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In vitro behaviour of nodal explants of *Portulaca grandiflora* under the influence of cytokinins

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Abstract

A method for the micropropagation of *Portulaca grandiflora* Hook is described to understand the *in vitro* behaviour of nodal explants in cytokinin-rich medium. Aseptic nodal explants were cultivated on Murashige and Skoog's medium containing different concentrations and combinations of 6-benzylaminopurine (BAP) and 6-furfurylaminopurine (kinetin, KIN). The nodal explants of mature plant can be stimulated to undergo multiple shoot formation on the medium supplemented with BAP and KIN. Direct organogenesis without callus formation from the nodal explants was observed on medium supplemented with cytokinins. Using BAP (2 to 10 μM) alone had a significant effect on the formation of multiple shoots, which were stout and robust. Similar concentrations of KIN (2 to 10 μM) showed a weaker response. It was observed that when the optimum concentration of KIN (8 μM) was supplemented with low concentration of BAP (2 μM or 4 μM) there was a synergistic effect on the explant in terms of shoot regeneration. Single shoots were rooted on a half strength medium with 2 μM indole-3-butyric acid.

Key words: cytokinin, multiple shoots, nodal explants, *Portulaca grandiflora*, synergistic effect.

Introduction

Plants are a traditional source for many chemicals used as pharmaceuticals, biochemicals, fragrance, food colour and flavours (Leung 1980). Betalains are one such commonly used food additive. The interest of the food industry on this compound has grown after they were identified as antifungal (Kimler 1995), antioxidant (Escribano et al. 1998) and having positive health effects on humans (Tesoriere et al. 2004). Its wide range of pH stability also makes it an important natural colour in the juice industry.

The family Portulacaceae is characterized by the occurrence of betalains. *Portulaca grandiflora* Hook is a popular ornamental plant rich in betaxanthins, a subclass of betalains. Portulal, a root-promoting substance, has also been reported from this plant (Mitsuhansh et al. 1969) with a potential applicability in tissue culture.

An efficiently reproducible and rapid *in vitro* regeneration system is a prequisite for improving the conventional plant breeding programmes. Genetic transformation is one of the promising methods where specific traits can be added with minimal alteration of the target plant genome. Therefore, direct shoot morphogenesis from the primary tissue is more desirable than via an intermediate callus phase (Larkin, Scrowcroft 1981). In

this regard a method for the large scale propagation of *Portulaca grandiflora* needs to be developed. The aim of the present work was to study the influence of cytokinins on shoot formation from nodal explants of *P. grandiflora*, with the aim to obtain a fast and efficient regeneration.

Materials and methods

Murashige and Skoog (1962) culture medium supplemented with 3 % sucrose and the gelling agent (0.8 % agar; w/v) was used. Different concentrations of kinetin (KIN) (2 to 10 μM) and 6-benzylaminopurine (BAP) (2 to 10 μM) individually and in combination were added to this medium. The pH of the medium was adjusted to 5.8. The medium (20 mL) was dispensed in culture tubes which were closed with cotton bunks and further capped with paper. The culture vessels containing the media and instruments were autoclaved at 121 °C for 30 min.

Nodal explants were obtained from a healthy population of mature plants of *Portulaca grandiflora* growing in the botanical garden of the Maharaja Sayajirao University, Vadodara. The explants were kept in running water for one hour, washed with a plant detergent (Tepol) for 5 min, and again rinsed thoroughly with sterile distilled water four to five times. Explants were surface sterilized with 0.1 % (w/v) $HgCl_2$ for 3 min and rinsed with autoclaved double distilled water four to five times. The nodal explants were cut to appropriate size (2.5 to 3.5 cm) with three to four nodes and inoculated vertically on the culture medium. A single explant was placed in each culture tube. In total 15 replicates were used per treatment and the experiment was repeated twice. All cultures were maintained at 25 ± 2 °C under a 12 h photoperiod of irradiance provided with white florescent tube. The experiment was monitored for a period of 6 weeks and data for number of shoots per explant and number of leaves per explant was recorded. Standard error of the mean for each value was calculated.

For root induction, the shoots were harvested from culture tubes and transferred on half strength MS medium supplemented with 2 μM indole-3-butyric acid.

Results

Nodal explants of *P. grandiflora* were cultivated on MS medium supplemented with different concentrations and combinations of two cytokinins, BAP and KIN (2 to 10 μM). Withn three to four days after explanting the axillary bud proliferated and young leaves were observed. All the concentrations of individual BAP and KIN as well as their combinations showed enhanced shoot formation. The young multiple shoots formed were pale yellow, turning pink due to formation of betalain pigment. Axillary branching and multiplication of shoots occurred without a callus phase. Shoot tips showed necrosis after five to six days of cultivation. To avoid this effect, shoots were subcultured frequently on fresh medium of the same combination.

Of the two cytokinins BAP was found to be more effective in inducing multiple shoot formation as compared to KIN (Table 1, Fig. 1A and B). It was also observed that the number of shoots increased with an increase in concentration of BAP, while the number of shoots/explants decreased with increase of KIN concentration (Table 1).

New shoots initiated from each developing bud adjacent to the primary axillary shoot

Table 1. Effect of cytokinins kinetin (KIN) and 6-benzylaminopurine (BAP) on development of nodal explants of *Portulaca grandiflora* after four weeks in tissue culture

Cytokinin concentration (μM)		Number of shoots per explant ± SE	Number of leaves per explant ± SE
KIN	BAP		
-	-	0	0
2	-	2.8 ± 0.2	9.7 ± 1.0
4	-	2.2 ± 0.3	7.2 ± 0.9
8	-	2.5 ± 0.3	9.1 ± 1.1
10	-	2.7 ± 0.3	9.3 ± 1.1
-	2	2.7 ± 0.9	7.2 ± 1.8
-	4	3.2 ± 1.1	8.5 ± 3.0
-	8	3.5 ± 0.7	7.5 ± 1.0
-	10	3.7 ± 0.3	9.5 ± 1.0
2	2	2.8 ± 0.4	8.0 ± 0.6
2	4	2.5 ± 0.3	8.6 ± 0.4
2	8	2.4 ± 0.6	8.5 ± 1.2
2	10	3.1 ± 0.6	8.5 ± 1.4
4	2	3.3 ± 0.2	10.5 ± 1.0
4	4	3.1 ± 0.4	7.0 ± 1.2
4	8	3.6 ± 0.6	8.8 ± 1.6
4	10	3.6 ± 0.6	9.6 ± 0.8
8	2	4.0 ± 0.6	11.8 ± 2.8
8	4	3.8 ± 1.7	12.0 ± 1.9
8	8	3.2 ± 0.4	7.1 ± 0.7
8	10	3.0 ± 0.8	9.5 ± 2.2
10	2	2.7 ± 0.5	6.5 ± 1.0
10	4	3.7 ± 0.5	9.5 ± 1.3
10	8	2.7 ± 0.5	7.5 ± 1.8
10	10	2.0 ± 0.4	7.3 ± 0.8

within a week. All regenerated shoots were free from callus tissues at their proximal ends. For BAP alone the maximum effect was observed at 10 μM, while for KIN at 2 μM (Table 1). Similar results were observed for leaf formation. The number of leaves per explant increased with anincrease in BAP concentration with the maximum response at 10 μM. The number of leaves per explant decreased with increase in KIN concentration (Table 1).

When KIN was combined with BAP, it was found that KIN stimulated faster BAP-dependent shoot growth. When 2 μM KIN was used with increasing concentration of BAP (2 to 10 μM) the response was similar to that of KIN alone (Table 1). With 4 μM KIN and varying concentration of BAP (2 to 10 μM) there was an increase in the number of shoots per explant and the number of leaves per explant (Table 1). The maximum effect on shoot formation was achieved with 8 μM KIN and BAP (2 to 10 μM). When KIN was kept constant at 8 μM and concentration of BAP was increased gradually, it was observed

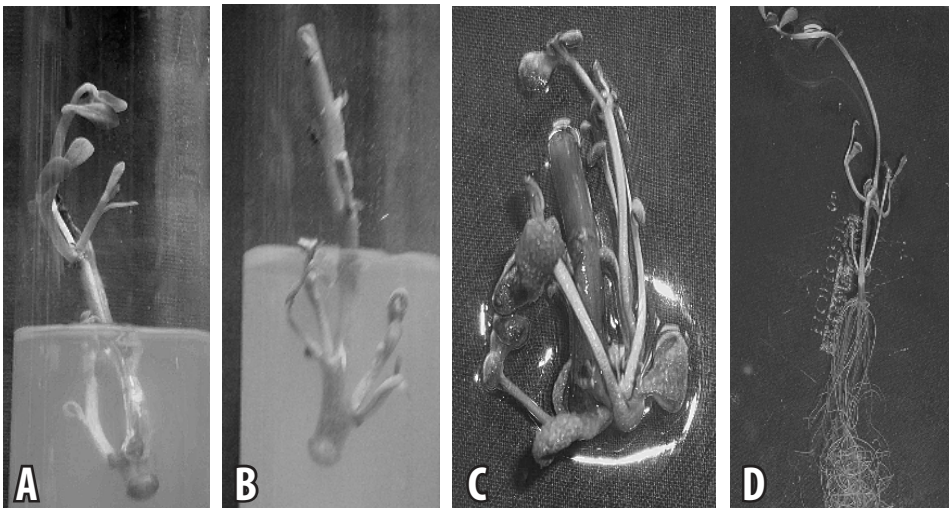

Fig. 1. Multiple shoot formation in *Portulaca grandiflora*. A, xillary bud proliferation in 2 μM kinetin. B, induction of multiple shoots in 2 μM 6-benzylaminopurine. C, synergistic effect of cytokinin (8 μM kinetin and 2 μM benzylaminopurine) D, rooting of the regenerated shoots in the presence of 2 μM indole-3-butyric acid.

that the frequency of multiple shoot formation and average number of leaves per explant decreased (Table 1). This suggests that at optimum concentration of KIN (8 μM) a low concentration of BAP (2 μM or 4 μM) is best suited for multiple shoot formation and for elongation of shoots. Further increase in KIN concentration (10 μM) lowered the number of shoots and a minimum was reached when the concentration of BAP also increased to 10 μM (Table 1). Thus the best response was obtained at 8 μM KIN plus 2 μM BAP (Fig. 1C) allowing to consider synergistic effect of the two cytokinins.

The regenerated axillary shoots were excised and transferred to ½ strength MS medium with sucrose (1 %) supplemented with 2 μM indole-3-butyric acid for induction of root formation. Root formation (Fig. 1D) was observed after 8 to 10 days. Initially roots were thin and slender. However, within three weeks they developed into slightly stout roots which were ready for hardening.

Discussion

In the present investigation cytokinins BAP and KIN were used for multiple shoot formation in *Portulaca grandiflora in vitro*. In general, bud break and development of shoots from stem node explants is a function of cytokinin activity (Sahoo, Chand 1998). In the present study axillary shoot buds were formed under the influence of both cytokinins. Further more the axillary branching and multiplication of shoots occurred without a callus phase. Similar findings have been reported in *Pterocarpus* on treatment with BAP and KIN (Anuradha, Pullaiah 1999).

Shoot tip necrosis was encountered in this experiment, which was augmented by subculturing on the same medium frequently. The use of cytokinins as plant growth regulators for cultures frequently is associated with the problem of shoot tip necrosis,

which can be solved by frequent subculture. Stem necrosis was also found in *Pterocarpus*, which was also reduced by subculture (Anuradha, Pullaiah 1999).

It was found that of the two individual cytokinins BAP was better than KIN in inducing multiple shoot formation. Cytokinins, especially BAP, are reported to overcome apical dominance, release lateral buds from dormancy and promote shoot formation (George 1993). Superiority of BAP in inducing multiple shoot formation has also been reported for a number of plants e.g. *Tridax procumbens* (Sahoo, Chand 1998), *Cypripedium flavum* (Yan 2006) and *Medicago truncatula* (Neves et al. 2001). For BAP alone, the best effect was obtained at 10 μM and a similar optimal concentration was also reported for *Ceropegia sahyadrica* (Nikam, Savant 2007) and *Andrographis paniculala* (Purkayastha et al. 2008).

KIN alone was not very efficient in inducing shoot bud multiplication in the present experiments. Low rate of multiplication in medium containing KIN has been observed in a number of plants e.a. *Bambusa balcooa* (Mudai, Borthakur 2009), *Ocimum gratissimum* (Gopi et al. 2006) and *Mentha arvensis* (Chishti et al. 2006). Thus, individually both cytokinins were found to induce multiple shoot formation, but the effect of BAP was more pronounced than that of KIN. Further it was noted that when the low concentration of KIN was supplemented with BAP, a synergistic influence on nodal cultures of *Portulaca grandiflora* was found in terms of number of shoots. A similar response is typical for a cytokinin combined with an auxin, which has been reported in a number of plants.

It was found in the present study that when the low KIN concentration was supplemented with BAP, both the number of shoots per explant and the number of leaves per explant increased. Optimum response was obtained at 8 μM KIN plus 2 μM BAP, supporting the synergistic effect of high concentration of KIN together with low concentration of BAP in *Portulaca grandiflora*. This type of effect has also been reported in many plants e.a. *Legenaria siceraria* (Shyamali, Kazumi 2007). Synergism of BAP and KIN was also reported in rootstock 1613C *Vitis solonis* × *V. labrusca* (Kumar et al. 2008).

The regenerated shoots were rooted in half strength MS medium with 1 % sucrose, supplemented with 2 μM IBA. Similarly, best rooting at half strength MS medium supplemented with IBA was obtained in *Vitex* (Balaraju et al. 2008), *Pappea capensis* (Mngomba et al. 2007), and *Bambusa balcooa* (Das, Pal 2005). Thus in the present investigation the development of nodal explants of *Portulaca grandiflora* in respect to multiple shoot formation *in vitro* was better in the presence of two cytokinins (BAP and KIN).

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