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 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 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 mg L^{-1} concentration. The use of 2 mg L^{-1} kinetin and 1 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 mg L^{-1}) and IBA or NAA at (1 and 2 mg L^{-1}) was investigated. The use of 2 mg L^{-1} kinetin and 2 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.

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

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ORIGINAL PAPER

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

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

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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 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 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 mg L^{-1} concentration. The use of 2 mg L^{-1} kinetin and 1 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 mg L^{-1}) and IBA or NAA at (1 and 2 mg L^{-1}) was investigated. The use of 2 mg L^{-1} kinetin and 2 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.

Keywords Sodium alginate · Encapsulation · Curd · Meristems · Artificial seeds

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Introduction

The outermost layer of cauliflower curd is made of several million meristems (Kieffer et al. 2001) and the use of curd meristematic tissue for in vitro culture has been investigated for micropropagation and the production of virus free plants (Grout and Crisp 1977; Kumar et al. 1993). The use of the meristematic layer of cauliflower curd is considered to be superior compared with the use of conventional protocols using seedling or leaf explants which have been found to be labour intensive (Pow 1969; Kumar et al. 1993). Kieffer et al. (2001) designed an effective protocol for the production of cauliflower propagules from fractionated and graded curd and these propagules were suggested to be suitable for encapsulation in sodium alginate matrices for the production of artificial seeds. The technique of artificial seed has been widely studied and works with various plant species including fruits, cereals, medicinal plants, vegetables, ornamentals, forest trees and orchids (Germanà et al. 2011, Sundararaj et al. 2010, Singh et al. 2009, Rai et al. 2008, Pintos et al. 2008, Micheli et al. 2007, Antonietta et al. 2007, Naik and Chand 2006, Malabadi and Staden 2005, Chand and Singh 2004, Nyende et al. 2003, Mandal et al. 2000). The use of microshoots has been widely reported for the production of artificial seeds in different plant species such as *Cineraria maritime* (Sandoval-Yugar et al. 2009), *Musa* sp. (Sandoval-Yugar

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

Cauliflower is an open pollinated species and there are technical challenges to producing in-bred lines with reliable self-incompatability or male sterility for producing F1 hybrids particularly amongst the winter-heading maturity sub-group. The production of cauliflower clones multiplied by tissue culture and distributed as artificial seeds could be useful alternative to F1 hybrids varieties and could also be useful in the context of the maintenance of elite clonal germplasm in cauliflower breeding programmes. The current study aimed to further optimize the production of cauliflower propagules and to investigate the ability of using cauliflower microshoots for the production of artificial seeds. Moreover, the investigation of cauliflower artificial seeds capacity to be grown on in commercial substrates was one of the main aims of this study.

Materials and methods

Plant materials

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

Cauliflower microshoot production

Large pieces (1–5 cm) of cauliflower curds were sterilized by immersion in 10% by volume un-thickened domestic bleach (0.06% sodium hypochlorite) for 15 min followed by a double wash with sterile distilled water. Explants were produced mechanically by eliminating the mass of non-responsive tissue (stem branches) and shaving off the upper meristematic layer using a sterilized scalpel working in a laminar flow cabinet. The meristimatic clusters were then homogenized using a commercial blender (Waring model 800) at approximately 1,700 rev min^{-1} in maintenance S23 (4.4 g L^{-1} Sigma^{TM} MS (Murashige and Skoog 1962) and 3% w/v sucrose) liquid media. Eight blending durations in the range 15–120 s were assessed in terms of their effects on the amount and subsequent growth of the micro-explants produced after sieving through precision sieves (212, 300 and 600 μm) (Endecotts Ltd, London, UK). The micro-explants were collected off the sieves, weighed and converted to aliquots of explants using small precision volumetric measures (74 or 240 ± 2 μL). Six containers (150 mL plastic pots), each containing 30 mL liquid S23 culture medium supplemented with 2 mg L^{-1} Kinetin and 1 mg L^{-1} IBA, were cultured with a constant volume of micro-explants (74 μL) and used with every blending duration in order to assess the effects of blending treatments. The pots were constantly shaken (150 rev min^{-1}) during culture at 20°C and exposed to 16 h photoperiod. The proportion of micro-explants having meristimatic domes was determined by taking 4 mL samples of cultures at 8 days from each pot and observing them under a zoom binocular microscope (Nikon SMZ-2T). The growth ability of micro-explants during culture was determined after 20 days as the number and fresh weight of microshoots produced.

The effects of several PGR combinations were evaluated in terms of their ability to induce micro-explant development. Nine PGR combinations consisting of various combination of the cytokinin (Kinetin) (1 and 2 mg L^{-1}) with the auxins IBA (1 and 2 mg L^{-1}) or NAA (1 and 2 mg L^{-1}) were evaluated in the first stage and another 4 combinations consisting of various combinations of Kinetin (1 and 2 mg L^{-1}) with IAA (1 and 2 mg L^{-1}) compared with the use of Kinetin (2 mg L^{-1}) and IBA (1 mg L^{-1}) were evaluated in the second stage. Four containers, each with 30 mL of culture medium, were cultivated with a constant volume of explants (74 μL) of the 300–425 μm explant size class and used with every treatment. In order to preserve culture sterility the culture media was supplemented 1 mL L^{-1} PPM^{TM} (Plant Preservative Mixture) and used with all the treatments.

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

Cauliflower artificial seed production

Micro-explants of the 212–300 μm size class were used for artificial seed production. Microshoots were mixed with sterilized (by tyndallisation) sodium alginate 2% (w/v) and dropped into a sterilized (autoclaved) solution of calcium chloride 15 g L^{-1} using a sterilized pipette. Microshoots were left in the calcium chloride for 30 min for full complexion. The artificial seeds were then transferred to a S23 liquid media (without PGRs) for 30 min followed by a quick wash with sterile distilled water. The optimal age

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

Five replications of 6 artificial seeds were placed onto different sterilized substrates (compost, vermiculite, perlite and sand) to assess their suitability for conversion and establishment using 11 day old microshoots produced from 212 to 300 µm micro-explants and cultivated in S23 liquid medium supplemented with 2 mg L^{-1} Kinetin and 1 mg L^{-1} IBA. Ten different irrigation solutions were assessed with each substrate as follows, (1) SDW. (2) S23 PGRs free. (3) Eight combinations of S23 supplemented with various combinations of kinetin (1 and 2 mg L^{-1}) with IBA or NAA (1 and 2 mg L^{-1}). Each pot was irrigated with 75 mL of the irrigation solution. Artificial seed conversion rate and the fresh weight of plantlets produced were evaluated after 50 days of culture.

Statistical analysis

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

Results and discussion

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

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

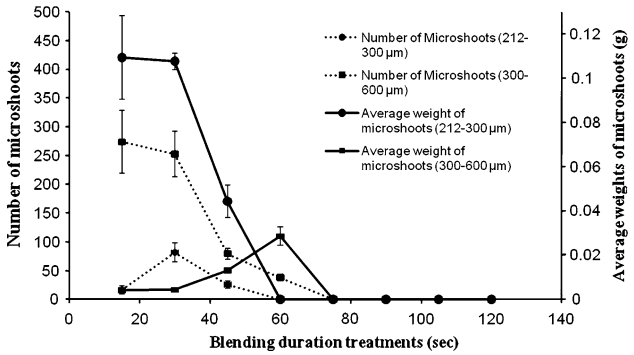

Fig. 1 The effect of the blending duration treatments on the number of growing microshoots (Number of microshoots (LSD = 19.58 for 212–300 µm size class, LSD = 70.52 for 300–600 µm size class)) and their average weights (Average weights of microshoots (LSD = 0.022 for 212–300 µm size class, LSD = 0.0045 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; Janssens 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 producing 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^{-1} Kinetin and 1 mg L^{-1} IBA and 2 mg L^{-1} Kinetin and 1 mg L^{-1} IBA treatments gave the optimal results in terms of the number of growing microshoot ($P < 0.001$), the use of 2 mg L^{-1} Kinetin and 1 mg L^{-1} 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^{-1} Kinetin and 1 mg L^{-1} IBA treatment was found to be better than all combination of Kinetin with IAA. Therefore, the use of 2 mg L^{-1} Kinetin and 1 mg L^{-1} IBA is recommended (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 essentially made in the root apex (Komor et al. 1993) and because the cauliflower explants have no roots, the main

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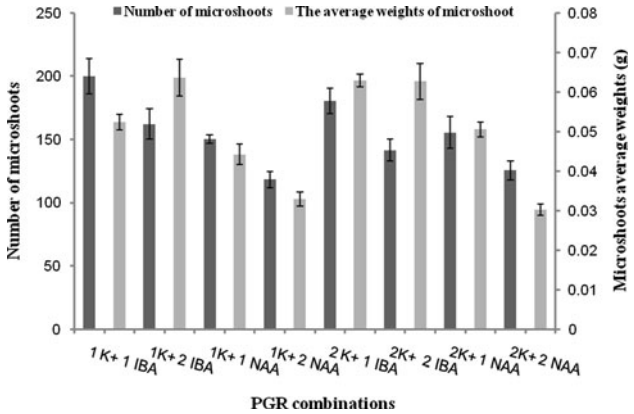

Fig. 2 The effect of the various combination of Kinetin (1, 2) mg L^{-1} incorporated with IBA (1, 2) mg L^{-1} or NAA (1, 2) mg L^{-1} added to S23 liquid medium on the number (Number of microshoots (LSD = 28.85)) and average weight (Average weights of microshoots (LSD = 0.0083)) of developing microshoots

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^{-1} 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, Böttgor 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.

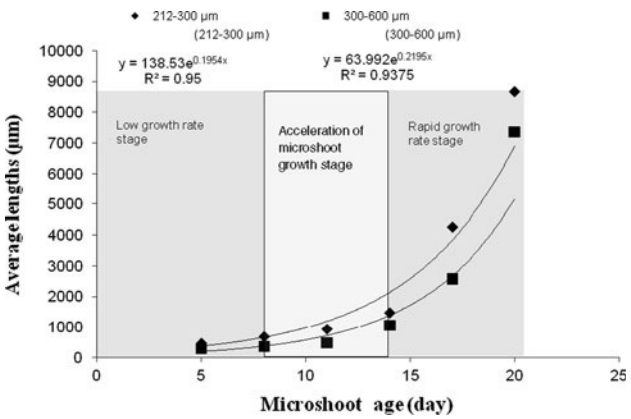

Fig. 3 Cauliflower explants growth assessed by measured length changes over time

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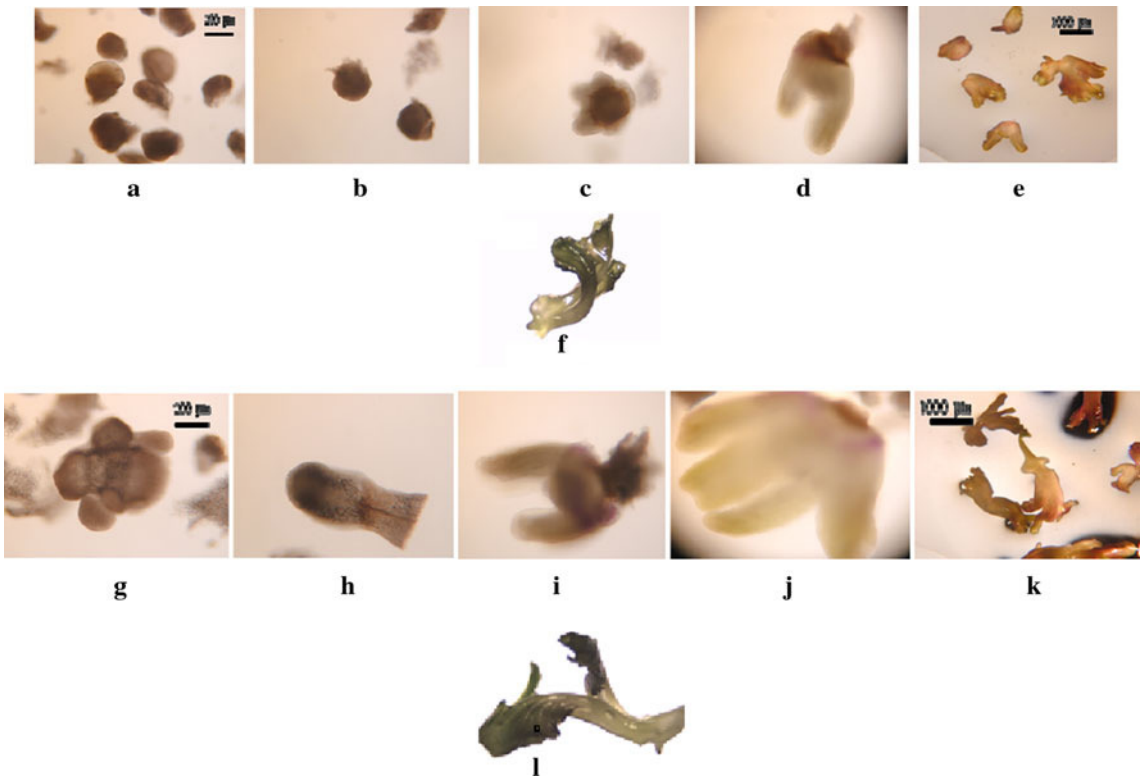

Fig. 4 Cauliflower microshoots at different developmental stages. **a**, **b**, **c**, **d**, **e** and **f** were taken from 212 to 300 μm size class and **g**, **h**, **i**, **j**, **k** and **l** from 300 to 600 μm. Photos were taken at 3, 6, 9, 12, 15, 17 and 20 days old respectively. **a**, **b**, **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

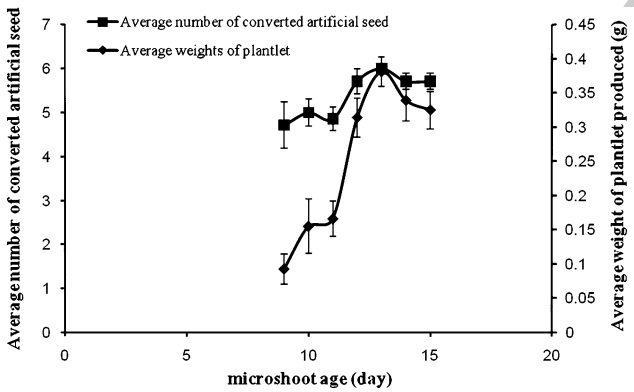

Fig. 5 The effect of the optimal microshoot age suitable for encapsulation (LSD = 0.687 for the conversion rate and LSD = 0.0675 for artificial seeds viability)

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). 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. Moreover, some of the PGR combinations such as the use of S23, 1 mg L^{-1} Kinetin and 2 mg L^{-1} IBA and the use of S23, 1 mg L^{-1} Kinetin and 2 mg L^{-1} NAA significantly reduced the conversion rate. However, the use of S23, 2 mg L^{-1} Kinetin and 2 mg L^{-1} NAA treatment was recommended resulting in the optimal artificial seed conversion rate and the best fresh weight of plantlets (Tables 1 and 2).

The type of auxin used with the culture substrates showed that 2 mg L^{-1} NAA was better than those used with semi solid culture media supplemented with 2 mg L^{-1} 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

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

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

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

[1] 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)

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

The optimal fresh weight of plantlets produced was obtained using compost as a culture substrate (Table 2). It seemed that the conversion rate and viability of artificial seed depends not only on the irrigation liquid mixture but also on the physical structure of the culture substrates. It might be good idea to investigate the result of using a mixture of compost and perlite and examining the effects on the conversion rate and viability of artificial seeds since the optimal conversion rate and viability were obtained using perlite and compost respectively. A high interaction between the moistening/irrigation solution compositions and the culture substrates was observed in terms of artificial seeds conversion rate and fresh weights of plantlets produced. While the use of perlite moistened with S23 or S23 supplemented with 2 mg L^{-1} Kinetin and 2 mg L^{-1} NAA irrigation solutions gave the best artificial seed conversion rate, the use of compost supplemented with S23, 1 mg L^{-1} Kinetin and 1 mg L^{-1} NAA was optimal in terms of fresh weight of plantlets. However, the use of S23, 2 mg L^{-1} Kinetin and 2 mg L^{-1} NAA as an irrigating solution was recommended with both perlite and compost resulting in the optimal conversion rate and producing the best plantlets fresh weights respectively since the only significantly better plantlets fresh weights than this treatment was obtained using S23, 1 mg L^{-1} Kinetin and 1 mg L^{-1} NAA and since the conversion rate was observed to be quite low when S23, 1 mg L^{-1} Kinetin and 1 mg L^{-1} NAA was used as irrigating solution (Tables 1 and 2). Several studies have investigated the possibilities of sowing

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

Conclusion

The production of microshoots from cauliflower curd was optimized. This study showed that the use of a commercial blender was a useful methodology for micro-explant generation and a 30 s blending duration treatment was found to be the best in terms of the number and viability of subsequent microshoots produced. The use of PGRs was essential for microshoot development and the best PGR combination was found to be 2 mg L^{-1} Kinetin and 1 mg L^{-1} IBA. The use of the described protocol is considered a cost effect methodology for cauliflower micropropagation to produce huge number of microshoots per curd.

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

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