Devernalization in Cultures of Brassica oleracea var. Botrytis Curd Tissues

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Abstract

Small pieces of cauliflower (***Brassica oleracea*** Var. Botrytis) curds were cultured on MS medium supplemented with auxins and cytokinins. Callus was induced on such curd pieces when cultured on MS medium supplemented with kinetin at concentrations of 0.1, 0.5 or 1.0 mg/l in combination with 0.5, 1.0 or 1.5 of the auxin 2, 4-D. Devernalization in curd florets gave rise to a large number of shoots. Maximum number of shoots obtained when the medium was supplemented with 1.0 mg/l 2, 4-D only reaching 32 shoots/explant. Shoots were rooted better in the presence of IBA. Maximum number of roots appeared on shoots cultured on MS medium supplemented with 0.5 or 1.0 mg/l IBA reaching 12 roots/shoot. A reversal relationship between root number and root length was noticed. Survival rate after acclimatization reached up to 80% when plantlets transplanted in a mixture of sand and compost (1:1). It is concluded that devernalization in cauliflower curds produces high number of plants. This is extremely important for mass production of plants from hybrids with distinct traits via devernalization of curd explants.

Introduction

One of the important *Brassica* varieties is cauliflower *Brassica oleracea* Var. Botrytis which is normally propagated by seeds. Hybrid seeds are expensive and seed companies monopolize production. Cauliflower propagated vegetatively *in vitro* by grafting curd material onto young plants was first reported by Watts and George (1963). They were able to obtain flowering shoots and seed from the grafted plant. Curd cultures were found to devernalize when placed in liquid Linsmaeir and Skoog (1965) medium with a relatively high concentrations of auxins and cytokinins (8 mg/l IAA and 2.56 mg/l kinetin). Shake cultures were also used by Walkey and Woolfitt (1970) for rapid clonal multiplication of cauliflower. Crisp and Walkey (1974) induced shoot formation (caulogenesis) which subcultured at least 10 times, and established plants in agar containing cultures. Buiatti and Bennici (1974) and Buiatti., *et al* (1974) established a relationship between growth, differentiation, hormone and genotype implementing a diallel crosses.Cauliflower shoots were regenerated from internodal sections and curds from greenhouse grown plants on a complicated HMHH medium with several auxins and cytokinins (Trimboli *et al.* 1977). They noted that adventitious buds initiated from callus tissue derived from secondary phloem and inner cortical cells. Rooting was achieved again on HMHH medium with high level of IBA.Somatic embryos were induced on cauliflower leaf derived callus on MS medium (Murashige and Skoog, 1962) supplemented with less than 0.1 mg/l of IAA (Pareek and Chandra, 1978). Mass production of somatic embryos can be indispensable source for synthetic (artificial) seed production. However, most of the resulted somatic embryos were found to be abnormal. Regeneration from suspension cultures has not been reported. Protoplasts were isolated by Vatsya and Bhaskaran (1982) from leaves of *in vitro* and *in vivo* plants and from cotyledons. Plant regeneration from cauliflower hypocotyl protoplasts was reported by Glimelius (1984). The effect of ventilation and CO2 supply on the growth and photosynthetic rate of mixotrophic plantlets of cauliflower were investigated by Abe, *et al.* (2005). They found that cauliflower mixotrophic plantlets *in vitro* grew rapidly with enhanced photosynthetic rate under ventilated culture conditions.

The objective of the current study is to induce devernalization in cauliflower avoiding complicated media previously reportd.

Materials and methods

Seeds of cauliflower hybrid (***Brassica oleracea*** Var. Botrytis) were sown in clay pots (12 cm in diameter) till plants reached maturity under glasshouse conditions. When immature curds reached 10 cm in diameter, three pieces (approximately 10 mg) were dissected and surface sterilized with sodium hypochlorite at a concentration of 1% for 5 min., cultured on a medium consisted of MS salts, 3% sucrose and the pH was adjusted to 5.7. Plant growth regulators were added (2, 4-D at 0, 0.1, 0.5, 1.0 1.5 mg/l), (Kinetin at 0, o.1, 0.5, 1.0, 1.5). 15 ml aliquots of the medium were dispensed in 75 ml jars then subjected to autoclaving at 120° C for 15 minutes and pressure of 1.02 kg/cm^2. Callusing, number of shoots per explant and shoot heights were recorded after 3 weeks. Curd pieces were cultured on MS medium supplemented with different concentrations of 2, 4-D. Number of shoots and shoot height were recorded 30 days after culture. Shoots were transferred onto of MS medium with different concentrations of IBA at concentrations (0, 0.1, 0.5, 1.0, 2.0) for rooting. Number of roots and mean root length were recorded after one month in

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culture. Plantlets were then ready for acclimatization, taken out, washed thoroughly and acclimatized according to the method of Roy, *et al.* (2004) with some modifications. *In vitro* rooted plantlets were then transplanted in clay pots containing sand, compost or a mixture of sand and compost (1:1) and covered with transparent polyethylene bags. Survival percentage was recorded 21 days after transplanting.

A completely randomized design with ten replicates for each treatment was conducted. Means and standard deviations were calculated (Goenz and Goenz, 1984).

Results and Discussion

Table 1 shows that callus induction occurred on MS medium supplemented with a combination of 2, 4-D at concentrations 0.5, 1.0 and 1.5 mg/l and kinetin at 0.1, 0.5 and 1.0 mg/l. Other concentrations did not induce callusing. The most important factor governs callus induction and subsequent organogenesis *in vitro* is plant hormones. This factor was enunciated by the experiments of Skoog and Miller (1957) on cultured stem tissues of tobacco. They showed that varying the concentrations of auxins and cytokinins in the culture media, has a direct effect on the type of tissue appears on cultural explant. When they used intermediate concentrations of auxins and cytokinins, the tissues grow as unorganized callus. It seems in our experiment that the right combination of auxin and cytokinins had induced callusing on cauliflower floret explants.

Table (1): Callusing at different concentrations of 2, 4-D and kinetin (mg/l) on dissected cauliflower florets taken from immature curds (n=10)

Kinetin / 2, 4-D	0.0	0.1	0.5	1.0	1.5
0.0	-	-	-	-	-
0.1	-	-	−	−	+
0.5	-	-	−	−	+
1.0	-	-	−	−	+
1.5	-	-	-	-	-

+ callusing occurred in more than half the cultures
- No callusing

Data shown in Table 2 indicate that the highest number of shoots obtained was at 1.0 mg/l 2, 4-D. Increasing or decreasing the level of this auxin led to a reduction in shoot number. Minimum shoot number obtained was in the treatment deficient in 2, 4-D (2 shoots). The later treatment, however achieved the maximum shoot height (22 mm) and this trait correlated inversely with the number of shoots. Fig. 1 shows cauliflower shoots initiated on MS medium supplemented with 1.0 mg/l 2, 4-D after one month in culture. It is well known that the right balance between auxins and cytokinins favors caulogenesis (shoot formation), callusing or rooting. Cauliflower curd explants may contain endogenous cytokinins which led to achieve such balance and ultimately shoot proliferation. These findings are unlike those of Walkey and Woolfitt (1970). They reported proliferation in cauliflower shoots from pieces of curd tissues cultured on medium supplemented with 0.5 mg/l BAP and 0.25 mg/l IBA using shake cultures, although in both experiments exogenous auxins were necessary for triggering proliferation.

Table (2): Direct regeneration from cauliflower curd pieces (approximately 10 mg) 30 days after culture on MS medium containing different concentrations of 2, 4-D (± SD for n=10)

2, 4-D (mg/l)	No. of shoots	Mean shoot height (mm)/shoot
0.0	3–0.8	22 ± 3.7
0.1	9 ± 2.3	20 ± 3.6
0.5	23 ± 5.8	18 ± 4.2
1.0	32 ± 7.1	16 ± 4.1
1.5	17 ± 5.3	20 ± 3.9
2.0	7 ± 2.1	21 ± 3.7

The potentia. of cauliflower florets when devernalized, seems to give rise to a large number of shoots. Commercially, the number of shoots obtained from 1.0 mg/l 2, 4-D treatment is a reasonable during 30 days only. This may be considered a high multiplication rate compared with other crops (Zimmerman, 2005).

Table 3 shows that shoots rooted even in the absence of plant growth regulators, but the roots are too little that may not support plantlets during acclimatization. Maximum number of roots, obtained was at IBA concentrations 0.5 and 1.0 mg/l (12 roots/plantlet each). Mean root length decreased as the level of IBA increased. These values were 11, 8, 5, 2 and 1.3 for the concentrations 0.0, 0.1, 0.5, 1.0 and 2.0 mg/l IBA respectively. It is well known that IBA encourages rooting *in vivo* and *in vitro* (Hartmann *et al.* 1997). This is due to the ability of IBA to encourage cell division and its role in root initiation. However, as the number of roots increases, a cute competition on the medium constituents may occur, causing a reduction in root length.

Plantlets were ready for acclimatization (Fig. 2) and transfer to glasshouse conditions.

Table (3): Rooting of shoots regenerated from devernalized curd pieces at different concentrations of IBA (± SD) n = 10

IBA (mg/l)	No. of shoots	Mean shoot height (mm)/shoot
0.0	2 ± 0.3	11 ± 2.8
0.1	7 ± 1.2	8 ± 2.1
0.5	12 ± 2.3	5 ± 0.9
1.0	12 ± 2.7	2 ± 0.4
1.5	8 ± 2.5	1.3 ± 0.3
2.0	2 ± 0.3	11 ± 2.8

Among the three propagation media tested for weaning plantlets, a mixture of sand and compost at a ratio 1:1 achieved the highest survival rate reached 80% (Table 4). This type of medium may provide a good balance between aeration and moisture content which led to a higher survival rate (Ibrahim and Majeed, 2001).

Table 4. Survival rate of cauliflower plantlets grown in three types of propagation media during acclimatization (± SD) n = 10

Type of medium	% Survival
Sand only	60 ± 5
Compost only	70 ± 5
Mixture (1:1)	80 ± 5

It is concluded from this study that devernalization occurs in cauliflower curds by supplementing the medium with 2, 4-D at a concentration of 1.0 mg/l is the most stimulatory among the investigated ones. Furthermore, it is possible to produce cauliflower plants year round utilizing of this phenomenon

Figure (1): Shoot regeneration from cauliflower curd pieces grown on MS medium containing 1.0 mg/l 2, 4-D after 30 days. (Picture represents the actual size)

Figure (2): Cauliflower plantlet ready for weaning 21 days after transferring shoots on rooting medium (Picture represents the actual size)

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الخلاصة

زرعت قطع صغيرة من الاقراص الزهرية لنبات القرنابيط ***Brassica oleracea*** Var. Botrytis على وسط MS المجهز بالاوكسينات والسايتوكاينينات. تم تحفيز التفاف على التشوء عند تداخل تراكيز 0.1، 0.5 او 1.0 ملغم/لتر من الكاينتين مع 0.5، 1.0 او 1.5 ملغم/لتر من 2,4-D على وسط MS. تبين بان تحول البراعم الزهرية الى خضرية قد حصل بمعدلات عالية منتجا اعدادا كبيرة من النموات الخضرية وصل اعلى معدل لها على الوسط الحاوي على 1ملغم/لتر من الاوكسين 2,4-D حيث وصل الى 32 فرعا للجزء النباتي الواحد. تم تجذير النموات على نفس الوسط بعد ان اضيف اليه 0.5 أو 1.0 ملغم/لتر من الاوكسين IBA حيث وصل عدد الجذور الى 12 جذرا لكل فرع. وظهرت علاقة عكسية بين عدد الجذور واطوالها. وصل معدل بقاء الشتلات حيه بعد الاقلمة الى 80% بعد ان نقلت الى خليط مكون من الرمل والكومبوست بنسبة 1:1. استنتج من هذا البحث بان ظاهرة تحول البراعم الزهرية الى خضرية في الاقراص الزهرية للقرنابيط قد يكون مصدرا مهما لانتاج الشتلات على مدار السنة خاصة من الاصناف البعيدة ذات الصفات المميزة.