevated CO_2 (Ainsworth and Long, 2005).

In contrast to this study, under conditions where nitrogen stress decreases leaf area, assimilation and sugar levels and increased allocation of biomass to non-photosynthetic tissue, the photosynthetic acclimation to elevated CO_2 can be

more pronounced in high compared to low nitrogen supply (Jifon and Wolfe, 2002). But this was clearly not an issue with safflower in these experiments.

Drought is one of the most important environmental factors limiting the growth and productivity of crop species worldwide and is probably the most important of the plant physical stresses in the global environment (Luo *et al*., 1999). Drought and salinity are considered to be the major environmental stresses for land loss and reduced crop yield with a predicted reduction in average yield of up to 50% in the world by the year 2050 as a result of associated climate change events (Wang *et al*., 2003). Plant biomass and productivity of a wide range of crops is reduced under drought, for example, in sunflower (Nezami *et al*., 2008), peanut (Chapman *et al.,* 1993) wheat (Kang *et al*., 2002), sunflower (Schittenhelm, 2010) rapeseed (Istanbulluoglu *et al*., 2010) bean (Acosta Gallegos and Kohashi Shibata, 1989; Boutraa and Sanders, 2001) and field grown safflower (Eslam, 2011; Istanbulluogh *et al*., 2009; Jalali *et al*., 2011; Kar *et al*., 2007; Pasban Eslam and Sadeqi, 2008; Sharghi and Bagheri, 2011) and it is widely documented that the direct effect of drought (short and long term drought) on photosynthetic rate would be through stomatal closure. It has been frequently established and supported by the work here that water stress has no effect on the variation of the maximum fluorescence ratio (Fv/Fm) indicating that drought has no effect on the primary photochemistry of PSII in most C_3 crops including sunflower (Cornic and Fresneau, 2002; Panković *et al*., 1999; Pastenes *et al*., 2005; Subrahmanyam *et al*., 2006). This shows that PSII is quite resistant to water stress (Shangguan et al., 2000). For safflower in this work, the maximum quantum yield of PSII photo-chemistry for all water regimes (including well water plants) was constant until day 120 and then declined at

day 125 but recovered back. The reason for this sudden decline is not entirely clear but high temperature inside the glass-house (above 40 ^{0}C) might be responsible for this decline. A similar decline in variable fluorescence (Fv) was also reported in the literature when plant leaves were exposed to a high heat shock temperature of 40 ^{0}C (Feierabend *et al*., 1992). Similarly, a combination of high temperature more than (40 ^{0}C) and water deficit in Phaseolus bean decreased the Fv/Fm ratio and CO_2 uptake but the crop could then recover as seen here too (Yordanov *et al*., 1997). In contrast, other researchers have cited both stomatal and non-stomatal limitation of photosynthesis through decreasing Rubisco and adenosine triphosphate (ATP) under severe drought (Flexas and Medrano, 2002). Moreover, recently, during an experiment on field grown safflower in Isfahan, Iran, using three irrigation treatments across six safflower genotypes, chlorophyll fluorescence Fv/Fm decreased under the maximum evaporation level (135 mm) when measured at both heading and at pollination stages (Miladi and Ehsanzadeh, 2010).

As stomatal conductance is reduced under the effect of drought the plants biomass, seed yield and yield components (capitula number) were significantly decreased (Eslam, 2011; Sharrifmoghaddasi and Omidi, 2010). The highest level of reduction recorded was when plants were exposed to drought throughout their development (from rosette to maturity) compared to the control and other drought regimes. This was probably due to the fact that safflower translocates a large percentage of its pre-anthesis carbohydrate accumulation to the seed during late season drought stress (Koutroubas *et al*., 2004). Also Jalali *et al*., (2011) revealed that a long period of water stress during stem

elongation (terminal drought) severely affected safflower growth and yield while a moderate drought at the same stage had no significant effect.

Nonsignificant reductions in above ground biomass, biological yield and harvest index were recorded for safflower here when exposed to mid-season drought. This was because safflower exhibited recovery when plants were relieved of water stress at earlier growth stages (rosette) and similarly other oil crops such as rapeseed have also shown recovery after relief of drought in the same way (Ahmadi and Bahrani, 2009).

The most important factors determining seed oil content and fatty acid composition is the genotype (Dajue, 1993) but other environmental factors such as drought have also been reported to affect the seed oil content in safflower and other oilseeds. Ashrafi and Razmjoo (2010) found that as water stress levels increased the oil content decreased. As reported in this study, terminal drought and long term drought reduced seed oil content by 14% but mid-season drought only reduced oil content by 5% compared with the well watered control.

7.3. General Conclusions

Despite being cited as a drought-resistant crop, Safflower positively responded to water availability and drought negatively affected safflower physiology, growth, seed yield and seed oil content. The reduction in biomass and seed yield could be mainly attributed to stomatal conductance indicating a closure of stomata. The Fv/Fm ratio was not affected and did not contribute to the reduction in CO_2 uptake and CO_2 fixation capacity as it was stable under all watering regimes. Since safflower growth recovered after early (mid-season) drought, this study suggested that the most sensitive stages for safflower over which it should be supplied with full irrigation is from stem elongation to maturity. For safflower to optimize assimilation rate and LAI more than 100 kg N ha^{-1} should be supplied and increases in both assimilation rate and leaf area can be expected to contribute to the increase in seed yield. Seed yield reached its maximum of 175 kg N ha^{-1} and this indicated the higher the nitrogen input the higher the partitioning to seed occurred. Seed oil content and fatty acid profile did not change with changes in nitrogen fertilizer availability. Seed yield also markedly increased and was associated with a noticeable increase in above ground biomass and assimilation rate of elevated CO_2. Elevated CO_2 was shown to increase both assimilation rate and partitioning of biomass. Seed oil content might be reduced and the seed fatty acid might be altered only slightly by elevated CO_2 and the result suggests that safflower would perform well under conditions of increasing CO_2 predicted for later this century. Since the photosynthetic rate still increased significantly under the lowest nitrogen in response to elevated CO_2 compared to ambient CO_2, it can be concluded that the negative impact of nitrogen deficiency on photosynthesis can be

ameliorated by elevated CO_2 and doubling CO_2 was equivalent to applying an extra 100 kg N ha^{-1}. At the same time Safflower had a large sink capacity that allowed a high photosynthetic rate at elevated CO_2 to be maintained. A nitrogen availability level equivalent of 125 kg N ha^{-1} was sufficient to avoid photosynthetic down regulation. If other factors optimized for safflower seed set are overcome it can be concluded that the seed yield positively responds to elevated CO_2 and nitrogen level of 175 kg N ha^{-1}.

7.4. Limitations of the study and future work

The results from this study are from pot grown plants inside a glass-house in enclosed chambers where some of the environment factors are limited. The enclosed chamber led to a limitation of radiation and coupled with low wind speed led to high humidity (chamber effect). The size of glass-house and chambers also limited the number of plants that could be grown and as a consequence did not allow an investigation of all relevant parameters. Furthermore, growing plants in pots led to a restriction in root growth and a possible limitation of nutrient availability to support plant growth. Re-establishment of the same experiments in free-air CO_2 enrichment (FACE) and open top chamber (OTC) along with growing plants in the soil are necessary to reflect the field microclimate, as they allow more natural condition and more detailed analysis required to represent a more exact picture as natural field conditions.

In this study, the effect of water stress, nitrogen, elevated CO_2 and elevated CO_2 in conjunction with nitrogen were only investigated on one cultivar and others should be studied to determine if there is genotypic variance in these responses. Four doses of nitrogen fertilizer in the form of ammonium nitrate were supplied at four monthly intervals in the nitrogen experiment in the nitrogen interaction with elevated CO_2 and it would desirable to further study increase doses of nitrogen especially during seed filling. Seed set was a problem in all of the experiments and this needs further experimentation to determine what is limiting pollination/fertilization. Other limiting factors such as radiance and high temperature appear to be the most likely to investigate in this respect. The physiological parameters were only measured at 50% anthesis

and these parameters should be tested at other growth stages to provide a full picture of safflower's response over time. In addition, multiple stresses will occur in the field as the climate changes in the future, for example elevated atmospheric CO_2 is usually combined with increased temperature (IPCC. 2007) and a collapse of water resources may occur. It will be particularly important to study various recovery processes after the relief of water stress for the effective use of water (Miyashita *et al*., 2005). Thus, the interaction of these factors on safflower growth, seed set and productivity and the molecular and biochemical changes associated with tolerance and susceptibility of responses should be the main focus of future work so as to provide a wide knowledge for plant breeders to develop or select safflower genotypes with adaptation to such environmental change. The use of safflower as an alternative agricultural crop in regions facing drastic climatic change, especially water shortage, can be expected.

Appendices

Appendix (A)

Hydroponic growth solution (VITALINK Max Grow)

This solution is constituted of two parts; part A and part B.

Each part comprised of the following macro and micro nutrients (manufacturers guaranteed analysis

Nitrogen (Total) 5.125%

Nitrate nitrogen 4.75 %

Ammoniacal nitrogen 0.37%

Potassium K_2O 7.8 %

Phosphorus (P_2 O_5) 2.6 %

Calcium 2.625 (SW 3.625%)

Magnesium 0.75% (SW 1%)

Sugar 0.7% (SW 0.64)

Boron 0.0087%

Copper 0.002%

Iron 0.068

Manganese 0.0145

Molybdenum 0.0013%

Since 0.0098%

Cobalt 0.0013%

Nickel 0.0013%

All macro and micro nutrients are in a chelated from apart from molybdenum which is supplied as Ammonium molybdate, cobalt as sulphate and nickel as sulphate.

Directions of use

The solutions should be shaken thoroughly before use and always add equal quantities of part A and B

The desired volume of water was added to the nutrient solutions and added the following rates:

Young vegetative add 1-2 mL part A per litre and add 1-2 mL part B per litre (CF 4.8) pH 5.2 - 6.5

Mature plant 3-4 mL of part A and 3-4 mL of part B per litre (CF 12-18) pH 5.2 - 6.5.

References

Abbadi J., Gerendás J. (2009) Nitrogen use efficiency of safflower as compared to sunflower. Journal of Plant Nutrition, **32**,929 - 945.

Abbadi J., Gerendas J., Sattelmacher B. (2008) Effects of nitrogen supply on growth, yield and yield components of safflower and sunflower. Plant Soil, 167-180.

Abdulhabip O., Demirbilek T., Atillagur M., Copur O.(2004) Effects of different sowing date intrarow spacing on yield and some agronomic traits of safflower (*Carthamus tinctorius* L.) under Harran Plain arid conditions. Turkey Journal of Agriculture, 413-419.

Abdurahman D.M., Seeling B., Rego J.T., Reddy B. (1999) The root system and nitrogen up take of a safflower crop (*Carthamus tinctorius* L.).Crop Research, **17**, 22- 27.

Abel G., Driscoll M. (1976) Sequential trait development and breeding for high yields in safflower. Crop Science **16**, 213-216.

Abrol Y.P., Raghuram N. (2007) Agriculture nitrogen use and its environmental implication. I.K. International Pvt Ltd.

Ackman R. (1980) Fish lipids, part 1. Advances in fish science and technology, 86-103. Fishing News Books Ltd.

Addiscott T.M. (2005) Nitrate, agriculture and the environment. CABI Publishing Series, CAB Books, Wallingford,UK.

Ahmadi M., Bahrani M. (2009) Yield and yield components of rapeseed as influenced by water stress at different growth stages and nitrogen levels.

American-Eurasian Journal of Agricultural and Environmental Sciences, **5**,755-761.

Ahmed F.E., Suliman A.S.H. (2010) Effect of water stress applied at different stages of growth on seed yield and water-use efficiency of Cowpea.

Aiken R.,Lamm F. (2006) Irrigation of oilseed crops. K-State Northwest Research-extension centre.105 Experimental Farm Road,Calby,Kansas.

Ainsworth E., Davey P., Hymus G., Osborne C., Rogers A., Blum H., Nösberger J., Lateong S. (2003) Is stimulation of leaf photosynthesis by elevated carbon dioxide concentration maintained in the long term? A test with Lolium perenne grown for 10 years at two nitrogen fertilization levels under Free Air CO_2 Enrichment (FACE). Plant, Cell and Environment , 26, 705-714.

Ainsworth E.A., Long S.P. (2005) What have we learned from 15 years of free air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytologist .**165**, 351-372.

Ainsworth E.A., Rogers A. (2007) The response of photosynthesis and stomatal conductance to rising CO_2: mechanisms and environmental interactions. Plant, Cell and Environment, **30**, 258-270.

Ainsworth E.A., McGrath J.M. (2010) Direct effects of rising atmospheric carbon dioxide and ozone on crop yields. Climate Change and Food Security, 109-130.

Ainsworth E.A., Davey P.A., Bernacchi C.J., Dermody O.C., Heaton E.A., Moore D.J., Morgan P.B., Naidu S.L., Yoo Ra H., Zhu X. (2002) A meta-

analysis of elevated CO_2 effects on soybean (*Glycine max*) physiology, growth and yield. Global Change Biology, **8**, 695-709.

Alizadeh K. (2005) Evaluation of safflower germplasm by some agronomic characteristics and their relationships on grain yield production in the cold dry lands of Iran. International Journal of Agriculture. Biology, **7**, 389-391.

Alam S.M., Pessarakli M. (1999) Nutrient uptake by plants under stress conditions. Handbook of plant and crop stress. 285-313.

Allen H.L. (1998) Carbon dioxide and other Atmospheric Gases, Principal of Ecology in plant production, edsT.R. Sinclair and F.P. Gardner, 169-184.

Allen j., H.I. (1999) Evapotranspiration response of plants and crops to carbon dioxide and temperature. Journal of crop production, **2**, 37-70.

Andrews T.J., Hudson G.S., Mate C.J., von Caemmerer S., Evans J.R., Arvidsson Y.B.C. (1995) Rubisco: the consequences of altering its expression and activation in transgenic plants. Journal of Experimental Botany, **46**, 1293-1300.

Anten N., Hirose T., Onoda Y., Kinugasa T., Kim H., Okada M., Kobayashi K. (2004) Elevated CO2 and nitrogen availability have interactive effects on canopy carbon gain in rice. New Phytologist, **161**, 459-471.

Anyia A., Herzog H. (2004) Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. European Journal of Agronomy, **20**, 327-339.

Armah-Agyeman G., Loiland J., Karrow R., Hang A. (2002) Safflower. [online] available at: http://hdl.handle.net/1957/20205.

Arp W. (1991) Effects of source‐sink relations on photosynthetic acclimation to elevated CO_2. Plant, Cell and Environment, **14**, 869-875.

Aslam M., Nelson M., Kailis S., Bayliss K., Speijers J., Cowling W. (2009) Canola oil increases in polyunsaturated fatty acids and decreases in oleic acid in drought‐stressed Mediterranean‐type environments. Plant Breeding, **128**, 348-355.

Austin A.T., Yahdjian L., Stark J.M., Belnap J., Porporato A., Norton U., Ravetta D.A., Schaeffer S.M. (2004) Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia, **141**, 221-235.

Bagheri H. (2011a) The study of morphophysiological characteristics of spring safflower varieties in different moisture regimes. Advances in Environmental Biology, **5**, 3854-3857.

Bagheri H. (2011b) The response of morphological traits in safflower cultivar to drought stress (*Carthamus tinctorius* L.). Research Journal of Fisheries and Hydrobiology , **6**, 533-536.

Baker J., Hartwell Allen L., Boote K., Pickering N. (1997) Rice responses to drought under carbon dioxide enrichment. 1. Growth and yield. Global Change Biology , **3**, 119-128.

Baker J., Allen Jr L., Boote K., Jones P., Jones J. (1989) Response of soybean to air temperature and carbon dioxide concentration. Crop Science, **29**, 98-105.

Baker J.T., Boote K. (1996) 4. The CO_2 Fertilization Effect: Higher carbohydrate production and retention as Biomass and Seed Yield. Global climate change

and agricultural production: direct and indirect effects of changing hydrological, pedological, and plant physiological processes,65.

Bannayan M., Tojo Seller C., Garcia A., Guerra L., Hoogenboom G. (2009) Interactive effects of elevated CO_2 and temperature on two long and short season peanut cultivars growth and development. Climatic Change, **96**, 389-406.

Bassil E.S., Kaffka S.R., Hutmacher R.A. (2002) Response of safflower (Carthamus tinctorius L.) to residual soil N following cotton (*Gossypium* spp.) in rotation in the San Joaquin Valley of California. The Journal of Agricultural Science,**138**, 395 - 402.

Bassiri A., Khosh-Khui M., Rouhani I. (1977) The influences of simulated moisture stress conditions and osmotic substrates on germination and growth of cultivated and wild safflowers. Journal of Agricultural Sciences, **88**, 95-100.

Bates L., Hall A. (1982) Diurnal and seasonal responses of stomatal conductance for cowpea plants subjected to different levels of environmental drought. Oecologia, **54**, 304-308.

Baxter R., Bell S., Sparks T., Ashenden T., Farrar J. (1995) Effects of elevated CO_2 concentrations on three montane grass species. Journal of Experimental Botany, **46**, 917-929.

Beering J.D. (2005) ((Eds.) Evolution responses of land plant to atmospheric CO_2, Springer Science + Business Media, Inc, New York,USA.

Beerling D., Woodward F. (1995) Leaf stable carbon isotope composition records increased water-use efficiency of C_3 plants in response to atmospheric CO_2 enrichment. Functional Ecology, 394-401.

Berendse F., Aerts R. (1987) Nitrogen-use-efficiency: a biologically meaningful definition?, JSTOR. 293-296.

Berglund D.R., Riveland N., Bergman J. (2010) Safflower production. [online] available at: http://hdl.handle.net/10365/9154.

Bernacchi C.J., Leakey A.D.B., Heady L.E., Morgan P.B., Dohleman F.G., McGrath J.M., Gillespie K.M., Wittig V.E., Rogers A., Long S.P. (2006) Hourly and seasonal variation in photosynthesis and stomatal conductance of soybean grown at future CO_2 and ozone concentrations for 3 years under fully open - air field conditions. Plant, Cell and Environment, **29**, 2077-2090.

Blumenthal C., Rawson H., McKenzie E. (1996) Changes in wheat grain quality due to doubling the level of atmospheric CO_2, Cereal Chemistry, **73**, 762-766.

Boogaard R., Veneklaas E.J., Lambers H. (1996) The association of biomass allocation with growth and water use efficiency of two *Triticum aestivum* cultivars. Functional Plant Biology, **23**, 751-761.

Booker F.L., Shafer S.R., Wei C.M., Horton S.J. (2000) Carbon dioxide enrichment and nitrogen fertilization effects on cotton (*Gossypium hirsutum* L.) plant residue chemistry and decomposition. Plant and Soil, **220**, 89-98.

Boote K., Jones P., Baker J., Jones J., Allen L. (1989) Response of soybean to air temperature and carbon dioxide concentration. Crop Science, 29, 98-105.

Bowes. (1996)(Eds.) Photosynthetic responses to changing atmospheric carbon dioxid concentration, Springer Netherlands.

Bowes G. (2004) Photosynthetic responses to changing atmospheric carbon dioxide concentration. Photosynthesis and the Environment, 387-407.

Bowler J.M., Press M.C. (1996) Effects of elevated CO_2, nitrogen form and concentration on growth and photosynthesis of a fast‐and slow‐growing grass. New Phytologist, **132**, 391-401.

Bray E.A. (1997) Plant responses to water deficit. Trends in Plant Science, 2, 48-54.

Bréda N.J.J. (2003) Ground based measurements of leaf area index: a review of methods, instruments and current controversies. Journal of Experimental Botany, 54, 2403-2417.

Bremner J., Sparks D., Page A., Helmke P., Loeppert R., Soltanpour P., Tabatabai M., Johnston C., Sumner M. (1996) Nitrogen-total. Methods of soil analysis. Part 3-chemical methods, 1085-1121.

Broadley M.R. (2000) What are the effects of nitrogen deficiency on growth components of lettuce? New Phytologist, **147**, 519-526.

Brooks A., Farquhar G. (1985) Effect of temperature on the CO_2:O_2 specificity of ribulose-1, 5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light. Planta , **165**, 397-406.

Brooks T.J., Wall G.W., Pinter P.J., Kimball B.A., LaMorte R.L., Leavitt S.W., Matthias A.D., Adamsen F.J., Hunsaker D.J., Webber A.N. (2000) Acclimation

response of spring wheat in a free-air CO_2 enrichment (FACE) atmosphere with variable soil nitrogen regimes. 3. Canopy architecture and gas exchange. Photosynthesis Research, **66**, 97-108.

Cabrera-Bosquet L., Albrizio R., Araus J.L., Nogués S. (2009) Photosynthetic capacity of field-grown durum wheat under different N availabilities: A comparative study from leaf to canopy. Environmental and Experimental Botany, **67**, 145 -152.

Campbell C.S., Heilman J.L., McInnes K.J., Wilson L.T., Medley J.C., Wu G., Cobos D.R. (2001) Seasonal variation in radiation use efficiency of irrigated rice. Agricultural and Forest Meteorology, **110**, 45-54.

Caporn S.J.M., Bridget A. (2009) 3 Threats from air pollution and climate change to upland systems. Drivers of Environmental Change in Uplands, **2**, 34.

Carlson R., Bazzaz F. (1980) The effects of elevated CO_2 concentrations on growth, photosynthesis, transpiration, and water use efficiency of plants, Academic Press, 609-623.

Cechin I., de Fátima Fumis T. (2004) Effect of nitrogen supply on growth and photosynthesis of sunflower plants grown in the greenhouse. Plant Science, **166**, 1379-1385.

Change I.P.O.C. (2007) Climate change 2007: the physical science basis. Report from Intergovernmental Panel on climate Change. Agenda.06. 07.07.

Chapin F.S., Bloom A.J., Field C.B., Waring R.H. (1987) Plant responses to multiple environmental factors. BioScience, **37**, 49-57.

Chaves M., Pereira J., Maroco J., Rodrigues M., Ricardo C., Osorio M., Carvalho I., Faria T., Pinheiro C. (2002) How plants cope with water stress in the field? photosynthesis and growth. Annals of Botany, **89**, 907-916.

Cheng W., Sakai H., Yagi K., Hasegawa T. (2009) Interactions of elevated CO_2 and night temperature on rice growth and yield. Agricultural and Forest Meteorology, **149**, 51-58.

Cheng W., Sims D.A., Luo Y., Coleman J.S., Johnson D.W. (2000) Photosynthesis, respiration, and net primary production of sunflower stands in ambient and elevated atmospheric CO_2 concentrations: an invariant NPP: GPP ratio? Global Change Biology , **6**, 931-941.

Chiariello R.N., Gulmon S.L. (1991) (Eds.) Stress effect on plant reproduction. Acadmic Press,

Christie W. (2003) Lipid Analysis: Isolation. Separation, Identification and Structural Analysis of Lipids . Barnes, Bridgwater, UK.

Cipollini M.L., Drake B.G., Whigham D. (1993) Effects of elevated CO_2 ongrowth and carbon/nutrient balance in the deciduous woody shrub *Lindera benzoin* (L.)Blume (Lauraceae). Oecologia, **96**,339-346.

Ciompi S., Gentili E., Guidi L., Soldatini G.F. (1996) The effect of nitrogen deficiency on leaf gas exchange and chlorophyll fluorescence parameters in sunflower. Plant Science, **118**,177-184.

Comstock J.P. (2002) Hydraulic and chemical signalling in the control of stomatal conductance and transpiration. Journal of Experimental Botany, **53**, 195-200.

Conley M.M., Kimball B., Brooks T., Pinter Jr P., Hunsaker D., Wall G., Adam N., LaMorte R., Matthias A., Thompson T. (2001) CO_2 enrichment increases water - use efficiency in sorghum. New Phytologist, **151**, 407- 412.

Cornic G., Fresneau C. (2002) Photosynthetic carbon reduction and carbon oxidation cycle are the main electron sinks for photosystem II activity during a mild drought. Juornal of Annals of Botany, **89**, 887-894.

Cornic G., Massacci A. (2004) Leaf photosynthesis under drought stress. Photosynthesis and the Environment, 347-366.

Coşge B., Gürbüz B., Kiralan M. (2007) Oil content and fatty acid composition of some safflower (*Carthamus tinctorius* L.) varieties sown in spring and winter. International Journal of Natural and Engineering Sciences, **1**, 11-15.

Cotrufo M.F., Ineson P., Scott A.Y. (1998) Elevated CO_2 reduces the nitrogen concentration of plant tissues. Global Change Biology, **4**, 43-54.

Crafts-Brandner S.J., Salvucci M.E. (2000) Rubisco activities constrains the photosynthetic potential of leaves at high temperature and CO_2. Proceedings of the National Academy of Sciences, **97**, 13430.

Cruz C., Lips H., Martins-Loução M.A. (2003) Nitrogen use efficiency by a slow-growing species as affected by CO_2 levels, root temperature, N source and availability. Journal of Plant Physiology , 160, 1421-1428.

Cure J.D., Israel. D.W., Rufty. T. W. (1988) Nitrogen stress effects on growth and seed yield of non nodulated soybean exposed to elevated CO_2. Crop Science, **28**, 671- 677.

Dajue I.L., Munde H.H. (1996)(Eds.) Safflower. Carthamus tinctorius, international plant Genetic Resources Institute, Rom,Italy.

Desai R., Bhatia C. (1978) Nitrogen uptake and nitrogen harvest index in durum wheat cultivars varying in their grain protein concentration. Euphytica, **27**, 561-566.

Del Pozo A., Pérez P., Morcuende R., Alonso A., Martínez-Carrasco R. (2005) Acclimatory responses of stomatal conductance and photosynthesis to elevated CO_2 and temperature in wheat crops grown at varying levels of N supply in a Mediterranean environment. Plant Science, **169**, 908-916.

Del Pozo A., Pérez P., Gutiérrez D., Alonso A., Morcuende R., Martínez-Carrasco R. (2007) Gas exchange acclimation to elevated CO_2 in upper-sunlit and lower-shaded canopy leaves in relation to nitrogen acquisition and partitioning in wheat grown in field chambers. Environmental and Experimental Botany , **59**, 371-38.

Delucia E.H., Sasek T.W., Strain B.R. (1985) Photosynthetic inhibition after long-term exposure to elevated levels of atmospheric carbon dioxide. Photosynthesis Research, **7**, 175-184.

Dermody O., Long S.P., DeLucia E.H. (2006) How does elevated CO_2 or ozone affect the leaf ‐ area index of soybean when applied independently? New Phytologist, **169**, 145-155.

Dordas A.C., Sioulas C. (2007) Safflower yield, chlorophyll content, photosynthesis, and water use efficiency response to nitrogen fertilizer under rain fed conditions. Industrial Crops and Products, **27**, 75-85.

Dordas A.C., Sioulas C. (2008) Safflower yield, chlorophyll content, photosynthesis, and water use efficiency response to nitrogen fertilizer under rain fed condition. Industrial Crops and Products, **27**, 75- 85.

Dordas C. (2009) Dry matter, nitrogen and phosphorus accumulation, partitioning and remobilization as affected by N and P fertilization and source-sink relations. European Journal of Agronomy, **30**, 129-139.

Drake B., Leadley P. (1991) Canopy photosynthesis of crops and native plant communities exposed to long‐term elevated CO_2. Plant, Cell and Environment, **14**, 853-860.

Drake B.G., Gonzàlez-Meler M.A., Long S.P. (1997) More efficient plants: a consequence of rising atmospheric CO2? Annual Review of Plant Biology, **48**,609-639.

Dreccer M., Van Oijen M., Schapendonk A., Pot C., Rabbinge R. (2000) Dynamics of vertical leaf nitrogen distribution in a vegetative wheat canopy. Impact on canopy photosynthesis. Annals of Botany, **86**, 821-831.

Dwivedi S.L., Nigam S.N., Nageswara Rao R.C., Singh U., K.V.S R. (1996) Effect of drought on oil, fatty acids and protein contents of groundnut (Arachis hypogaea L.). Field Crops Research, **48**, 125-133.

Ehleringer J. (2005) The influence of atmospheric CO_2, temperature, and water on the abundance of C3/C 4 taxa. A History of Atmospheric CO_2 and Its Effects on Plants, Animals, and Ecosystems, 214-231.

Eichhorn E.R. (1999) (Eds.) Biology of plants Sixth Edition. Freeman and company /Worth publishers.

Eksilinge B.S., Sondge V.D., Raikiielkar S.V. (1993) Effect of advance application of fertilizer on oil content and oil productivity in safflower (Carthamus tinctorius) varieties. Indian journal of Agronomy,**38**, 661-663.

Elfadl E., Reinbrecht C., Frick C., Claupein W. (2009) Optimization of nitrogen rate and seed density for safflower (*Carthamus tinctorius* L.) production under low-input farming conditions in temperate climate. Field Crops Research, **114**, 2-13.

Ellis R., Summerfield R., Roberts E. (1988) Effects of temperature, photoperiod and seed vernalization on flowering in faba bean Vicia faba. Annals of Botany, **61**, 17-27.

Emongor V. (2010) Safflower (*Carthamus tinctorius* L.) the underutilized andneglected crop: A Review. Asian Journal of Plant Science, **9**, 299-306.

Eslam B.P. (2011) Evaluation of physiological indices for improving water deficit tolerance in spring safflower. Journal of Agricultural Science and Technology, **13**, 327-338.

Evans J.R. (1989) Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia, **78**, 9-19.

Evans R., Belnap J. (1999) Long-term consequences of disturbance on nitrogen dynamics in an arid ecosystem. Ecology, **80**, 150-160.

Ewert F. (2004) Modelling plant responses to elevated CO_2: how important is leaf area index? Annals of Botany, **93**, 619.

Ewert F., Pleijel H. (1999) Phenological development, leaf emergence, tillering and leaf area index, and duration of spring wheat across Europe in response to CO_2 and ozone. European Journal of Agronomy, **10**, 171-184.

Fageria N.K., Baligar V.C., Jones C.A. (2010) Growth and mineral nutrition of field crops CRC Press, Taylor &Francis Group.

Fallovo C., Colla G., Schreiner M., Krumbein A., Schwarz D. (2009) Effect of nitrogen form and radiation on growth and mineral concentration of two Brassica species. Scientia Horticulturae, **123**, 170-177.

Fangmeier A., Grüters U., Hertstein U., Sandhage-Hofmann A., Vermehren B., Jäger H.J. (1996) Effects of elevated CO_2 nitrogen supply and tropospheric ozone on spring wheat. I. Growth and yield. Environmental Pollution, **91**, 381-390.

Fangmeier A., De Temmerman L., Mortensen L., Kemp K., Burke J., Mitchell R., van Oijen M., Weigel H.J. (1999) Effects on nutrients and on grain quality in spring wheat crops grown under elevated CO_2 concentrations and stress conditions in the European, multiple-site experiment ESPACE-wheat'. European Journal of Agronomy,1**0**,215-229.

Farage P.K., McKee I.F., Long S.P. (1998) Does a low nitrogen supply necessarily lead to acclimation of photosynthesis to elevated CO_2? Plant Physiology **, 118**, 573.

Farooq M., Basra S., Wahid A., Cheema Z., Cheema M., Khaliq A. (2008) Physiological role of exogenously applied glycinebetaine to improve drought

tolerance in fine grain aromatic rice (*Oryza sativa* L.). Journal of Agronomy and Crop Science, **194**, 325-333.

Feierabend J., Schaan C., Hertwig B. (1992) Photoinactivation of catalase occurs under both high-and low-temperature stress conditions and accompanies photoinhibition of photosystem II. Plant Physiology, **100**, 1554.

Ferasat M., Sajed N., Mirzakhani M. (2008) Response of Agriculture traits to drought stress condition in four safflower genotypes. New finding in agriculture. Scientific Information Database (SID), Iran.

Fei H., Tsang E., Cutler A.J. (2007) Gene expression during seed maturation in *Brassica napus* in relation to the induction of secondary dormancy. Genomics,**89**,419-428.

Field C., Mooney H. (1983) Leaf age and seasonal effects on light, water, and nitrogen use efficiency in a California shrub. Oecologia , **56**, 348-355.

Field C., Mooney H.A. (1986) 1 The photosynthesis-nitrogen relationship in wild plants, Cambridge University Press, 25.

Flexas J., Medrano H. (2002a) Drought-inhibition of photosynthesis in C_3 plants: Stomatal and non stomatal limitation revisited. Journal of Botany, **89**, 183-189.

Flexas J., Medrano H. (2002b) Energy dissipation in C_3 plants under drought. Functional Plant Biology, **29**, 1209-1215.

Flexas J., Escalona J.M., Evain S., Gulias J., moya I., Osmod C.B., Medrano H. (2002) Steady state chlorophyll fluorescence(Fs) measurments as a tool to follow variation of net CO_2 assimilation and stomatal conductance during water-stress in C_3 plant. Journal of Physiologia Plantarum, **114**, 231-240.

Forde B.G., Clarkson D.T., Callow J.A. (1999) Nitrate and Ammonium Nutrition of Plants: Physiological and Molecular Perspectives, Advances in Botanical Research, Academic Press, 1-90.

Foyer C.H., Ferrario-Mery S., Noctor G. (2001) Interactions between carbon andnitrogen metabolism. Plant Nitrogen, 237-254.

Franzaring J., Högy P., Fangmeier A. (2008) Effects of free-air CO_2 enrichment on the growth of summer oilseed rape (*Brassica napus* cv. Campino). Agriculture Ecosystems and Environment, **128**,127-134.

Fredeen A., Gamon J., Field C. (1991) Responses of photosynthesis and carbohydrate‐partitioning to limitations in nitrogen and water availability in field‐grown sunflower. Plant, Cell and Environment, **14**, 963-970.

Garcia R., Long S., Wall G., Osborne C., Kimball B., Nie G., Pinter P., LaMorte R., Wechsung F. (1998) Photosynthesis and conductance of spring‐wheat leaves: field response to continuous free‐air atmospheric CO_2 enrichment. Plant, Cell and Environment, **21**, 659-669.

Gastal F., Lemaire G. (2002) N uptake and distribution in crops: an agronomical and ecophysiological perspective. Journal of Experimental Botany, 53:789.

Gecgel U., Demirci M., Esendal E., Tasan M. (2007) Fatty acid composition of the oil from developing seeds of different varieties of safflower (*Carthamus tinctorius* L.). Journal of the American Oil Chemists' Society, **84**, 47-54.

Geiger M., Haake V., Ludewig F., Sonnewald U., Stitt M. (1999) The nitrate and ammonium nitrate supply have a major influence on the response of

photosynthesis, carbon metabolism, nitrogen metabolism and growth to elevated carbon dioxide in tobacco. Plant, Cell and Environment, **22**, 1177-1199.

Ghannoum O., Seneweera S.P., Conroy J.P., Ishimaru K., Okada M., Lieffering M., Kim H.Y., Kobayashi K. (2002) Changes in source–sink relations during development influence photosynthetic acclimation of rice to free air CO_2 enrichment (FACE). Functional Plant Biology, **29**, 947-955.

Ghioni C., Bell J., Sargent J. (1996) Poly unsaturated fatty acids in neutral lipids and phospholipids of some fresh water insects. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology,**114**, 161-170.

Gifford R.M., Evans L. (1981) Photosynthesis, carbon partitioning, and yield. Annual Review of Plant Physiology,**32**, 485-509.

Gifford R.M., Barrett D.J., Lutze J.L. (2000) The effects of elevated CO_2 on the C: N and C: P mass ratios of plant tissues. Plant and Soil, 224, 1-14.

Gimenez C., Connor D.J., Rueda F.(1994) Canopy development, photosynthesis and radiation-use efficiency in sunflower in response to nitrogen. Field Crops Research, **38**, 15-27.

Givnish T.J. (1986) (Eds.) On the economy of plant form and function: Proceeding of the sixth maria. Biddles short run books, Cambridge University Great Britain.

Greenwood D.J., Gastal F., Lemaire G., Draycott A., Millard P., Neeteson J.J. (1991) Growth rate and % N of field grown crops: theory and experiments. Annals of Botany, **67**,181.

Gregory P.J., Marshall B., Biscoe P.V. (1981) Nutrient relations of winter wheat: 3. Nitrogen uptake, photosynthesis of flag leaves and translocation of nitrogen to grain. The Journal of Agricultural Science, **96**, 539-547.

Grindlay D.J.C. (1997) Reviews Towards an explanation of crop nitrogen demand based on the optimization of leaf nitrogen per unit leaf area. The Journal of Agricultural Science, **128**,377-396.

Grossman-Clarke S., Pinter Jr P., Kartschall T., Kimball B., Hunsaker D., Wall G., Garcia R., LaMorte R. (2001) Modelling a spring wheat crop under elevated CO_2 and drought. New Phytologist, **150**,315-335.

Golombek S., Rolletschek H., Wobus U., Weber H. (2001) Control of storage proteinaccumulation during legume seed development. Journal of plant physiology, **158**, 457-464.

González F.G., Slafer G.A., Miralles D.J. (2003) Grain and floret number in responseto photoperiod during stem elongation in fully and slightly vernalized wheats. Field Crops Research, **81**, 17-27.

Gurusamy C., Thiagarajan C. (1998) The pattern of seed development and maturation in clauliflower (*Brassica oleracea* L. var. botrytis). *Phytohorn*, **38**,259-268.

Hampton J., Boelt B., Roleston M., Chastain T. (2012) Effects of elevated CO_2 and temperature on seed quality. The Journal of Agricultural Science, **1**, 1-9.

Harmens H., Stirling C.M., Marshall C., Farrar J.F. (2000) Does down‐regulation of photosynthetic capacity by elevated CO_2 depend on N supply in *Dactylis glomerata*? Physiologia Plantarum, **108**, 43-50.

Hartz - Rubin J.S., DeLucia E.H. (2001) Canopy development of a model herbaceous community exposed to elevated atmospheric CO_2 and soil nutrients. Physiologia Plantarum, **113**, 258-266.

Hatami B., Khajehali J., Sabzalian M. (2008) The effect of drought Stress on population density and damage of safflower fly (*Acanthiophilus helianthi*), aphid (*Uroleucon carthami*) and *Leafhopper Empoasca decipiens*. JWSS-Isfahan University of Technology, **12**, 699-709.

Hatfield J.L., Follett R.F. (2008) Nitrogen in the environment: Sources, problems, and management Academic Press.

Hebeisen T., Luscher A., Zanetti S., Fischer B., Hartwig U., Frehner M., Hendrey G., Blum H. (1997) Growth response of *Trifolium repens* L. and Lolium perenne L. as monocultures and bi - species mixture to free air CO_2 enrichment and management. Global Change Biology, **3**, 149-160.

Heimann M., Reichstein N. (2008) Terrestrial ecosystem carbon dynamics and climate feedback. Nature, **451**, 289-292.

Heinemann A.B., Maia A.H.N., Dourado-Neto D., Ingram K., Hoogenboom G. (2006) Soybean (*Glycine max* (L.) Merr.) growth and development response to CO_2 enrichment under different temperature regimes. European Journal of Agronomy, **24**, 52-61.

Herdrich N. (2001) (Eds.) Safflower Production tips, Washington State University, College of Agriculture and Home Economy.

Hershey D.R. (1995) Plant biology science project. New York: Wiley.

Hikosaka K., Kinugasa T., Oikawa S., Onoda Y., Hirose T. (2011) Effect of elevated CO_2 concentration on seed production in C3 annual plants. Journal of Experimental Botany, **62**, 1523- 1530.

Hill A.B., Knowles P.F. (1968) Fatty acid composition of the oil of developing seeds of different varieties of safflower. Journal of Crop Science,**8**, 275-277.

Hirose T., Ackerly D., Traw M., Ramseier D., Bazzaz F. (1997) CO_2 elevation, canopy photosynthesis, and optimal leaf area index. Ecology, **78**, 2339-2350.

Hocking P., Meyer C. (1991) Effects of CO_2 Enrichment and Nitrogen Stress on Growth, and Partitioning of Dry Matter and Nitrogen in Wheat and Maize. Functional Plant Biology, **18**, 339-356.

Hogy P., Fangmeier A. (2008) Effects of elevated atmospheric CO_2 on grain quality of wheat. Journal of Cereal Science, **48**, 580-591.

Högy P., Franzaring J., Schwadorf K., Breuer J., Schütze W., Fangmeier A. (2010) Effects of free-air CO_2 enrichment on energy traits and seed quality of oilseed rape. Agriculture, Ecosystems and Environment, **139**, 239-244.

Högy P., Wieser H., Köhler P., Schwadorf K., Breuer J., Franzaring J., Muntifering R., Fangmeier A. (2009) Effects of elevated CO_2 on grain yield and quality of wheat: results from a 3 ‐ year free ‐ air CO_2 enrichment experiment. Plant Biology, **11**, 60-69.

Högy P., Brunnbauer M., Koehler P., Schwadorf K., Breuer J., Franzaring J., Zhunusbayeva D., Fangmeier A. (2011) Grain quality characteristics of spring wheat (*Triticum aestivum*) as affected by free-air CO_2 enrichment. Environmental and Experimental Botany.

Hsiao T.C., Jackson R.B., Yiqi L., Harold A.M. (1999) Interactive effects of water stress and elevated CO_2 on growth, photosynthesis, and water use efficiency, Carbon Dioxide and Environmental Stress, Academic Press, San Diego, 3-31.

Huber S.C., Sugiyama T., Alberte R.S. (1989) Photosynthetic determinants of growth in maize plants: Effect of nitrogen nutrition on growth, carbon fixation and photochemical features. Plant Cell Physiology, **30**,1063-1072.

Ibrahim A., Abusteit E., El ‐ Metwally E. (1989) Response of rapeseed (Brassica napus L.) growth, yield, oil content and its fatty acids to nitrogen rates and application times. Journal of Agronomy and Crop Science, **162**, 107-112.

IPCC. (2007). Climate Change 2007- The physical science Basic.,contribution of working Group 1 to the fourth Assessment Report of the Intergovernmental panel on Climate Change.Cambridge University Press.

Istanbulluogh A., Gocmen E., Gezer E., Pasa C., Konukcu F. (2009) Effect of water stress at different development stages on yield and water productivity of winter and summer safflower (*Carthamus tinctorius* L.). Journal of Agriculture Water Mangement , **96**, 1429-1434.

Istanbulluoglu A. (2009) Effects of irrigation regimes on yield and water productivity of safflower (*Carthamus tinctorius* L.) under Mediterranean climatic conditions. Agricultural Water Management, **96**,1792-1798.

Izquierdo N.G.A., Andrade L.A.N., Cantarero F.H., Marcelo G. (2006) Modelling the response of fatty acid composition to temperature in a traditional sunflower hybrid. Agronomy Journal, **98**,451.

Jablonski L.M., Wang X., Curtis P.S. (2002) Plant reproduction under elevated CO_2 conditions: a meta‐analysis of reports on 79 crop and wild species. New Phytologist, **156**, 9-26.

Jalali A.H., Salehi F., Bahrani M.J. (2011) Effects of different irrigation intervals and weed control on yield and yield components of safflower (*Carthamus tinctorius* L.), Aechives of Agronomy and Soil Science, **53**, 11, 2012.

Jaleel C.A., Manivannan P., Wahid A., Farooq M., Al-Juburi H.J., Somasundaram R., Panneerselvam R. (2009) Drought stress in plants: a review on morphological characteristics and pigments composition. International Journal of Agriculture Biology, **11**,100-105.

Janick J., Whipky A. (2007) (Eds.) Issues in new crops and new uses, ASHS Press,Alexandria,VA.

Jefferies R. (1995) Physiology of crop response to drought. Potato ecology and modelling of crops under conditions limiting growth. Kluwer Academic Publishers, the Netherlands, 61-74.

Jifon J.L., Wolfe D.W. (2002) Photosynthetic acclimation to elevated CO_2 in *Phaseolus vulgaris* L. is altered by growth response to nitrogen supply. Global Change Biology, **8**,1018-1027.

Johnson D., Ball J., Griffin K., Thomas R., Walker R., Strain B., Tissue D. (1998) Effects of carbon dioxide and nitrogen on growth and nitrogen uptake in ponderosa and loblolly pine. Journal of Environmental Quality, **27**, 414-425.

Johnson D.W., Ball T., Walker R.F. (1995) Effects of elevated CO_2 and nitrogen on nutrient uptake in ponderosa pine seedlings. Plant and Soil, **168**, 535-545.

Johnston A.M., Tanaka D.L., Miller P.R., Brandt S.A., Nielsen D.C., Lafond G.P., Riveland N.R. (2002) Oilseed crops for semiarid cropping systems in the northern Great Plains. Agronomy Journal, **94**, 231-240.

Kaffka S.R., Kearney T.E. (1998) (Eds.) Safflower production in California, Communication services, Oakland ,California 94608-1239.

Kanemoto K., Yamashita Y., Ozawa T., Imanishi N., Nguyen N.T., Suwa R., Mohapatra P.K., Kanai S., Moghaieb R.E., Ito J. (2009) Photosynthetic acclimation to elevated CO_2 is dependent on N partitioning and transpiration in soybean. Plant Science, **177**,398-403.

Kang S., Zhang F., Hu X., Zhang J. (2002a) Benefits of CO_2 enrichment on crop plants are modified by soil water status. Plant and Soil, **238**, 69-77.

Kang S., Zhang L., Liang Y., Hu X., Cai H., Gu B. (2002b) Effects of limited irrigation on yield and water use efficiency of winter wheat in the Loess Plateau of China. Agricultural Water Management, **55**, 203-216.

Kar G., Kumar A., Martha M. (2007) Water use efficiency and crop coefficients of dry season oilseed crops. Agricultural Water Management, **87**,73-82.

Kazemeini S., Edalat M., Mohazabieh S., Hejazi A. (2010) Effect of drought stress on yield and yield components of safflower under redroot pigweed densities, Iranian Society of Weed Science, 437-440.

Kendall W.A. (1952) The effect of intermittently varied petiole temperature on carbohydrate translocation from bean leaves. *Plant Physiology,* **27**, 631.

Körner C., Asshoff R., Bignucolo O., Hättenschwiler S., Keel S.G., Peláez-Riedl S.,Pepin S., Siegwolf R.T.W., Zotz G. (2005) Carbon flux and growth in mature

deciduous forest trees exposed to elevated CO_2. Science, **309**,1360-1362.

Kim Y.H., Liffering M.S., Miura, Kobayashi K., Okada M. (2001) Growth and nitrogen uptake of CO_2 enrichment rice under field condition. New Phytologist, **150**, 223-229.

Kim Y.H., Lieffering M., Kobayashi K., Okada M., Mitchell W.M., Gumpertz M. (2003) Effect of free air CO_2 enrichment and nitrogen supply on the yield of temperate paddy rice crops. Field Crop Research, 83.

Kimball B., Morris C., Pinter Jr P., Wall G., Hunsaker D., Adamsen F., LaMorte R., Leavitt S., Thompson T., Matthias A. (2001) Elevated CO_2, drought and soil nitrogen effects on wheat grain quality. New Phytologist, **150**, 295-303.

Kimball B.A., Kobayashi K., Bindi M. (2002) Responses of agricultural crops to free-air CO_2 enrichment, Advances in Agronomy, Academic Press, 293-368.

Knowles P. (1985) Oilseed crop breeding: past and future. Oil Crops: Sesame and Safflower (A. Omran, ed.). Manuscript Report, IDRC-MR,**105**, 199-209.

Knowles P.F. (1976) Evolution of crop plants. Longman Group Ltd.

Koutroubas S.D., Papakosta D.K., Doitsinis A. (2004) Cultivar and seasonal effects on the contribution of pre-anthesis assimilates to safflower yield. Field Crops Research, **90**, 263-274.

Koutroubas S.D., Papakosta D.K., Doitsinis A. (2008) Nitrogen utilization efficiency of safflower hybrids and open -pollinated varieties under Mediterranean condition. Field Crops Research, **107**, 56- 61.

Larigauderie A., Hilbert D.W., Oechel W.C. (1988) Effect of CO_2 enrichment and nitrogen availability on resource acquisition and resource allocation in a grass, *Bromus mollis*. Oecologia, **77**, 544-549.

Lartey R., Caesar-TonThat T., Caesar A., Shelver W., Sol N., Bergman J. (2005) Safflower: a new host of *Cercospora beticola*. Plant Disease, **89**, 797-801.

Latiri-Souki K., Nortcliff S., Lawlor D.W. (1998) Nitrogen fertilizer can increase dry matter, grain production and radiation and water use efficiencies for durum wheat under semi-arid conditions. European Journal of Agronomy, **9**, 21-34.

Lawlor D., Mitchell R. (1991) The effects of increasing CO_2 on crop photosynthesis and productivity: a review of field studies. Plant, Cell and Environment, **14**,807-818.

Lawlor D.W. (1995) Photosynthesis, productivity and environment. Journal of Experimental Botany, **46**,1449.

Lawlor D.W. (2002) Limitation to photosynthesis in water-stressed Leaves: Stomata vs. Metabolism and the role of ATP. Annals of Botany, **89**, 871-885.

Lawlor D.W., Lemaire G., Gastal F. (2001) Nitrogen, plant growth and crop yield. Plant nitrogen, 343-367.

Lea P.J., Francois J., Gaudry M. (2001) (Eds.) Plant nitrogen, Springer verlag, Berlin Heidlberg, INRA Paris.

Leakey A.D.B., Ainsworth E.A., Bernacchi C.J., Rogers A., Long S.P., Ort D.R. (2009) Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. Journal of Experimental Botany, **60**, 2859-2876.

Lee E.H., Pausch R.C., Rowland R.A., Mulchi C. (1997) Responses of field-grown soybean (cv. Essex) to elevated SO_2 under two atmospheric CO_2 concentrations. Environmental and Experimental Botany, **37**:85-93.

Li F., Kang S., Zhang J. (2004) Interactive effects of elevated CO_2, nitrogen and drought on leaf area, stomatal conductance, and evapotranspiration of wheat. Agricultural Water Management,**67**, 221-233.

Lieffering M., Han-Yong, Kobayashi K., Okada M. (2004) The impact of elevated CO_2 on the elemental concentration of field - grown rice grains. Field Crop Research, **88**, 279-286.

Long S. (1991) Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: Has its importance been underestimated? Plant, Cell and Environment, **14**, 729-739.

Long S.P., Ainsworth E.A., Rogers A., Ort D.R. (2004) Rising atmospheric carbon dioxide: plants FACE the Future. Annual Review. Plant Biology, **55**,591-628.

Long S.P., Zhu X.I.N.G., Naidu S.L., Ort D.R. (2006) Can improvement in photosynthesis increase crop yields. Plant, Cell and Environment, **29**, 315-330.

Lu C., Zhang J. (1998) Effects of water stress on photosynthesis, chlorophyll fluorescence and photo inhibition in wheat plants. Functional Plant Biology, **25**,883-892.

Lyon D., Baltensperger D., Sall R., Kerr E. (1991) Growing safflower in Nebraska. Univ. Nebraska, Ext. Neb Fact, 91-36.

Lozovaya V.V., Lygin A.V., Ulanov A.V., Nelson R.L., Daydé J., Widholm J.M.

(2005)Effect of temperature and soil moisture status during seed development on soybeanseed isoflavone concentration and composition. Crop science, **45**, 1934-1940.

Mafakheri A., Siosemardeh A., Bahramnejad B., Struik P., Sohrabi E. (2010) Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Australian Journal of Crop Science, **4**,580-585.

Mall R., Singh R., Gupta A., Srinivasan G., Rathore L. (2006) Impact of climate change on Indian agriculture: A review. Climatic Change, **78**,445-478.

Manderscheid R., Burkart S., Bramm A., Weigel H.J. (2003) Effect of CO_2 enrichment on growth and daily radiation use efficiency of wheat in relation to temperature and growth stage. European Journal of Agronomy, **19**, 411-425.

Manivannan P., Jaleel C.A., Sankar B., Kishorekumar A., Somasundaram R., Lakshmanan G.M.A., Panneerselvam R. (2007) Growth, biochemical modifications and proline metabolism in *Helianthus annuus* L. as induced by drought stress. Colloids and Surfaces B: Bio interfaces, **59**,141-149.

Marco G., Massacci A., Gabrielli R. (1988) Drought effects on photosynthesis and fluorescence in hard wheat cultivars grown in the field. Physiologia Plantarum, **74**, 385-390.

Mardanov A., Samedovam A., Shirvany T. (1998) Root-shoot relationships in plant adaptation to nitrogen deficiency. Developments in Plant and Soil Sciences, **82**,147-154.

Martínez-Carrasco R., Pérez P., Morcuende R. (2005) Interactive effects of elevated CO_2, temperature and nitrogen on photosynthesis of wheat grown

under temperature gradient tunnels. Environmental and Experimental Botany, **54**, 49-59.

Maxwell K., Johnson G.N. (2000) Chlorophyll fluorescence a practical guide. Journal of Experimental Botany, **51**, 659-668.

McDonald A., Davies W. (1996) Keeping in touch: responses of the whole plant to deficits in water and nitrogen supply. Advances in Botanical Research, **22**,229-300.

McDonald E.P., Erickson J.E., Kruger E.L. (2002) Research note: Can decreased transpiration limit plant nitrogen acquisition in elevated CO_2. Functional Plant Biology, **29**,1115-1120.

McKee I.,Woodward F. (1994) CO_2 enrichment responses of wheat: interactions with temperature, nitrate and phosphate. New Phytologist, 447-453.

McPherson M.A., Good A.G., Topinka A.K.C., Hall L.M. (2004) Theoretical hybridization potential of transgenic safflower (*Carthamus tinctorius* L.) with weedy relatives in the New World. Canadian Journal of Plant Science, **84**, 923-934.

Medlyn B., McMurtrie R. (2005) Effects of CO_2 on plants at different timescales. A History of Atmospheric CO_2 and Its Effects on Plants. Animals, and Ecosystems, 441-467.

Medrano H., Escalona J., Bota J., Gulias J., Flexas J. (2002) Regulation of photosynthesis of C_3 plants in response to progressive drought: stomatal conductance as a reference parameter. Annals of Botany, **89**, 895-905.

Miflin B.J., Habash D.Z. (2002) The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in the nitrogen utilization of crops. *Journal of Experimental Botany*, **53**, 979-987

Miladi L.A., Ehsanzadeh P. (2010) The negative effect of drought on safflower grain yield through impact on photosynthetic surfaces and on efficiency. Iranian Journal of Field Crop Science.

Miyashita K., Tanakamaru S., Maitani T., Kimura K. (2005) Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. Environmental and Experimental Botany, **53**, 205-214.

Mokhtassi Bidgoli A., Akbari G.A., Mirhadi M.J., Zand E., Soufizadeh S. (2006) Path analysis of the relationships between seed yield and some morphological and phenological traits in safflower (*Carthamus tinctorius* L.). Euphytica, **148**, 261-268.

Mooney H.A. (1999) Carbon dioxide and environmental stress Academic Press.Sandiego,California.

Morison J., Lawlor D. (1999) Interactions between increasing CO_2 concentration and temperature on plant growth. Plant, Cell and Environment , **22**, 659-682.

Mott K.A. (1988) Do stomata respond to CO_2 concentrations other than intercellular? Plant Physiology, **86**,200.

Mozaffari K., Asadi A.A. (2006) Relationships among traits using correlation, principal components and path analysis in safflower mutants sown in irrigated and drought stress condition. Asian Journal of Plant Sciences, **5**, 977-983.

Mulholland B., Craigon J., Black C., Colls J., Atherton J., Landon G. (1997) Impact of elevated atmospheric CO_2 and O_3 on gas exchange and chlorophyll content in spring wheat (*Triticum aestivum* L.). Journal of Experimental Botany, **48**,1853.

Mwale S., Azam-Ali S., Massawe F. (2007) Growth and development of bambara ground nut in response to soil moisture: 1. Dry matter and yield. European Journal of Agronomy, **26**, 345-353.

Nakano H., Makino A., Mae T. (1997) The effect of elevated partial pressures of CO_2 on the relationship between photosynthetic capacity and N content in rice leaves. Plant Physiology, **115**, 191-198.

Nasser R.R., Fuller M., Jellings A. (2008) Effect of elevated CO_2 and nitrogen levelson lentil growth and nodulation. Agronomy for sustainable development, **28**,175-180.

Nelson G.C. (2009) Climate change: Impact on agriculture and costs of adaptation Intl Food Policy Research Inst.

Nikabadi S., Soleimani A., Dehdashti S., Yazdanibakhsh M. (2008) Effect of sowing dates on yield and yield components of spring safflower (*Carthamus tinctorius* L.) in Isfahan region. Pakistan Journal of Biological Sciences,PJBS, **11**,1953.

Nimbkar N. (2002) Safflower rediscovered. Times Agricultural Journal, **2**, 32-36.

Nimbkar N. (2008) Issues in safflower production in India. Proceeding of the Seventh International Safflower.

Nimbkar N., Singh V. (2005) (Eds.) Safflower (*Carthamus tinctorrius* L.), CRC Press.

Novoa R., Loomis R.S. (1981) Nitrogen and plant production. Plant and Soil, **58**, 177-204.

Oad F.C., Sohu G.N., Qayyum S.M., Gandahi A.W., Chandio G.Q., Oad N.L. (2001) Comparative performance of sunflower varieties in response to different fertility regimes. Pakistan Journal of Applied Science, **1**,397- 399.

Oelke A.E., Oplinger S.E., Teynor M.T., Putnam H.D., Doll D.J., Kelling A.K., Durgan R.B., Noetzel M.D. (1992) Safflower. Alternative Field Crop Manual, University of Wisconsin-Extension, Cooperative Extension.

Olesen J.E., Bindi M. (2002) Consequences of climate change for European agricultural productivity, land use and policy. European Journal of Agronomy,**16**,239-262.

Omidi A., Sharifmogadas M. (2010) Evaluation of Iranian Safflower Cultivars Reaction to Different Sowing Dates and Plant Densities. World Applied Sciences Journal, 8, 953-958.

Omidi A.H., Khazaei H., Hongbo S. (2009) Variation for some important agronomic Traits in 100 Spring Safflower (*Carthamus tinctorius* L.) Genotypes. American-Eurasian Journal of Agricultural and Environmental Science, **5**, 791-795.

Öztürk E., Özer H., Polat T. (2008) Growth and yield of safflower genotypes grown under irrigated and non-irrigated conditions in a highland environment. Plant Soil Environment, **54**, 453-460.

Pankovic D., Sakac Z., Kevresan S., Plesuica M. (1999) Acclimation to long term water deficit in the leaves of two sunflower hybrids: photosynthesis, electron transport and carbon metabolism. Journal of Experimental Botany, **50**,127-138.

Pasban Eslam B., Sadeqi A.T. (2008) Evaluation of physiological and agronomic characteristics of spring safflower genotypes for terminal drought tolerance. Tabriz, Iran.

Pastenes C., Pimentel P., Lillo J. (2005) Leaf movements and photo inhibition in relation to water stress in field-grown beans. Journal of Experimental Botany, **56**, 425-433.

Patil V., Reddy M., Nerkar Y. (1994) Efficiency of early generation selections for yield and related characters in safflower (*Carthamus tinctorius* L.). TAG Theoretical and Applied Genetics, **89**, 293-296.

Pennisi E. (2005) Sky-High Experiments. *Science,***309**, 1314-1315. DOI: 10.1126/science.309.5739.1314.

Pettersson R., McDonald A.J.S. (1994) Effects of nitrogen supply on the acclimation of photosynthesis to elevated CO_2. Photosynthesis Research, **39**,389-400.

Pinter Jr P.J., Kimball B.A., Garcia R.L., Wall G.W., Hunsaker D.J., LaMorte R.L. (1996) Free-air CO_2 enrichment: responses of cotton and wheat crops Academic Press, San Diego, CA, USA.

Polly,W.H. (2002) Implication of atmospheric and climatic change for yield and water use efficiency. Crop Science, **42**, 131-140.

Poorter H. (1993) Interspecific variation in the growth response of plants to an elevated ambient CO_2 concentration. Plant Ecology, **104**, 77-97.

Poorter H., Perez-Soba M. (2002) Plant growth at elevated CO_2. The Earth system: biological and ecological dimensions of global environmental change, **2**, 489-496.

Poorter H., Navas M.L. (2003) Plant growth and competition at elevated CO_2: on winners, losers and functional groups. New Phytologist,**157**,175-198.

Porra R.J. (2002) The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. Photosynthesis Research, **73**,149-156.

Pospisilova J., Catsky. (1999) Development of water stress under increased atmospheric CO2 concentration. Biologia Plantarum, **42**, 1-24.

Poulson M.E., Boeger M.R.T., Donahue R.A. (2006) Response of photosynthesis to high light and drought for *Arabidopsis thaliana* grown under a UV-B enhanced light regime. Journal of Photosynthetica Research, **90**, 79-90.

Prasad P., Staggenborg S., Ristic Z. (2008) Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. Response of crops to limited water: understanding and modelling water stress effects on plant growth processes. American Society of Agronomy/Crop Science Society of America/Soil Science Society of America, Madison, WI, 301-356.

Prasad P., Boote K.J., Allen Jr L.H., Thomas J.M.G. (2002) Effects of elevated temperature and carbon dioxide on seed - set and yield of kidney bean (*Phaseolus vulgaris* L.). Global Change Biology, **8**, 710-721.

Prasad P.V.V., Allen Jr L., Boote K. (2005) Crop responses to elevated carbon dioxide and interaction with temperature: grain legumes. Journal of Crop Improvement, **13**, 1-2.

Prasad P.V.V., Boote K.J., Allen Jr L.H., Thomas J.M.G. (2003) Super-optimal temperatures are detrimental to peanut (*Arachis hypogaea* L.) reproductive processes and yield at both ambient and elevated carbon dioxide. Global Change Biology, **9**,1775-1787.

Prieto P., Penuelas J., Llusia J., Asensio D., Estiarte M. (2009) Effects of long-term experimental night-time warming and drought on photosynthesis, Fv/Fm and stomatal conductance in the dominant species of a Mediterranean shrubland. Acta Physiologiae Plantarum, **31**, 729-739.

Prior S., Runion G., Rogers H., Arriaga F. (2010) Elevated Atmospheric Carbon Dioxide Effects on Soybean and Sorghum Gas Exchange in Conventional and No-Tillage Systems.

Quiroga A.R., Díaz-Zorita M., Buschiazzo D.E. (2001) Safflower productivity as related to soil water storage and management practices in semiarid regions. Communications in Soil science and Plant analysis, **32**, 2851-2862.

Radoglou K., Jarvis P. (1992) The effects of CO_2 enrichment and nutrient supply on growth morphology and anatomy of *Phaseolus vulgaris* L. seedlings. Annals of Botany, **70**, 245-256.

Radoglou K., Aphalo P., Jarvis P. (1992) Response of photosynthesis, stomatal conductance and water use efficiency to elevated CO_2 and nutrient supply in acclimated seedlings of *Phaseolus vulgaris* L. Annals of Botany, **70**,257-264.

Rathke G.W., Christen O., Diepenbrock W. (2005) Effects of nitrogen source and rate on productivity and quality of winter oilseed rape (*Brassica napus* L.) grown in different crop rotations. Field Crops Research, **94**,103-113.

Rathke G.W., Behrens T., Diepenbrock W. (2006) Integrated nitrogen Management strategies to improve seed yield, oil content and nitrogen efficiency of winter oilseed rape (*Brassica napus* L.): A review. Agriculture Ecosystem and Environment, **117**,80-108.

Reddy A.R., Rasineni G.K., Raghavendra A.S. (2010) The impact of global elevated CO_2 concentration on photosynthesis and plant productivity. Current Science, **99**, 46-54.

Reddy G.K.M., Dangi G.K.M., Kumar S.S., Reddy.A.V. (2001) Effect of moisture stress on seed yield and quality in sunflower (*Helianthus annuus* L.). Journal of Oil Seeds Research, **20**, 282-283.

Reddy K., Robana R., Hodges H.F., Liu X., McKinion J.M. (1998) Interactions of CO_2 enrichment and temperature on cotton growth and leaf characteristics. Environmental and Experimental Botany, **39**, 117-129.

Reddy K.R., Koti S., Davidonis G.H., Reddy V.R. (2004) Interactive effects of carbon dioxide and nitrogen nutrition on cotton growth, development, yield, and fibre quality. Agronomy Journal, **96**, 1148-1157.

Reddy V., Reddy K., Hodges H. (1995) Carbon dioxide enrichment and temperature effects on cotton canopy photosynthesis, transpiration, and water-use efficiency 1. Field Crops Research, **41**, 13-23.

Rerkasem B., Netsangtip R., Lordkaew S., Cheng C. (1993) Grain set failure in boron deficient wheat. Plant and Soil, **155**, 309-312.

Röbbelen S., Downey R.K., Ashri A. (1989) Oilcrops of the world: their breeding and utilization McGraw-Hill Publishing company. CAB Direct.

Rodriguez D., Ewert F., Goudriaan J., Manderscheid R., Burkart S., Weigel H. (2001) Modelling the response of wheat canopy assimilation to atmospheric CO_2 concentrations. New Phytologist, **150**, 337-346.

Rogers A., Humphries S.W. (2000) A mechanistic evaluation of photosynthetic acclimation at elevated CO_2. Global Change Biology, **6**, 1005-1011.

Rogers A., Fischer B.U., Bryant J., Frehner M., Blum H., Raines C.A., Long S.P. (1998) Acclimation of Photosynthesis to Elevated CO2under Low-Nitrogen Nutrition Is Affected by the Capacity for Assimilate Utilization. Perennial Ryegrass under Free-Air CO_2 Enrichment. Plant Physiology, **118**, 683-689.

Rogers G., Milham P., Thibaud M., Conroy J. (1996a) Interactions between rising CO2 concentration and nitrogen supply in cotton. I. Growth and leaf nitrogen concentration. Functional Plant Biology, **23**, 119-125.

Rogers G., Milham P., Gillings M., Conroy J. (1996b) Sink strength may be the key to growth and nitrogen responses in N-deficient wheat at elevated CO_2. Functional Plant Biology, **23**, 253-264.

Rogers H.H., Dahlman R.C. (1993) Crop responses to CO_2 enrichment. Vegetatio, 104/105, 117-131.

Rogers H.H., Cure J.D., Smith J.M. (1986) Soybean growth and yield response to elevated carbon dioxide. Agriculture, Ecosystems and Environment, **16**, 113-128.

Rogers H.H., Runion B.G., Stephan A.P., Torbert A.A. (1999) (Eds.) Response of plant to elevated atmospheric CO_2: Root growth, Mineral nutrition, and soil Carbon. Ademic press, San Diego, California 92101-4495,USA.

Rogers H.H., Sionit N., Cure J.D., Smith J.M., Bingham G.E. (1984) Influence of elevated carbon dioxide on water relations of soybeans. Plant Physiology, **74**, 233.

Romano B., Frenguelli G., Ferranti F. (1984) Changes in protein and carbohydratecontent during development of seeds and siliquas of rapeseed (*Brassica napus* L.).*Plant Biosystem*,**118**, 137-145.

Rosenzweig C., Liverman D. (1992) Predicted effects of climate change on agriculture: A comparison of temperate and tropical regions. Global Climate Change: Implications, Challenges and Mitigation Measures. Philadelphia: Pennsylvania Academy of Sciences. 342-361.

Rudmann S.G., Milham P.J., Conroy J.P. (2001) Influence of High CO_2 Partial Pressure on Nitrogen Use Efficiency of the C4 Grasses *Panicum coloratum* and *Cenchrus ciliaris*. Annals of Botany, **88**, 571-577.

Sage R.F. (1994) Acclimation of photosynthesis to increasing atmospheric CO_2: the gas exchange perspective. Photosynthesis Research , **39**,351-368.

Sage R.F., Pearcy R.W. (1987) The nitrogen use efficiency of C3 and C4 plants: II. leaf nitrogen effects on the gas exchange characteristics of *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). Plant Physiology, **84**, 959.

Samancı B., Özkaynak E. (2003) Effect of Planting Date on Seed Yield, Oil Content and Fatty Acid Composition of Safflower (*Carthamus tinctorius*) Cultivars Grown in the Mediterranean Region of Turkey. Journal of Agronomy and Crop Science, **189**, 359-360.

Schenk M. (1996) Regulation of nitrogen uptake on the whole plant level. Plant andSoil , **18**, 131-137.

Sanz-Sáez Á., Erice G., Aranjuelo I., Nogués S., Irigoyen J.J., Sánchez-Díaz M. (2010) Photosynthetic down-regulation under elevated CO_2 exposure can be prevented by nitrogen supply in nodulated alfalfa. Journal of plant physiology, **167**, 1558-1565.

Sardans J., Peñuelas J., Prieto P., Estiarte M. (2008) Drought and warming induced changes in P and K concentration and accumulation in plant biomass and soil in a Mediterranean shrubland. Plant and Soil, **306**, 261-271.

Sator C. (1999) Oilseed rape grown in different gaseous environments. 10th International Rapeseed Congress. Canberra, Australia.

Schenk U., Jager H.J., Weigel H.J. (1997) The response of perennial ryegrass/white clover swards to elevated atmospheric CO_2 concentrations. 1. Effects on competition and species composition and interaction with N supply. New Phytologist, 67-79.

Schlesinger W.H., Reynolds J.F., Cunningham G.L., Huenneke L.F., Jarrell W.M., Virginia R.A., Whitford W.G. (1990) Biological feedbacks in global desertification. Science, **247**, 1043-1048.

Sehgal D., Raina S.N. (2011) Carthamus. Wild Crop Relatives: Genomic and Breeding Resources, 63-95.

Seneweera S., Norton R. (2011) Plant Responses to Increased Carbon Dioxide. Crop Adaptation to Climate Change, 198-217.

Shah N., Paulsen G. (2003) Interaction of drought and high temperature on photosynthesis and grain-filling of wheat. Plant and Soil, **257**,219-226.

Shangguan Z., Shao M., Dyckmans J. (2000a) Effects of nitrogen nutrition and water deficit on net photosynthetic rate and chlorophyll fluorescence in winter wheat. Journal of Plant Physiology, **156**, 46-51.

Shangguan Z.P. (1997) Regulation of nitrogen nutrition on photosynthetic characteristics of winter wheat in dryland. Plant Nutrition and Fertilizer Science, 3, 105-110.

Shangguan Z.P., Shao M.A., Dyckmans J. (2000b) Nitrogen nutrition and water stress effects on leaf photosynthetic gas exchange and water use efficiency in winter wheat. Environmental and Experimental Botany, **44**, 141-149.

Sharghi Y., Bagheri H. (2011) The Study of Biological Yield, Grain Yield and Oil Yield in Spring Safflower Varieties Under of Condition Water Streets. Research Journal of Fisheries and Hydrobiology, **6**, 563-566.

Sharrifmoghaddasi M., Omidi A. (2010) Study of interrupting irrigation effects at different growth stages on grain and oil yields of new safflower varieties. Advances in Environmental Biology, 4, 387-391.

Shaw M.R., Huxman T.E., Lund C.P. (2005) Modern and future semi-arid and arid ecosystems, in: J. R. Ehleringer, et al. (Eds.), A history of atmospheric CO_2 and its effects on plants, animals, and ecosystems, Springer Science + Business Media, Inc., New York, USA.

Shi W., Wang S., Yang Q. (2010) Climate change and global warming. Reviews in Environmental Science and Biotechnology, **9**, 99-102.

Siddiqui M., Oad F. (2006) Nitrogen requirement of safflower (*Carthamus tinctorius* L.) for growth and yield traits. Asian Journal of Plant Sciences, **5**,563-565.

Simpson, Ogorzaly. (2001) (Eds.) Economic botany third edition plants in our world, megraw

Sims A.D., Cheng W., Luo Y., Seeman R.j. (1999) Photosynthetheic acclimation to elevated CO2 in aSunfflower canopy. Experimental botany, **50**, 645-653.

Sinaki J.M., Heravan E.M., ShiraniRad A.H., Noormohammadi G., Zarei G. (2007) The effect of water deficit during growth stage of canola (*Brassica napus* L.). American Eurasian journal Agriculture and Environment Science, **2**, 417-422.

Sinclair T.R. (1998) Historical changes in harvest index and crop nitrogen accumulation. Crop science, 38, 638- 643.

Singh V., Nimbkar N. (2006) Safflower (*Carthamus tinctorius* L.). Chapter, 6, 167-194.

Singh V., Nimbkar N., Rajvanshi A., Bergman J., Henning Mündel H. (2001) Safflower research & development at Nimbkar Agricultural Research Institute (NARI), Department of Plant Pathology, North Dakota State University. 117-121.

Sinha P.G., Saradhi P.P., Uprety D.C., Bhatnagar A.K. (2011) Effect of elevated CO_2 concentration on photosynthesis and flowering in three wheat species belonging to different ploidies. Agriculture, Ecosystems and Environment.

SionitI N., Strain B., Flint E. (1987) Interaction of temperature and CO_2 enrichment on soybean: growth and dry matter partitioning. Canadian Journal of Plant Science, **67**, 59-67.

Smith H.V., Jimmerson J. (2005) Safflower. Brieffing No. 58 in Agriculture Marketing / policy center.

Smith R.J. (1996) (Eds.) Safflower, United State of America with vegetable oil - based inks. AOCS press.

Soleimani R. (2010) Variability of grian and oil yield in spring safflower as affected by nitrogen application. Journal of Plant Nutrition, **33**,1744-1750.

Souza R.P., Machado E.C., Silva J.A.B., Lagôa A.M.M.A., Silveira J.A.G. (2004) Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (*Vigna unguiculata*) during water stress and recovery. Environmental and Experimental Botany, **51**, 45-56.

Springer C.J., Ward J.K. (2007) Flowering time and elevated atmospheric CO_2. New Phytologist, **176**, 243-255.

Starner D.E., Hamama A., A, Bhardwaj H.L. (1999) (Eds.) Perspective on new crops and new uses,, ASHS press, Alexandra.

Steer B., Harrigan E. (1986) Rates of nitrogen supply during different developmental stages affect yield components of safflower (*Carthamus tinctorius* L.). Field Crops Research, **14**,221-231.

Steer B.T., Seiler G.J. (1990) Changes in fatty acid composition of sunflower (Helianthus annuus) seeds in response to time of nitrogen application, supply rates and defoliation. Journal of the Science of Food and Agriculture, **51**, 11-26.

Stitt M., Krapp A. (1999) The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. Plant, Cell and Environment, **22**, 583-621.

Strasil Z., Vorlicek Z. (2002) The effect of nitrogen fertilizer, sowing rates and site on yields and yield components of selected varieties of safflower (*Carthamus tinctorius* L.). Rostlinna vyroba, **48**, 307 - 311.

Streck N.A. (2005) Climate change and agro ecosystems: the effect of elevated atmospheric CO_2 and temperature on crop growth, development, and yield. Ciência Rural, **35**,730-740.

Stulen I., Hertog J. (1993) Root growth and functioning under atmospheric CO_2 enrichment. Plant Ecology, **104**, 99-115.

Subrahmanyam D., Subash N., Haris A., Sikka A. (2006) Influence of water stress on leaf photosynthetic characteristics in wheat cultivars differing in their susceptibility to drought. Photosynthetica, **44**, 125-129.

Svecnjak Z., Rengel Z. (2006) Canola cultivars differ in nitrogen utilization efficiency at vegetative stage. Field Crops Research, **97**, 221-226.

Taiz, Zeiger. (2002) (Eds.) Plant Physiology .Third Edition, Sinaur Association, Sunderland, MA, USA.

Taub D.R., Wang X. (2008) Why are nitrogen concentrations in plant tissues lower under elevated CO2? A critical examination of the hypotheses. Journal of Integrative Plant Biology, **50**,1365-1374.

Taub D.R., Miller B., Allen H. (2008) Effects of elevated CO_2 on the protein concentration of food crops: a meta-analysis. Global Change Biology, 1**4**, 565-575.

Tezara W., Fernández M., Donoso C., Herrera A. (1998) Seasonal changes in photosynthesis and stomatal conductance of five plant species from a semiarid ecosystem. Photosynthetica, **35**, 399-410.

Thompson G., Woodward F. (1994) Some influences of CO_2 enrichment, nitrogen nutrition and competition on grain yield and quality in spring wheat and barley. Journal of Experimental Botany, **45**, 937-942.

Tóth V.R., Mészáros I., Veres S., Nagy J. (2002) Effects of the available nitrogen on the photosynthetic activity and xanthophyll cycle pool of maize in field. Journal of Plant Physiology, **159**, 627-634.

Tuncturk M., Yildirim B. (2004) Effects of different forms and doses of nitrogen fertilizers on safflower (*Charthamus tinctorius* L.). Pakistan Journal of Biological Sciences, **7**,1385-1389.

Tuncturk M., Çiftçi V. (2007) Relationships between yield and some yield components in rapeseed (*Brassica napus* ssp. oleifera L.) cultivars by using correlation and path analysis. Pakistan Journal of Botany, 39, 81.

Uslu N. (2003) Safflower seed quality response to sowing date and head position. Sesame and Safflower. Newsletter.107-110.

Vara Prasad P., Boote K.J., Hartwell Allen Jr L., Thomas J.M.G. (2003) Super‐optimal temperatures are detrimental to peanut (*Arachis hypogaea* L.) reproductive processes and yield at both ambient and elevated carbon dioxide. Global Change Biology, **9**, 1775-1787.

Vernon L.P. (1960) Spectrophotometric determination of chlorophylls and pheophytins in plant extracts. Analytical chemistry, **32**, 1144-1150.

Vu J.C.V. (2005) Acclimation of peanut (*Arachis hypogaea* L.) leaf photosynthesis to elevated growth CO_2 and temperature. Environmental and Experimental Botany, **53**, 85-95.

Vu J.C.V., Allen Jr L.H., Bowes G. (1989) Leaf ultrastructure, carbohydrates and protein of soybeans grown under CO_2 enrichment. Environmental and Experimental Botany, **29**, 141-147.

Vu J.C.V., Gesch R.W., Pennanen A.H., Allen Hartwell L. (2001) Soybean photosynthesis, Rubisco, and carbohydrate enzymes function at supra optimal temperatures in elevated CO_2. Journal of plant physiology, **158**, 295-307.

Wang W., Vinocur B., Altman A. (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta, **218**, 1-14.

Warren C.R., Adams M.A., Chen Z.L. (2000) Is photosynthesis related to concentrations of nitrogen and Rubisco in leaves of Australian native plants? Functional Plant Biology, **27**,407-416.

Wheeler R.M., M ackowiak C.L., Yorio N.C., Sager J.C. (1999) Effects of CO_2 on Stomatal Conductance: Do Stomata Open at Very High CO_2 Concentrations? Annals of Botany, **83**, 243.

Wheeler T., Batts G., Ellis R., Hadley P., Morison J. (1996a) Growth and yield of winter wheat (Triticum aestivum) crops in response to CO_2 and temperature. The Journal of Agricultural Science, **127**, 37-48.

Wheeler T., Hong T., Ellis R., Batts G., Morison J., Hadley P. (1996b) The duration and rate of grain growth, and harvest index, of wheat (*Triticum aestivum* L.) in response to temperature and CO_2. Journal of Experimental Botany, **47**, 623.

Williams M., Shewry P., Lawlor D., Harwood J. (1995) The effects of elevated temperature and atmospheric carbon dioxide concentration on the quality of grain lipids in wheat (*Triticum aestivum* L.) grown at two levels of nitrogen application. Plant, Cell and Environment , **18**, 999-1009.

Wolfe D.W., Gifford R.M., Hilbert D., Luo Y. (1998) Integration of photosynthetic acclimation to CO2 at the whole‐plant level. Global Change Biology, **4**, 879-893.

Wong S., Osmond C. (1991) Elevated Atmosphere Partial Pressure of CO_2 and Plant Growth. III. Interactions Between *Triticum aestivum* (C_3) and Echinochloa frumentacea (C_4) During Growth in Mixed Culture Under Different CO_2, N Nutrition and Irradiance Treatments, With Emphasis on Below-Ground

Responses Estimated Using the δ13C Value of Root Biomass. Functional Plant Biology, **18**,137-152.

Wright G.C., Hammer L.G. (1996) Distribution of nitrogen and radiation use efficiency in peanut canopies. Australian Journal of Agricultural Research, **45**, 565 - 574.

Wu D.X., Wang G.X., Bai Y.F., Liao J.X. (2004) Effects of elevated CO_2 concentration on growth, water use, yield and grain quality of wheat under two soil water levels. Agriculture, Ecosystems and Environment, **104**, 493-507.

Xu Z.Z., Zhou G.S. (2006) Combined effects of water stress and high temperature on photosynthesis, nitrogen metabolism and lipid peroxidation of a perennial grass *Leymus chinensis*. Planta, **224**, 1080-1090.

Yang L., Huang J., Yang H., Dong G., Liu G., Zhu J., Wang Y. (2006a) Seasonal changes in the effects of free-air CO_2 enrichment (FACE) on dry matter production and distribution of rice (*Oryza sativa* L.). Field Crops Research, **98**, 12-19.

Yang L., Huang J., Yang H., Zhu J., Liu H., Dong G., Liu G., Han Y., Wang Y. (2006b) The impact of free-air CO_2 enrichment (FACE) and N supply on yield formation of rice crops with large panicle. Field Crops Research, **98**, 141-150.

Yang L., Wang Y., Dong G., Gu H., Huang J., Zhu J., Yang H., Liu G., Han Y. (2007) The impact of free-air CO_2 enrichment (FACE) and nitrogen supply on grain quality of rice. Field Crops Research, **102**, 128-140.

Yau S. (2007) Winter versus spring sowing of rain-fed safflower in a semi-arid, high-elevation Mediterranean environment. European Journal of Agronomy, **26**,249-256.

Yau S.K., Ryan J. (2010) Response of rainfed safflower to nitrogen fertilization under Mediterranean conditions. Industrial Crops and Products.

Yoon S., Hoogenboom G., Flitcroft I., Bannayan M. (2009) Growth and development of cotton (*Gossypium hirsutum* L.) in response to CO_2 enrichment under two different temperature regimes. Environmental and Experimental Botany, **67**,178-187.

Yordanov I., Velikova V., Tsonev T. (2000) Plant responses to drought, acclimation, and stress tolerance. Photosynthetica, **38**, 171-186.

Yordanov I., Tsonev T., Merakchiiska-Nikolova M., Georgieva K., Goltsev V. (1997) Gas exchange and chlorophyll fluorescence during water and high temperature stresses and recovery. Probable protective effect of carbamide cytokinin 4-PU30. Photosynthetica, 33.

Yuan J.S., Himanen S.J., Holopainen J.K., Chen F., Stewart Jr C.N. (2009) Smelling global climate change: mitigation of function for plant volatile organic compounds. Trends in Ecology and Evolution, **24**,323-331.

Yuelin J., Qingguo Z., Wei Y., Yugang Y., Gongming W. (2005) Effects of Elevated Atmospheric CO2 Concentration on Growth and Yield of Soybean [J]. Chinese Agricultural Science Bulletin, **21**, 355-355.

Zaman A. (1988) Effect of N,P and K fertilizers on seed yield oil content of safflower in drought prone lateritic tract of West Bengal. Oil seeds Research, 179- 182.

Zaman A., Das P. (1990) Response of safflower to different moisture regimes and nitrogen levels in semi-arid tropics. Oilseed Research, **7**, 26-32.

Zerihun A., Gutschick V.P., Bassirirad H. (2000) Compensatory roles of nitrogen uptake and photosynthetic N-use efficiency in determining plant growth response to elevated CO2: evaluation using a functional balance model. Annals of Botany, **86**, 723-730.

Zhao D., Reddy K.R., Kakani V.G., Reddy V.R. (2005) Nitrogen deficiency effects on plant growth, leaf photosynthesis, and hyperspectral reflectance properties of sorghum. European Journal of Agronomy, **22**, 391-403.

Zheljazkov V.D., Vick B., Ebelhar M.W., Buehring N., Astatkie T. (2012) Nitrogen applications modify seed and oil yields and fatty acid composition of winter mustard. Industrial Crops and Products, **36**, 28-32.

Zimmerman L. (1973) Effect of photoperiod and temperature on rosette habit in safflower. Crop Science, **13**, 80-81.

Ziska L., Weerakoon W., Namuco O., Pamplona R. (1996) The influence of nitrogen on the elevated CO_2 response in field-grown rice. Functional Plant Biology, **23**,45-52.