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# Encapsulation of cauliflower (*Brassica oleracea* var *botrytis*) microshoots as artificial seeds and their conversion and growth in commercial substrates

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**Abstract**

An effective protocol for the mass production of cauliflower microshoots was refined using the meristematic layer of cauliflower curd. After the meristematic layer was surface sterilized and shaved off, a commercial blender was used for homogenization and several blending treatments were tested in the range 15–120 s and 30 s was found to be optimal in terms of the amount explants produced and their subsequent growth ability. Explants were cultivated in S23 liquid medium ( $4.4 \text{ g L}^{-1}$  MS (Murashige and Skoog) and 3% v/w sucrose) supplemented with several combinations of plant growth regulators (PGRs) including 1 and  $2 \text{ mg L}^{-1}$  of kinetin in combination with three types of auxins (indole butyric acid (IBA), Naphthaleneacetic acid (NAA) and Indole-3-acetic acid (IAA)), each at 1 and  $2 \text{ mg L}^{-1}$  concentration. The use of  $2 \text{ mg L}^{-1}$  kinetin and  $1 \text{ mg L}^{-1}$  IBA gave the best results in terms of its effects on explant induction. Microshoots of different sizes were encapsulated in a sodium alginate matrix and the optimal stage suitable for the production of artificial seeds was assessed in terms of both subsequent conversion and plantlet viability. The feasibility of cultivating cauliflower artificial seeds in commercial substrates (compost, vermiculite, perlite and sand) irrigated with different solution mixtures including sterilized distilled water (SDW), PGRs-free S23 medium and S23 medium supplemented with kinetin ( $1$  and  $2 \text{ mg L}^{-1}$ ) and IBA or NAA at ( $1$  and  $2 \text{ mg L}^{-1}$ ) was investigated. The use of  $2 \text{ mg L}^{-1}$  kinetin and  $2 \text{ mg L}^{-1}$  NAA applied with S23 gave the optimal response with both perlite and compost. This study showed high growth capacity of cauliflower artificial seeds in commercial substrates which is considered a promising step for their direct use in vivo.

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**Keywords (separated by '-')** Sodium alginate - Encapsulation - Curd - Meristems - Artificial seeds

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2 **Encapsulation of cauliflower (*Brassica oleraceae* var *botrytis*)**  
3 **microshoots as artificial seeds and their conversion and growth**  
4 **in commercial substrates**

5 **Hail Rihan · Mohammed Al-Issawi ·**  
6 **Stephen Burchett · Michael P. Fuller**

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36 2 mg L<sup>-1</sup> kinetin and 2 mg L<sup>-1</sup> NAA applied with S23  
37 gave the optimal response with both perlite and compost.

This study showed high growth capacity of cauliflower 38  
artificial seeds in commercial substrates which is consid- 39  
ered a promising step for their direct use in vivo. 40

**Keywords** Sodium alginate · Encapsulation · Curd · 42  
Meristems · Artificial seeds 43

**Introduction** 44

The outermost layer of cauliflower curd is made of several 45  
million meristems (Kieffer et al. 2001) and the use of curd 46  
meristematic tissue for in vitro culture has been investi- 47  
gated for micropropagation and the production of virus free 48  
plants (Grout and Crisp 1977; Kumar et al. 1993). The use 49  
of the meristematic layer of cauliflower curd is considered 50  
to be superior compared with the use of conventional 51  
protocols using seedling or leaf explants which have been 52  
found to be labour intensive (Pow 1969; Kumar et al. 53  
1993). Kieffer et al. (2001) designed an effective protocol 54  
for the production of cauliflower propagules from frac- 55  
tionated and graded curd and these propagules were sug- 56  
gested to be suitable for encapsulation in sodium alginate 57  
matrices for the production of artificial seeds. The tech- 58  
nique of artificial seed has been widely studied and works 59  
with various plant species including fruits, cereals, 60  
medicinal plants, vegetables, ornamentals, forest trees and 61  
orchids (Germanà et al. 2011, Sundararaj et al. 2010, Singh 62  
et al. 2009, Rai et al. 2008, Pintos et al. 2008, Micheli et al. 63  
2007, Antonietta et al. 2007, Naik and Chand 2006, Mal- 64  
abadi and Staden 2005, Chand and Singh 2004, Nyende 65  
et al. 2003, Mandal et al. 2000). The use of microshoots has 66  
been widely reported for the production of artificial seeds 67  
in different plant species such as *Cineraria maritime* 68  
(Sandoval-Yugar et al. 2009), *Musa* sp. (Sandoval-Yugar 69

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70 et al. 2009), *Gerbera jamesonii* (Taha et al. 2009) and  
71 *Picrorhiza kurrooa* (Mishra et al. 2010).

72 Cauliflower is an open pollinated species and there are  
73 technical challenges to producing in-bred lines with reli-  
74 able self-incompatibility or male sterility for producing F1  
75 hybrids particularly amongst the winter-heading maturity  
76 sub-group. The production of cauliflower clones multiplied  
77 by tissue culture and distributed as artificial seeds could be  
78 useful alternative to F1 hybrids varieties and could also be  
79 useful in the context of the maintenance of elite clonal  
80 germplasm in cauliflower breeding programmes. The cur-  
81 rent study aimed to further optimize the production of  
82 cauliflower propagules and to investigate the ability of  
83 using cauliflower microshoots for the production of artifi-  
84 cial seeds. Moreover, the investigation of cauliflower artifi-  
85 cial seeds capacity to be grown on in commercial  
86 substrates was one of the main aims of this study.

## 87 Materials and methods

### 88 Plant materials

89 Three F1 hybrid varieties of cauliflower Clemen, Mascaret  
90 and Broden previously found to be equally responsive in  
91 tissue culture were used. Plants were obtained from the  
92 field in Cornwall, courtesy of Simmonds & Sons Ltd, and  
93 replanted in raised beds at the University of Plymouth. The  
94 plants were grown according to good commercial practice  
95 and raised to maturity when the curds were harvested and  
96 stored at 2–4°C until required. The use of 3 varieties gave a  
97 continuous supply of cauliflower heads over the experi-  
98 mental period.

### 99 Cauliflower microshoot production

100 Large pieces (1–5 cm) of cauliflower curds were sterilized  
101 by immersion in 10% by volume un-thickened domestic  
102 bleach (0.06% sodium hypochlorite) for 15 min followed  
103 by a double wash with sterile distilled water. Explants were  
104 produced mechanically by eliminating the mass of non-  
105 responsive tissue (stem branches) and shaving off the upper  
106 meristematic layer using a sterilized scalpel working in a  
107 laminar flow cabinet. The meristematic clusters were then  
108 homogenized using a commercial blender (Waring model  
109 800) at approximately 1,700 rev min<sup>-1</sup> in maintenance S23  
110 (4.4 g L<sup>-1</sup> Sigma<sup>TM</sup> MS (Murashige and Skoog 1962) and  
111 3% w/v sucrose) liquid media. Eight blending durations in  
112 the range 15–120 s were assessed in terms of their effects  
113 on the amount and subsequent growth of the micro-  
114 explants produced after sieving through precision sieves  
115 (212, 300 and 600 µm) (Endecotts Ltd, London, UK). The  
116 micro-explants were collected off the sieves, weighed and

converted to aliquots of explants using small precision 117  
volumetric measures (74 or 240 ± 2 µL). Six containers 118  
(150 mL plastic pots), each containing 30 mL liquid S23 119  
culture medium supplemented with 2 mg L<sup>-1</sup> Kinetin and 120  
1 mg L<sup>-1</sup> IBA, were cultured with a constant volume of 121  
micro-explants (74 µL) and used with every blending 122  
duration in order to assess the effects of blending treat- 123  
ments. The pots were constantly shaken (150 rev min<sup>-1</sup>) 124  
during culture at 20°C and exposed to 16 h photoperiod. 125  
The proportion of micro-explants having meristematic 126  
domes was determined by taking 4 mL samples of cultures 127  
at 8 days from each pot and observing them under a zoom 128  
binocular microscope (Nikon SMZ-2T). The growth ability 129  
of micro-explants during culture was determined after 130  
20 days as the number and fresh weight of microshoots 131  
produced. 132

The effects of several PGR combinations were evaluated 133  
in terms of their ability to induce micro-explant develop- 134  
ment. Nine PGR combinations consisting of various combi- 135  
nation of the cytokinin (Kinetin) (1 and 2 mg L<sup>-1</sup>) with 136  
the auxins IBA (1 and 2 mg L<sup>-1</sup>) or NAA (1 and 2 mg 137  
L<sup>-1</sup>) were evaluated in the first stage and another 4 combi- 138  
nations consisting of various combinations of Kinetin 139  
(1 and 2 mg L<sup>-1</sup>) with IAA (1 and 2 mg L<sup>-1</sup>) compared 140  
with the use of Kinetin (2 mg L<sup>-1</sup>) and IBA (1 mg L<sup>-1</sup>) 141  
were evaluated in the second stage. Four containers, each 142  
with 30 mL of culture medium, were cultivated with a 143  
constant volume of explants (74 µL) of the 300–425 µm 144  
explant size class and used with every treatment. In order 145  
to preserve culture sterility the culture media was supple- 146  
mented 1 mL L<sup>-1</sup> PPM<sup>TM</sup> (Plant Preservative Mixture) 147  
and used with all the treatments. 148

Microshoot development was assessed from the 2 mg 149  
L<sup>-1</sup> Kinetin, 1 mg L<sup>-1</sup> IBA and 1 mL L<sup>-1</sup> PPM treatment 150  
cultivated with 74 µL of micro-explants from each of the 151  
two size class 212–300 µm and 300–600 µm. Random 152  
microshoot samples (n = 15) were taken from each culture 153  
every 3 days and their length measured under a zoom 154  
binocular microscope. Measurements commenced when 155  
the microshoots were 5 days old and continued until 156  
20 days old. 157

### Cauliflower artificial seed production 158

Micro-explants of the 212–300 µm size class were used for 159  
artificial seed production. Microshoots were mixed with 160  
sterilized (by tyndallisation) sodium alginate 2% (w/v) and 161  
dropped into a sterilized (autoclaved) solution of calcium 162  
chloride 15 g L<sup>-1</sup> using a sterilized pipette. Microshoots 163  
were left in the calcium chloride for 30 min for full com- 164  
plexion. The artificial seeds were then transferred to a S23 165  
liquid media (without PGRs) for 30 min followed by a 166  
quick wash with sterile distilled water. The optimal age 167

168 suitable for microshoot encapsulation was determined for  
 169 9–15 day-old microshoots after which the microshoots were  
 170 too big to be encapsulated. Five replications of 10 artificial  
 171 seeds, were cultivated in plastic pots (10 × 10 × 8 cm)  
 172 containing 75 mL of semi-solid S23 supplemented with  
 173 2 mg L<sup>-1</sup> IBA. The conversion rate and fresh weight of  
 174 plantlets produced was evaluated after 20 days of culture.

175 Five replications of 6 artificial seeds were placed onto  
 176 different sterilized substrates (compost, vermiculite, perlite  
 177 and sand) to assess their suitability for conversion and  
 178 establishment using 11 day old microshoots produced from  
 179 212 to 300 μm micro-explants and cultivated in S23 liquid  
 180 medium supplemented with 2 mg L<sup>-1</sup> Kinetin and 1 mg  
 181 L<sup>-1</sup> IBA. Ten different irrigation solutions were assessed  
 182 with each substrate as follows, (1) SDW. (2) S23 PGRs  
 183 free. (3) Eight combinations of S23 supplemented with  
 184 various combinations of kinetin (1 and 2 mg L<sup>-1</sup>) with  
 185 IBA or NAA (1 and 2 mg L<sup>-1</sup>). Each pot was irrigated with  
 186 75 mL of the irrigation solution. Artificial seed conversion  
 187 rate and the fresh weight of plantlets produced were eval-  
 188 uated after 50 days of culture.

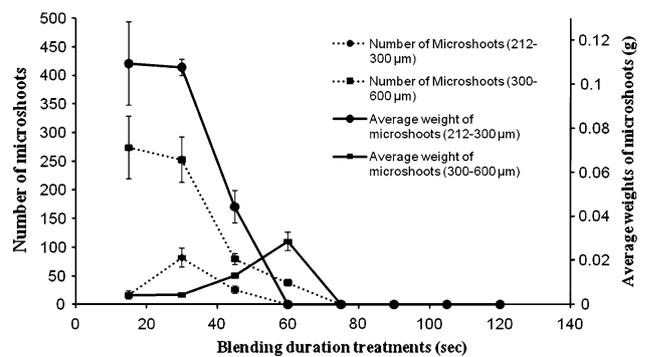
#### 189 Statistical analysis

190 Results are presented as means ± standard error (SE). All  
 191 data were subjected to analysis of variance (ANOVA)  
 192 using Minitab software (version 15) and comparisons of  
 193 means were made with least significant difference test  
 194 (LSD) at 5% level of probability.

#### 195 Results and discussion

196 There was an interaction between the number of micro-  
 197 explants produced and their subsequent development in  
 198 response to blending duration. In terms of the number of  
 199 growing explants during subsequent culture, the use of the  
 200 30 and 15 s treatments were found to be optimal for size  
 201 classes 212–300 μm and 300–600 μm respectively (Fig. 1).  
 202 However, while no significant difference was found  
 203 between these two treatments at size class 300–600 μm,  
 204 the number of developing microshoots was significantly higher  
 205 using 30 s treatment at size class 212–300 μm (Fig. 1). In  
 206 terms of microshoots fresh weight (fresh weight were  
 207 considered as a good indicator of microshoots viability  
 208 since it expresses the speed of growth), the use of 60 s for  
 209 size class 300–600 μm was found to be optimal but the  
 210 number of developing explants using this treatment was  
 211 significantly lower than the use of 30 s treatment  
 212 ( $P < 0.001$ ) (Fig. 1). The use of the 30 s blending treat-  
 213 ments is recommended as optimal.

214 It was observed that the proportion of micro-explants  
 215 which had meristematic domes and those that were classed  
 216 as debris increased with the duration of blending for both

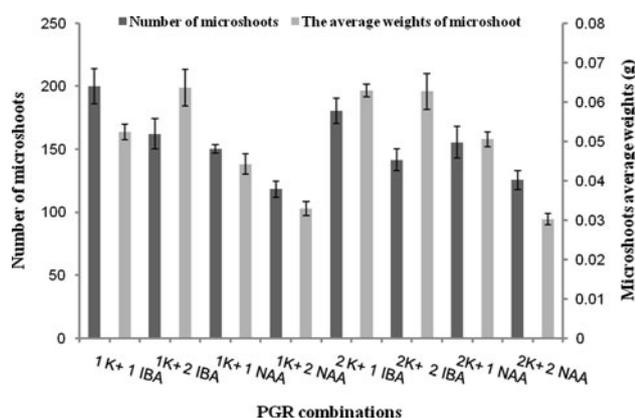


**Fig. 1** The effect of the blending duration treatments on the number of growing microshoots (Number of microshoots (LSD = 19.58 for 212–300 μm size class, LSD = 70.52 for 300–600 μm size class)) and their average weights (Average weights of microshoots (LSD = 0.022 for 212–300 μm size class, LSD = 0.0045 for 300–600 μm size class)) at two size classes, 212–300 μm and 300–600 μm

size classes (212–300 and 300–600 μm). The use of  
 blenders has also been described for mass production of  
 initial explants of fern (Knauss 1976; Cooke 1979; Jans-  
 sens and Sepelie 1989; Teng and Teng 1997) and for  
 separating meristamoid aggregates of several species (Ziv  
 and Ariel 1991; Standardi and Piccioni 1998; Ziv et al.  
 1998; Teng and Ngai 1999). The use of a blender for  
 cauliflower explants production was also previously  
 reported by Kieffer et al. (2001) and as shown in this work  
 the blender is a crude but effective way of producing small  
 micro-explants which remain viable and capable of pro-  
 ducing microshoots. Explant growth capacity however  
 quickly diminishes as the blending duration increases and  
 30 s gives the optimal response rate under the conditions  
 described here.

PGRs added to the liquid medium were found to have a  
 crucial role in the induction of development of the explants  
 since none of the explants developed in PGR-free S23.  
 Although the use of 1 mg L<sup>-1</sup> Kinetin and 1 mg L<sup>-1</sup> IBA  
 and 2 mg L<sup>-1</sup> Kinetin and 1 mg L<sup>-1</sup> IBA treatments gave  
 the optimal results in terms of the number of growing  
 microshoot ( $P < 0.001$ ), the use of 2 mg L<sup>-1</sup> Kinetin and  
 1 mg L<sup>-1</sup> IBA was found to be one of the best in terms of  
 the microshoot fresh weight ( $P < 0.001$ ) (Fig. 2). In the  
 second stage of this investigation, the use of 2 mg L<sup>-1</sup>  
 Kinetin and 1 mg L<sup>-1</sup> IBA treatment was found to be  
 better than all combination of Kinetin with IAA. Therefore,  
 the use of 2 mg L<sup>-1</sup> Kinetin and 1 mg L<sup>-1</sup> IBA is rec-  
 ommended (Fig. 2).

Cytokinins are reported to have a crucial role in the  
 organization of sink activity and nutrient partitioning  
 (Kuiper 1988; Kuiper et al. 1989). Cytokinins are essen-  
 tially made in the root apex (Komor et al. 1993) and  
 because the cauliflower explants have no roots, the main



**Fig. 2** The effect of the various combination of Kinetin (1, 2) mg L<sup>-1</sup> incorporated with IBA (1, 2) mg L<sup>-1</sup> or NAA (1, 2) mg L<sup>-1</sup> added to S23 liquid medium on the number (Number of microshoots (LSD = 28.85)) and average weight (Average weights of microshoots (LSD = 0.0083)) of developing microshoots

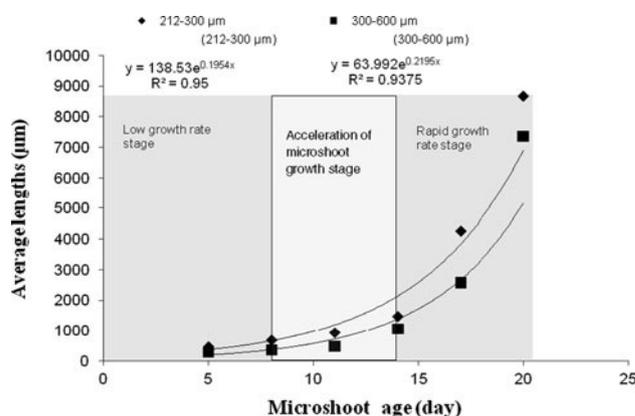
cytokinin source is provided by the culture medium. The current investigation also showed that the type of auxin was important and there were large differences between the number and the viability of microshoots depending on the type of auxin used and IBA was found to be best for the development of the explants. Despite these differences all of the auxin types used had a positive effect in the induction of cauliflower micro-explants. This is in contrast to Vandemoortele et al. (2001) who reported that it is difficult to associate endogenous auxin with a function in the induction of cauliflower curd explants. However, none of the growing microshoots showed the capacity for the development of roots irrespective of the combination of PGRs used. This limitation of microshoot rooting might be caused by an interaction with Kinetin since transferred microshoots to semi solid medium containing 2 mg L<sup>-1</sup> IBA displayed roots within a few days. The current observations are in contrast with those reported by Kieffer et al. (2001) who reported the capacity of NAA at low concentration to encourage early rooting of microshoots even in the presence of Kinetin in the culture medium. This may be a cauliflower varietal effect. It has been reported by others that the use of cytokinin decreases the number of lateral roots in other species (Hinchee and Rost 1986, Goodwin and Morris 1979, Böttger 1974). Eriksen (1974) working in peas mentioned that the presence of high concentrations of cytokinin could have negative effects on the initial step of rooting by deterring the activity of auxin. Rani Debi et al. (2005) indicated that cytokinin inhibits lateral root initiation in rice (*Oryza sativa*) and Nakashimada et al. (1995) also observed inhibition effects of kinetin presence in the culture media on the root elongation of horseradish hairy roots (*Armoracia rusticana*) plantlets. However, Hinchee and Rost (1986) reported that the

auxin:cytokinin ratio has an essential role in co-ordinating lateral root growth in pea seedlings. It is clear that there is no universal explanation for the variations in these observations indicating a strong genotypic effect.

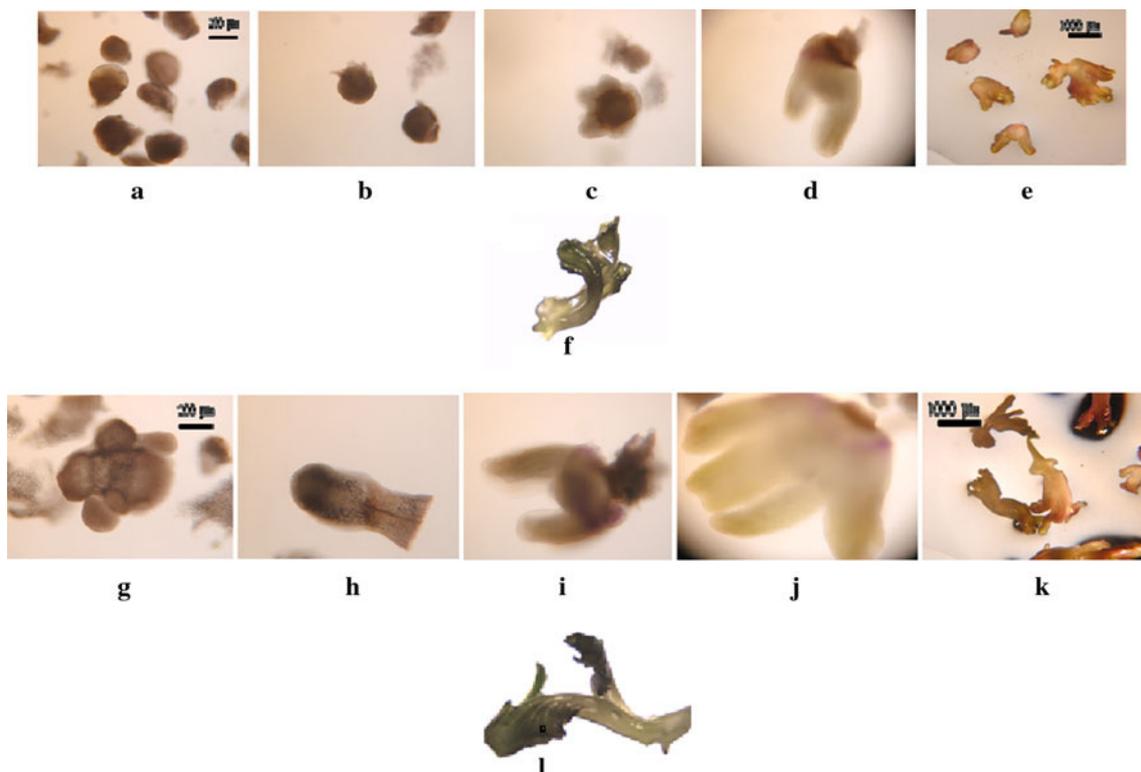
Explant development stages were determined for explants from the 212 to 300 μm and 300 to 600 μm size classes. The explants started growing very slowly for the first 11 days in both size classes but after that the growth rate increased exponentially with time (Fig. 3). The best growth rate was observed with size class 212–300 μm compared to the 300–600 μm size class and might be because of competition for nutrient supply since explants produced at size class 212–300 μm bear only 1 meristematic dome, giving one microshoot while the explants at size class 300–600 μm bear 2–3 meristematic domes giving 2–3 microshoots and more localized competition for nutrients (Fig. 4).

We divided microshoot development into three main stages: (a) 0–11 day stage when the growth rate was very low. (b) 11–15 day stage of culture when an acceleration of microshoot growth was observed. (3) A stage after 15 days when the growth rate was rapid (Fig. 3).

The optimal age for microshoot encapsulation was observed to be 13–14 days (Fig. 5). The encapsulation of both younger and older microshoots had a negative effect on the subsequent artificial seed conversion rate and fresh weights of plantlets produced. The growth of microshoots younger than 13 day-old was observed to be very slow and encapsulation seemed to be an inhibitor of microshoot growth at this sensitive stage. The growth of microshoots older than 14 days old was observed to be rapid in culture but this fast growth brought about metabolic activity which seemed to negatively affect the subsequent of microshoot encapsulation. It was concluded that microshoots derived in an accelerating stage of growth (i.e. stage 2) were optimal for encapsulation.

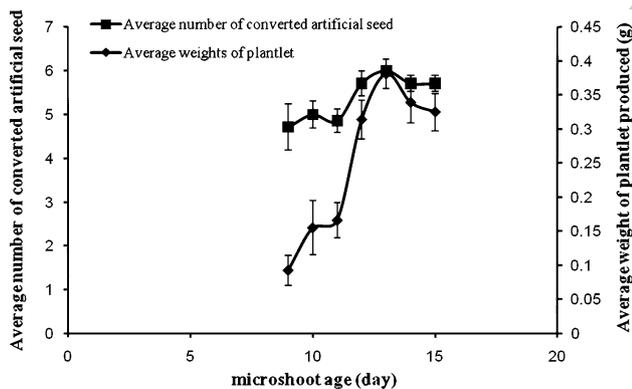


**Fig. 3** Cauliflower explants growth assessed by measured length changes over time



**Fig. 4** Cauliflower microshoots at different developmental stages. **a, b, c, d, e** and **f** were taken from 212 to 300 μm size class and **g, h, i, j, k** and **l** from 300 to 600 μm. Photos were taken at 3, 6, 9, 12, 15, 17

and 20 days old respectively. **a, b, c, d, g, h, i, and j** were taken at 100 times magnification, **e** and **k** were taken at 40 times magnification and **f** and **l** without any magnification



**Fig. 5** The effect of the optimal microshoot age suitable for encapsulation (LSD = 0.687 for the conversion rate and LSD = 0.0675 for artificial seeds viability)

321 For cauliflower artificial seeds to be a promising candidate for agriculture their capacity to be sown into commercial substrates such as compost, perlite, vermiculite or sand needs to be evaluated. It was clear from this investigation that the moistening/irrigation solution composition used during conversion was the key factor for the success of these substrates. No conversion of artificial seeds was observed when the culture substrates were irrigated with sterile distilled water and this is in common with other

species tested (Naik and Chand 2006) (Soneji et al. 2002). 330 The use of S23 PGR-free as an irrigating solution had a positive effect on the conversion rate and viability of the artificial seeds (Tables 1 and 2). The use of PGRs combinations had a significant influence on the fresh weight of plantlets produced 50 days after conversion but they did not improve the conversion rate of the artificial seeds. 336 Moreover, some of the PGR combinations such as the use of S23, 1 mg L<sup>-1</sup> Kinetin and 2 mg L<sup>-1</sup> IBA and the use of S23, 1 mg L<sup>-1</sup> Kinetin and 2 mg L<sup>-1</sup> NAA significantly reduced the conversion rate. However, the use of S23, 2 mg L<sup>-1</sup> Kinetin and 2 mg L<sup>-1</sup> NAA treatment was recommended resulting in the optimal artificial seed conversion rate and the best fresh weight of plantlets (Tables 1 and 2). 343

344 The type of auxin used with the culture substrates showed that 2 mg L<sup>-1</sup> NAA was better than those used with semi solid culture media supplemented with 2 mg L<sup>-1</sup> IBA (data not shown). This could be either due to the presence of Kinetin in the liquid media used for culture substrate moistening or because of the physical structure of culture substrates led to less transportation efficiency of PGRs to the cauliflower microshoots. However, although the optimal conversion rate was obtained using perlite, the viability of artificial seeds was negatively affected by this substrate and the growth of plantlets stopped at a certain 354

**Table 1** The effect of irrigation composition and culture substrates on artificial seed conversion rate (%)

PGRs (mg L <sup>-1</sup> ) <sup>1</sup>			Culture substrates				Treatment averages
Kin	IBA	NAA	Perlite	Sand	Compost	Vermiculite	
1	1	0	90 <sup>c</sup>	67 <sup>g</sup>	36 <sup>1</sup>	63 <sup>h</sup>	64 <sup>b</sup>
1	2	0	82 <sup>e</sup>	93 <sup>b</sup>	34 <sup>lm</sup>	33 <sup>m</sup>	60 <sup>bc</sup>
1	0	1	93 <sup>b</sup>	43 <sup>k</sup>	33 <sup>m</sup>	56 <sup>i</sup>	56 <sup>bc</sup>
1	0	2	83 <sup>e</sup>	63 <sup>h</sup>	26 <sup>o</sup>	30 <sup>n</sup>	50 <sup>c</sup>
2	1	0	93 <sup>b</sup>	97 <sup>a</sup>	73 <sup>f</sup>	30 <sup>n</sup>	73 <sup>ab</sup>
2	2	0	87 <sup>d</sup>	43 <sup>k</sup>	55 <sup>i</sup>	26 <sup>o</sup>	52 <sup>c</sup>
2	0	1	93 <sup>b</sup>	90 <sup>c</sup>	43 <sup>k</sup>	30 <sup>n</sup>	64 <sup>b</sup>
2	0	2	97 <sup>a</sup>	87 <sup>d</sup>	67 <sup>g</sup>	46 <sup>j</sup>	74 <sup>a</sup>
0	0	0	97 <sup>a</sup>	83 <sup>e</sup>	90 <sup>c</sup>	46 <sup>j</sup>	79 <sup>a</sup>
Average			91 <sup>a</sup>	74 <sup>b</sup>	50 <sup>c</sup>	40 <sup>d</sup>	

<sup>1</sup> S23 was supplemented with the described PGRs. (LSD = 10.528 for PGR combinations, LSD = 5.84 for culture substrates and LSD = 2.105 for PGR combinations × Culture substrates (interaction))

**Table 2** The effect of irrigation composition and culture substrate on the plantlet fresh weight (g)

PGRs (mg L <sup>-1</sup> ) <sup>1</sup>			Culture substrates				Average
Kin	IBA	NAA	Perlite	Sand	Compost	Vermiculite	
1	1	0	0.068 <sup>efghij</sup>	0.064 <sup>fghij</sup>	0.109 <sup>bcdefghi</sup>	0.050 <sup>ghij</sup>	0.072
1	2	0	0.051 <sup>ghij</sup>	0.075 <sup>efghij</sup>	0.178 <sup>ab</sup>	0.030 <sup>ij</sup>	0.083
1	0	1	0.071 <sup>efghij</sup>	0.025 <sup>j</sup>	0.234 <sup>a</sup>	0.050 <sup>ghij</sup>	0.095
1	0	2	0.073 <sup>efghij</sup>	0.12 <sup>bcdefgh</sup>	0.044 <sup>hij</sup>	0.033 <sup>ij</sup>	0.067
2	1	0	0.061 <sup>ghij</sup>	0.073 <sup>efghij</sup>	0.092 <sup>cdefghij</sup>	0.034 <sup>ij</sup>	0.065
2	2	0	0.087 <sup>defghij</sup>	0.054 <sup>ghij</sup>	0.167 <sup>abc</sup>	0.033 <sup>ij</sup>	0.085
2	0	1	0.061 <sup>ghij</sup>	0.141 <sup>bcdef</sup>	0.124 <sup>bedefg</sup>	0.031 <sup>ij</sup>	0.089
2	0	2	0.072 <sup>efghij</sup>	0.160 <sup>abcd</sup>	0.144 <sup>bcde</sup>	0.044 <sup>hij</sup>	0.105
0	0	0	0.052 <sup>ghij</sup>	0.072 <sup>efghij</sup>	0.059 <sup>ghij</sup>	0.027 <sup>ij</sup>	0.052
Average			0.066 <sup>b</sup>	0.087 <sup>b</sup>	0.128 <sup>a</sup>	0.037 <sup>c</sup>	

<sup>1</sup> S23 was supplemented with the described PGRs. (LSD = 0.022 for culture substrates and LSD = 0.080 for the interaction between the PGR combinations and culture substrates)

355 point. It was suggested that the cessation of growth could  
356 be caused by a lack of moistened liquid mixture supplied  
357 since the same volume of liquid mixture was used with the  
358 four culture substrates and the signs of dehydration were  
359 observed with perlite in comparison with other substrates.

360 The optimal fresh weight of plantlets produced was  
361 obtained using compost as a culture substrate (Table 2). It  
362 seemed that the conversion rate and viability of artificial  
363 seed depends not only on the irrigation liquid mixture but  
364 also on the physical structure of the culture substrates. It  
365 might be good idea to investigate the result of using a  
366 mixture of compost and perlite and examining the effects  
367 on the conversion rate and viability of artificial seeds since  
368 the optimal conversion rate and viability were obtained  
369 using perlite and compost respectively. A high interaction  
370 between the moistening/irrigation solution compositions  
371 and the culture substrates was observed in terms of

artificial seeds conversion rate and fresh weights of plant- 372  
373 lets produced. While the use of perlite moistened with S23  
374 or S23 supplemented with 2 mg L<sup>-1</sup> Kinetin and 2 mg L<sup>-1</sup>  
375 NAA irrigation solutions gave the best artificial seed con-  
376 version rate, the use of compost supplemented with S23,  
377 1 mg L<sup>-1</sup> Kinetin and 1 mg L<sup>-1</sup> NAA was optimal in  
378 terms of fresh weight of plantlets. However, the use of S23,  
379 2 mg L<sup>-1</sup> Kinetin and 2 mg L<sup>-1</sup> NAA as an irrigating  
380 solution was recommended with both perlite and compost  
381 resulting in the optimal conversion rate and producing the  
382 best plantlets fresh weights respectively since the only  
383 significantly better plantlets fresh weights than this treat-  
384 ment was obtained using S23, 1 mg L<sup>-1</sup> Kinetin and 1 mg  
385 L<sup>-1</sup> NAA and since the conversion rate was observed to be  
386 quite low when S23, 1 mg L<sup>-1</sup> Kinetin and 1 mg L<sup>-1</sup> NAA  
387 was used as irrigating solution (Tables 1 and 2). Several  
388 studies have investigated the possibilities of sowing

389 artificial seeds in soil or commercial substrates, for  
390 example, on the use vermiculite, sand and soil for the  
391 cultivation of mulberry artificial seeds (Machii and  
392 Yamanouchi 1993), the use of soil for as alfalfa artificial  
393 seed conversion substrate (Fujii et al. 1989), the use of  
394 perlite for M.26 apple rootstock (Micheli et al. 2002) and  
395 *Citrus reticulata* (Antonietta et al. 2007) and the use of  
396 sand for *elite indica* rice (Roy and Mandal 2008) and it is  
397 clear that the optimal conditions need to be derived  
398 empirically for each species examined.

## 399 Conclusion

400 The production of microshoots from cauliflower curd was  
401 optimized. This study showed that the use of a commercial  
402 blender was a useful methodology for micro-explant gen-  
403 eration and a 30 s blending duration treatment was found to  
404 be the best in terms of the number and viability of sub-  
405 sequent microshoots produced. The use of PGRs was  
406 essential for microshoot development and the best PGR  
407 combination was found to be 2 mg L<sup>-1</sup> Kinetin and 1 mg  
408 L<sup>-1</sup> IBA. The use of the described protocol is considered a  
409 cost effect methodology for cauliflower micropropagation  
410 to produce huge number of microshoots per curd.

411 This study also demonstrated the ability of encapsulat-  
412 ing cauliflower microshoots in a sodium alginate matrix for  
413 artificial seed production and was the first which has  
414 investigated the capacity of the cauliflower artificial seed  
415 growing in commercial substrates (compost, vermiculite,  
416 perlite and sand). Fully functional in vivo plantlets were  
417 obtained using these commercial substrates and this opens  
418 promising vistas for the direct use of cauliflower artificial  
419 seeds in in vivo situations.

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