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MICROPROPAGATION POTENTIAL OF *POLIANTHES TUBEROSA* L BULBS, SCALES AND LEAVES

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To enhance the rate of multiplication of *Polianthes tuberosa, in vitro* techniques were applied. The micropropagation potential of bulb and leaf tissues was explored. Twin scale and chip explants from the lower halves and single scale explants from the upper halves of the bulbs were cultured on MS medium supplemented with different concentrations of naphthalene acetic acid (NAA) and benzyl aminopurine (BAP). bulblet induction was pronounced on both twin scales and chips. Bulblet formation and gain in size was greater in the case of chips than in twin scales, indicating an influence of the size of the explant. Bulblet induction on single scale explants of tuberose bulbs was also observed. Leaf blade explants formed callus on MS medium supplemented with defined hormonal concentrations of BAP, *AA and Kin. Psuedoembryogenic bodies regenerated from the calli.

Key words: *Polianthes tuberosa* , Micropropagation, ; Bulblets, Psuedoembryogeny.

Introduction

Tuberose is a slow growing tender summer flowering bulb. The bulb multiplies at a rate of 5 - 8 daughter bulblets per annum. These bulblets take at leaset 3 - 4 seasons to mature and attain flowering size in favourable conditions. The mature bulb produces only one floral stalk and then dies off. Hence the rate of multiplication is quite low.

Propagators of bulbs have developed means of increasing the natural slow rate of daughter bulb production because seedling may have a juvenile phase lasting several years. Also they may not breed true - to - type in clonally propagated cultivars or they may be sterile. Hence biotechnology, a means of speeding up propagation of bulbous cultivars into commercial production, is required to save time and cost.

Utilization of the underground portion of ornamental monocots for micropropagation was first introduced by Stone and Hollings (1970). Bulblet formation was induced by planting the vertically divided segments of the bulbs on suitable culture media. Meanwhile the use of pieces of single scale with some basal plate tissue attached was also tried, but the percentage success was very low (Alkema 1971; Broertjes and Alkema 1971; Alkema 1975).

Chipping was introduced by Stone *et al* (1973). The whole bulbs were radially divided into 8 to 16 pieces, each piece consisting of 7 - 8 scales joined with a piece of basal plate. Initially chips were sown in soil in polyethene bags. Later, soil was replaced with defined culture media for *in vitro* propagated chips (Flint and Hanks 1982).

Twin scaling, the most advanced technique, was developed from earlier work on the propagation of *Hippeastrum, Narcissus* and other *Amarylids*. From one large bulb, 30 - 60 twin scales could be obtained. Each twin scale consisted of two scales with a small piece of basal plate. It has proved successful for a wide range of Amaryllidaceous species as well as *Irises* and certain other Liliaceaous species (Topsett 1972; Hussey 1982).

Organ culture of tuberose chipping and twin scaling was tried along with tissue culture procedures to enhance the rate of propagation.

Materials and Methods

Large single nosed round and healthy bulbs of tuberose were selected from local nursery stocks. The bulbs were prepared by removing the brown outer scales (tunic), dead roots and remaining foliage by trimming away the dirty outer crust of basal plate and by cutting off and discarding the top part of the bulb so as to give a flat top, using a sharp razor blade. The bulbs were placed downwards on a work surface and cut into segments.

Chips. Bulbs were cut into halves, quarters, eights or sixteens, ensuring that each segment retained a triangular piece of basal plate to which the bulb scales were attached. Each segment represented a chip.

Twin scales. For twin scale preparation, the upper two-thirds of each bulb was removed. Other procedures were the same as

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for chip preparation i.e. eight/sixteen segments were gained. Each segment was then carefully cut into twin scales with a scalpel, starting at the outside of each segment and gently peeling back the two outer-most scales from the remainder of the segments and cutting down through the basal plate tissue to separate the pairs of scales from the remaining segment. Repeated work provided at least 3 - 4 fold more twin scale explants than that of chips per bulb.

Explants were sterilized prior to inoculation, by dipping in alcohol for 30 seconds followed by immersion in 0.1%(wv^{-1}) $HgCl_2$ solution for 20 minutes. They were then washed three times with sterilized double-distilled water.

MS medium (Murshige 1962) was used throughout the study. Growth regulators and their concentrations added to the culture medium are given when required in text. The pH of the medium was adjusted with 0.1 N NaOH/HCl at 5.7. Agar was added @ 0.7%. The medium was autoclaved at 121°C at 15 psi for 20 minutes.

Results and Discussion

A mixed array of results was produced when chip explants made from tuberose bulbs were inoculated in media supplemented with NAA (0.0 - 4.0 mg l^{-1}) and BAP (0.0 - 16.0 mg l^{-1}). Bulblet formation was achieved in 10 out of 30 concentrations of NAA and/or BAP. Striking results were obtained in medium supplemented with 0.5 mg l^{-1} NAA alone. Bulblets with small green leaves appeared near the basal plate. The chip explants then formed voluminous callus with some browning and root regeneration (Table 1). Similar results, but without root regeneration were achieved in medium supplemented with 0.25 mg l^{-1} NAA and 0.8 mg l^{-1} BAP. Concentrations of NAA and BAP @ 4 and 16 mg l^{-1} respectively proved suitable for inducing and regenerating bulblets in the axils of scale leaves of the chip explants. No results were achieved in 13 out of 30 concentrations tested. Some other concentrations however produced only swelling in the explant and browning.

The responses of twin scales in media supplemented with NAA and BAP were also mixed. The experiment showed that the most concentrations tested either did not give positive results or only some swelling of the explants accompanied by browning, root regeneration and/or callusing (Table 2). Among the more significant results swelling, bulblet formation with the emergence of small green leaves and also some callusing from the twin scales was observed in medium supplemented with 2.0 mg l^{-1} NAA only. Bulblets formed at the basal plate of explants accompanied by the growth of small green leaves and also some callusing in medium supplemented with 1.0 mg l^{-1} NAA and 2.0 mg l^{-1} BAP. Similarly, small bulbs with emerging green leaves were also observed in medium supplemented with 2.0 mg l^{-1} NAA and 16.0 mg l^{-1} BAP.

Explants from leaf blade and leaf base without basal tissue were cultured on bulb-inducing media as for chips and twin scales and on the medium with 1.0 mg l^{-1} each NAA and IAA, 2.0 mg l^{-1} Kin and 7.0 mg l^{-1} BAP. Results are shown in Table 3 and 4.

Responses of the baseless scales of *Polianthes tuberosa* bulbs to media supplemented with NAA and BAP concentrations are shown in Table 3. Bulb scale segments showed an overall tendency to regenerate small bulblets in 3 out of 4 media tested. Swelling of the explants with no bulb formation was observed in medium supplemented with 16.0 mg l^{-1} NAA only, but the phenomenon of bulblet regeneration from a single leaf was observed in media supplemented with 4.0 mg l^{-1} only. Numerous small green bulblets upto 0.5 cm in length were formed in this medium (Table 3). Comparable results were also obtained using media supplemented with 2.0 mg l^{-1} NAA alone or 4.0 mg l^{-1} NAA along with 2.0 mg l^{-1} BAP.

Voluminous fast growing callus was observed from the leaf blade explants of *Polianthes tuberosa* grown on the MS medium supplemented with 1.0 mg l^{-1} each of NAA and IAA, 2.0 mg l^{-1} Kin and 7.0 mg l^{-1} BAP (Table 4). The calli were nodular and friable and a number of green spots appeared during culture. Extensive root regeneration was also observed from the calli in this medium. Moderately fast growing white and green calli with a nodular texture were observed in another medium tested which had a combination of 1.0 mg l^{-1} NAA and 5.0 mg l^{-1} BAP (Table 4).

Twin scales cultured on MS medium supplemented with NAA (0.0 - 2.0 mg l^{-1}) and BAP (0.0 - 16.0 mg l^{-1}) exhibited range of results. Effects of NAA were more pronounced than those of BAP. Increasing the concentration of NAA (1.0 - 2.0 mg l^{-1}) alone effectively induced callogenesis and bulblet formation. Bulblets formed the plantlets with green leaves. Swelling, browning and root regeneration of twin scale explants was noted at low and high concentrations of BAP alone. 1.0 - 2.0 mg l^{-1} NAA in combination with BAP also modified the behaviour, inducing green leaved bulblets.

Chips explants of *P. tuberosa* were also cultured on MS medium supplemented with auxin and cytokinins i.e. 0.0 - 4.0 mg l^{-1} NAA and 0.0 - 16.0 mg l^{-1}BAP. Responses of chips to BAP alone were similar to those of twin scales with signs of swelling and browning, whereas slightly changed in the case of NAA alone. Green leaved bulblets were formed at lower concentrations of NAA i.e. 0.5 and 1.0 mg l^{-1}. Bulblet formation and the production of green leaved plantlets with

occasional callusing and browning, seemed to be exhibited under the influences of lower concentrations of NAA (0.25, 0.5 and 1.0 mg l^{-1}).

A shift in the effective range of NAA towards lower concentrations for bulblet induction in chips (0.25 - 1.0 mg l^{-1}) to that required for twin scales (1.0 - 2.0 mgl^{-1}) was probably due to differences in the size of explants. Chips were usually double or triple the size of twin scales. The larger number of scale leaves and the larger meristematic area of the chip increased the chances of regeneration per explant. Secondary twin scales totally depended on the exogeneously supplied nutrients and growth regulators for their survival and regeneration potential.

Exogenously induced bulblet formation has been frequently reported in the literature. Direct somatic organogenesis from a variety of explants such as basal plate tissue and halved shoot bases (Pandey *et al* 1992; Stanilova *et al* 1994)and bulb scales and twin scales (Steinitz and Yahel 1982; Yang *et al* 1989; Huang and Liu 1989) of bulbous and cormous plants has been reported by several workers. According to an unpublished data on micropropagation of ornamental monocotyledonous plants propagating through somatic embryogenesis (SE), somatic organogenesis(SO) and direct somatic organogenesis(DSO), 63.1% culture media on which species exhibited direct somatic organogenesis contained NAA (0.25 - 3.0 mg l^{-1}) while 73.6% culture media contained BAP (0.1 - 3.0 mg l^{-1}) alone. Combination of NAA + BAP was also determined in 31.5% primary culture media employed for direct organogenesis. Twin scales and chips of *Polianthes tuberosa* responded to NAA and BAP in the range of 0.0 - 4.0 mg l^{-1} and 0.0 - 16.0 mg l^{-1}respectively. Their *in vitro* response is expressed in terms of % culture media - primary and/or secondary, incroporated with different concentrations and combination of growth regulators capable of inducing SE, SO or DSO.

Table 1

Response of the chip explants of *Polianthes tuberosa* bulbs to the media supplemented with various NAA and BAP concentrations

BAP (mg l^{-1})	NAA (mg l^{-1})					
	0.0	0.25	0.5	1.0	2.0	4.0
0.0	N	BN	GBuCBR	BuG	N	N
2.0	S	BuG	BuG	N	N	NB
4.0	SB	BuG	BG	Bu	SC	BuG
8.0	N	$_{B}$GuCB	BuGB	N	N	N
16.0	SB	GBuB	N	N	NB	BuGR

N,No response; S, Swelling of the explants; B,Browning of the explant/ callus; Bu, Bulb formation; G,Green leaves; R,Root regeneration; C, Callusing.

Callogenesis of the explants regenerating bulblets indicated that regeneration might not have been mediated through callus. Combination of NAA and BAP was reported in 18.5% and 11.1% primary & secondary culture media respectively inducing somatic organogenesis whereas 14.8% of primary and culture secondary media each contained 2,4-D with BAP, inducing somatic organogenesis i.e. shoot apices, which sometime regenerated roots on the same medium as in case of *Amaryllis* (Seabrook and Cumming 1977). NAA and BAP combination effectively induced bulblets in the bulb scale segments of *P. tuberosa* removed from the upper half of the bulb. Combination of NAA and BAP @ 4.0 mg l^{-1} and 2.0 mg l^{-1} respectively induced multiple bulblets. Peck and Cumming (1986) have reported somatic organogenesis from bulb scales of *Muscari armenicum* in presence of 0.5 mg l^{-1} BAP, 2.0 mg l^{-1} NAA and adenine in primary and 2.5 mg l^{-1}

Table 2

Response of the chip explants of *Polianthes tuberosa* bulbs to the media supplemented with various NAA and BAP concentrations

BAP (mg l^{-1})	NAA (mg l^{-1})				
	0.0	0.25	0.5	1.0	2.0
0.0	N	B	N	SC	SGBuC
2.0	SB	-	SG	BuCG	-
4.0	SB	R	SC	Bu	-
8.0	SB	N	-	-	CB
16.0	SBR	N	-	RS	BuG

N,No response; S, Swelling of the explants; B,Browning of the explant/ callus; Bu, Bulb formation; G,Green leaves; R,Root regeneration; C,Callusing.

Table 3

Response of the chip explants of *Polianthes tuberosa* bulbs to the media supplemented with various NAA and BAP concentrations

NAA (mg l^{-1})	BAP (mg l^{-1})	Bulb formation (+/-)	Remarks
0.0	16.0	-	Explants only swelled up
2.0	0.0	+	Explants turned green with the formation of green small bulblets. Turned brown after 9-10 weeks.
4.0	0.0	+	Green, small bulblets, 0.5 cm in length.
4.0	2.0	+	Single scale leaves formed number of bulblets

Table 4

Callusing from leaf explants of *Polianthes tuberosa*

Growth regulators (mgl^{-1})				Callus characteristics			
NAA	IAA	KIN	BAP	Quantity	Colour	Texture	Remarks
1.0	-	-	5.0	+ +	White and green	Nodular and friable	Moderately growing callus. Response not very good.
1.0	1.0	2.0	7.0	++ + +	White and green	Nodular and compact	Very voluminous callus growth. Number of green spots appeared on callus masses. Extensive root regenerations.

NAA in secondary culture medium. Bulblet regeneration in leaf segments of *Ornithogallum maculatum* in presence of NAA and BAP (2.0 mg l^{-1} each) was also reported by Rensburg *et al* (1988).

There was a distinct difference in response of chip, twin scales and bulb scale segment all obtained from the bulbous portions. Although chips and twin scales carried basal meristematic tissues of basal plate but formed single bulblet per explant; multiple bulblet induction per explant of bulb scale segment was due to the response of large wounded area or cut ends. The case is similar to bulblet differentiation in bulb scale segments of *Lilium longiflorum* being discussed by Ishioka and Tanimoto (1990). It was stated that the number of adventive bulblet formation on medium with auxin and cytokinin increased in proportion to the wounding area of the bulb scale when explants were treated with one of the preserved wounding hormones traumatic acid.

Leaf segment response indirectly routed regeneration different from the scale segments. The leaf explants formed callus on high concentration of NAA and BAP. So the explants were cultured on the medium supplemented with four growth regulator combination tried previously for somatic embryogenesis from floral stalk explants. Nodular and friable textured calli formed in voluminous quantities on 1.0 mg l^{-1} IAA and NAA, 2.0 mg l^{-1} Kin, and 7.0 mg l^{-1} BAP. Morphologically appearance of the established cultures resembled to those of filamentous ones (Zaidi *et al* 1994). Histomorphological analysis of the leaf segment culture was same as those of filament i.e. the formation of protocormaceous bodies as discussed by Tian and Yang (1984) in case of gynogenic embryoids and callus in ovary culture of *Oryza sativa.*

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