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Short Communication

Direct shoot generation from florets of chrysanthemum cultivars

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The National Botanical Research Institute, Lucknow has developed a series of unique chrysanthemum genotypes utilizing the existing germplasm stocks through conventional selective breeding and induced mutation breeding. Chrysanthemum can be propagated by seeds but due to extremely heterozygosity, vegetative propagation is preferred (cutting, suckers) for commercial purpose. But multiplication by this method is much too slow to be commercially practical. In vitro regeneration of chrysanthemum is well established from shoot apex [1-3] and nodal explants [4, 5]. Reports of adventitious shoot regeneration from floret explants of chrysanthemum are also available, but in all cases shoots were produced from floret derived callus [6-8] and there is always a loss of some genetic homogeneity with a lengthy callus phase [6, 8, 9]. Recently, a highly efficient direct adventitious shoot regeneration system from florets of chrysanthemum has been developed [10]. Moreover, contamination from floret explants are very low. In the present study an attempt has been made to develop protocol using direct shoot regeneration system from ray florets of 28 chrysanthemum genotypes for commercial exploitation.

For standardization of micropropagation, a wide range of varieties including different bloom types and colour were selected (Table 1). Six varieties [1-6] are already in international floriculture trade, ten [7-16] are highly valued standard type commercial varieties, five [17-21] are well- accepted cut flower varieties and the remaining seven [22-28] are unique 'no-pinch no-stake' mini chrysanthemum varieties.

Outer whorl florets from half-bloom flower-heads were collected from all the cultivars and were washed thoroughly under running tap water for 15 min and for another 5 min with a 5% aqueous solution of Teepol (liquid detergent). Florets were then repeatedly washed with single distilled water followed by a quick dip (30 sec.) in 70% ethanol and surface sterilized with 0.1% (w/v) $HgCl_2$ for 2 min followed by repeated rinsing with sterile double distilled water. MS medium [11] supplemented within 0.2 mg/l α-naphthaleneacetic acid (NAA), 1 mg/l 6-benzylaminopurine (BAP), sucrose (3%) and bacto-agar (0.8%) was used [10] for direct regeneration of shoot buds. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 15 min. The cultures were incubated under 16 h photoperiod at 25 ± 1°C.

Half strength MS medium supplemented with 0.1 mg/l NAA, 1.5% sucrose and 0.8% bacto-agar was used for rooting of about 2.5 cm. isolated shoots. Regenerated plants with well-established roots were transferred to potted soil containing a mixture of sand:soil:manure (1:1:1 v/v) and kept in a chamber with 80-90% relative humidity for 15 days before transfer to glasshouse conditions.

The basal portion of the florets became swollen after 3-6 days and callus formation was visible after 7 days along the cut surface. Shoot bud formation was visible under stereomicroscope (Figure 1a) earliest after 10 days (Haldighai) for latest after 24 days (Sonar bangla). Direct shoot regeneration always took place from the basal portion of the florets (Figure 1b). The results of shoot regeneration from florets of all the cultivars are shown in Table 1. Maximum regeneration potential was found in cv. "Regol Time" (70.0%) and the minimum was in 'Royal Mundial' (8.8). Out of shoot bud initiation, frequency of regeneration and average number of shoots per responding explant varied among the cultivars. However, four large flowered cvs. (Snow Ball, Silver Globe, Green Sleeve and Diamon Jubilee), one small flowered (White Stay Four) and one mini var. (Diana) showed no regeneration. Five large flowered cultivars were cultured on higher dose of NAA and BAP (MS + 1 mg/l NAA + 2mg/l BAP). Interestingly, all the large varieties showed higher regeneration, except

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Table 1. Shoot bud regeneration from ray florets of different chrysanthemum cultivars observed in the present study

S. No.	Name of the cultivar	Bloom type	Colour	Shoot formation (%)	No. of shoots/responding explant	Shoot bud initiation (days)
A. Small varieties (International commercial varieties)						
1.	Regol Time	Double Korean	Yellow	70.00	7.7	14
2.	Fun Shine	"	Pinkish white	30.00	7.5	27
3.	Royal Mundial	"	Light purple	6.6	8.8	20
4.	Bronze Mundial	"	Light purple	26.5	6.1	15
5.	Mundial	"	Pinkish white	16.6	8.0	17
6.	White Stay Four	"	White	0	0	0
B. Large varieties						
7.	Kiku Biori	Incurved, tubular 1/3 open at tip	Inner surface Green (Gr. 8B)	20.0	2.5	22
8.	William Turnar	Reflex, irregular tendency	White (Gr. 155-B)	35.5	3.0	23
9.	Gypsy Queen	Spider, tubular, tip opened hooked	White	30.0	5.1	21
10.	Sonar Bangla	Incurved, outer petal tubular, half petal open, spatula type	Yellow (Gr. 4C)	9.0	4.3	24
11.	Alfred Wilson	Reflex	Inner surface red (Gr. 53A)	11.1	2.3	23
12.	Ottome Zakura	Ball shaped, decorative	Purple (Gr. 69)	20.0	4.7	21
13.	Snow Ball	Incurve	White	0	0	0
14.	Silve Globe	Incurve	Deep pink	0	0	0
15.	Green Sleeve	Incurve	Whitish Green	0	0	0
16.	Diamon Jubilee	Quilled (Spider), tubular floret, tip open and hooked	Inner surface yellow (Gr. 9B) Outer surface yellow (Gr. 9c)	0	0	0
C. Small varieties (Developed at NBRI)						
17.	Sharad Har	Double Korean	Yellow (Gr. 22A)	25	2.6	19
18.	Vijay	Double Korean	Yellow orange (Gr. 22A)	25	2.6	19
19.	Kumkum	Decorative	Garnet brown	11.7	4.5	21
20.	Cotton Ball	Pompon	White	27.2	6.5	15
21.	Birbal Sahani	Pompon	White (Gr. 155B)	56.2	5.6	17
D. Mini varieties						
22.	DWS-19	Double Korean	Yellow seedling (bicoloured) tip red	62.1	8.3	11
23.	Haldighati	1-3 whorl, flat, disc prominent	Bright yellow	40.0	4.1	10
24.	Cameo	Decorative	Purple (Gr. 77C)	13.3	3.5	14
25.	Pancho	Single whorl, flat, disc prominent	Orange-yellow	40.0	5.5	15
26.	Swarn-Singar	Double korean	Yellow (Gr. 12a)	11.1	4.5	16
27.	Hemant Singar	Single whorl, flat, disc prominent	Deep pink	13.6	3.0	13
28.	Diana	1-3 whorl florets, disc prominent	Deep red	-	-	-
$C.D_{0.05}$				7.33	1.06	

Table 2. Shoot bud regeneration from ray florets of large flowered chrysanthemum medium : MS + 1 mg/l NAA + 2mg/l BAP + 30 gm/l Sucrose + 0.8% bacto-agar)

S.N.	Name of cultivar	Shoot bud formation (%)	No. of shoots/responding explants	Shoot initiation (days)
1.	Diamond Jubilee	40.0	10.8	24
2.	Kiku Biori	20.0	14.0	20
3.	Green Sleeve	80.0	16.6	24
4.	Snow Ball	30.0	16.0	27
5.	Silver Globe	0	0	0

Silver Globe where there was no regeneration (Table 2). Differential regeneration potential due to the physiological status of different genotypes is well established [10, 12-16]. The regenerated shoots were separated from the initial explants and cultured in the same medium for multiplication (Figure 1c). About 100% rooting was observed within one week of transfer of shoots to the rooting medium. Rooted plantlets were transferred to potted soil and kept in a humid chamber for hardening (Figure 1d) before their transfer to glasshouse. All the plants survived in the glasshouse conditions where they grew vigorously and flowered true to the mother floret colour genotype.

An attempt was made to estimate the plantlets production rate of cv. Cotton Ball. 22 explants (ray florets) were inoculated and six were regenerated and the total number of shoot buds were 39. Each shoot was inoculated into same medium for multiplication and the process was repeated upto 12 weeks. Average number of shoot buds per responding explant were 5.4, 5.5 and 6.0 after 4, 8 and 12 weeks, respectively. Extrapolating this rate of increase, approx. 68129.42 plantlets could be produced from one ray floret within 28 weeks (Table 3).

The results clearly indicated that direct floret regeneration protocol has tremendous potential to be exploited at an industrial level for commercial floriculture in chrysanthemum.

Table 3. Expected rate of multiplication of one ray floret of C. morifolium cv. Cotton Ball during 28 weeks of culture in an optimum standerdized media

Steps	No. of shoots produced		Period of time
I	1×1.77	= 1.77	4 weeks
II	1.77×5.4	= 9.55	8 weeks
III	9.55×5.5	= 52.56	12 weeks
IV	52.56×6.0	= 315.41	16 weeks
V	315.41×6.0	= 1892.48	20 weeks
VI	1892.48×6.0	= 11354.90	24 weeks
VII	11354.90×6.0	= 68129.42	28 weeks

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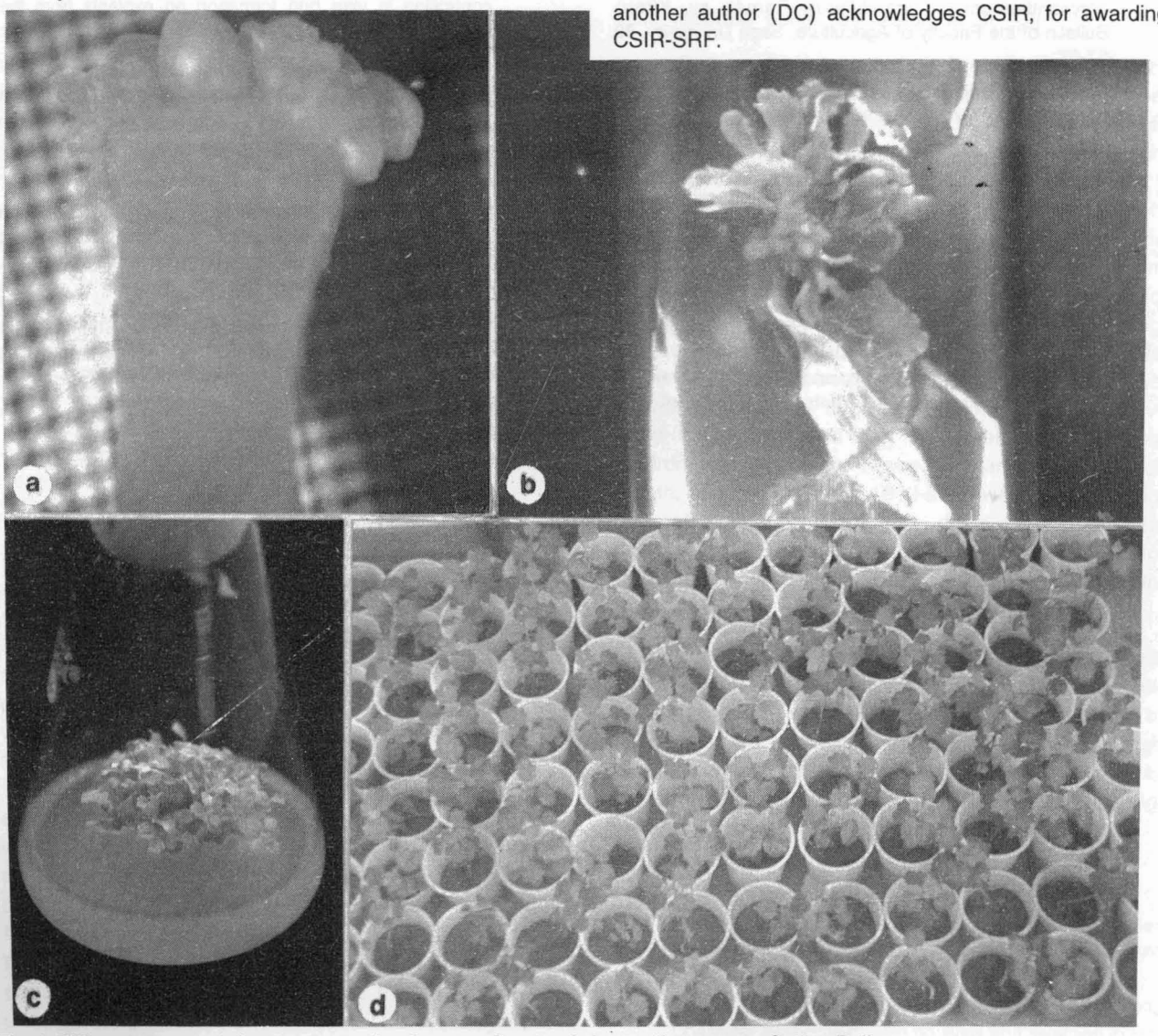

Fig. 1. Different stages of shoot regeneration from ray florets of chrysanthemum cv. Cotton Ball.
(a) Streomicrophotograph showing direct shoot regeneration from ray florets
(b) Direct shoot regeneration from ray florets after 28 days of culture
(c) Multiplication of regenerated shoots
(d) Hardening of regenerated chrysanthemum plantlets in large scale

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