



Standardization of regeneration protocol for small flower chrysanthemum (*Chrysanthemum morifolium*) cultivars*

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A different type of protocol for chrysanthemum tissue cultures has already been standardized and this technique has been widely exploited. Chrysanthemum is globally the third economically most important floricultural crop. Chrysanthemum *in vitro* culture technique was extremely useful for producing a huge number of explants in a short span of time (Dao *et al.* 2006). Tissue culture studies in chrysanthemum has been done as a tool for mutation induction in the Floriculture Section, National Botanical Research Institute, Lucknow (Misra *et al.* 2004), chimera management (Chakraborty *et al.* 2002) and as a means of micro propagation (Dwivedi *et al.* 2000).

The experiment was conducted during 2009–10. Ray florets explant were collected from flower head of five *C. morifolium* cultivars, viz Purnima, Pooja, Otome Zakura, Flirt

and Little Darling. Explants were surface sterilized and aseptically placed in Petri dishes containing 25.0 ml of liquid half strength MS medium. Ray florets were cut and inoculated on four different MS medium (Murashige and Skoog 1962) and kept in a growth chamber at 26 ± 2°C under 16 hr illuminations. Callus initiation was recorded after four weeks of incubation. Callus initiation, callus weight, percentage of somatic embryogenesis, shoot formation, number of shoots/explant and shoot length derived from the ray floret base explants were recorded. Data on production of callus of five cultivars of chrysanthemum (*C. morifolium*) were recorded after four weeks of incubation. Callus formation varied broadly among chrysanthemum cultivars (Fig 1) (Table 2). Our observations indicates that the protocols A and B gave the highest average of callus induction (Table 2).

Table 1 MS composition of four media used in chrysanthemum cultivars for callus induction, shoot differentiation and root induction

	Callus initiation	Shoot differentiation	Root induction	Callus initiation	Shoot differentiation	Root induction
MS components		(Medium A)			(Medium B)	
MS Media	1 X	1 X	½ X	1 X	1 X	½ X
NAA (mg/l)	0.5	0.5		0.5	0.5	0.025
BAP (mg/l)	1.25	1.25		0.75	0.75	
Sucrose (g/l)	30.0	30.0	15.0	30.0	30.0	15.0
Agar (g/l)	8.0	8.0	6.0	8.0	8.0	6.0
		(Medium C)			(Medium D)	
MS Media	1 X	1 X	½ X	1 X	1 X	½ X
IAA (mg/l)	0.75	0.75		0.1	0.1	0.25
BAP (mg/l)	0.25	0.25	0.25	0.2	0.2	0.025
NAA (mg/l)	0.2	0.2		0.2	0.2	
Kinetin(mg/l)	0.1	0.1		0.1	0.1	
Sucrose (g/l)	30.0	30.0	15.0	30.0	30.0	15.0
Agar (g/l)	8.0	8.0	6.0	8.0	8.0	6.0

*Short note

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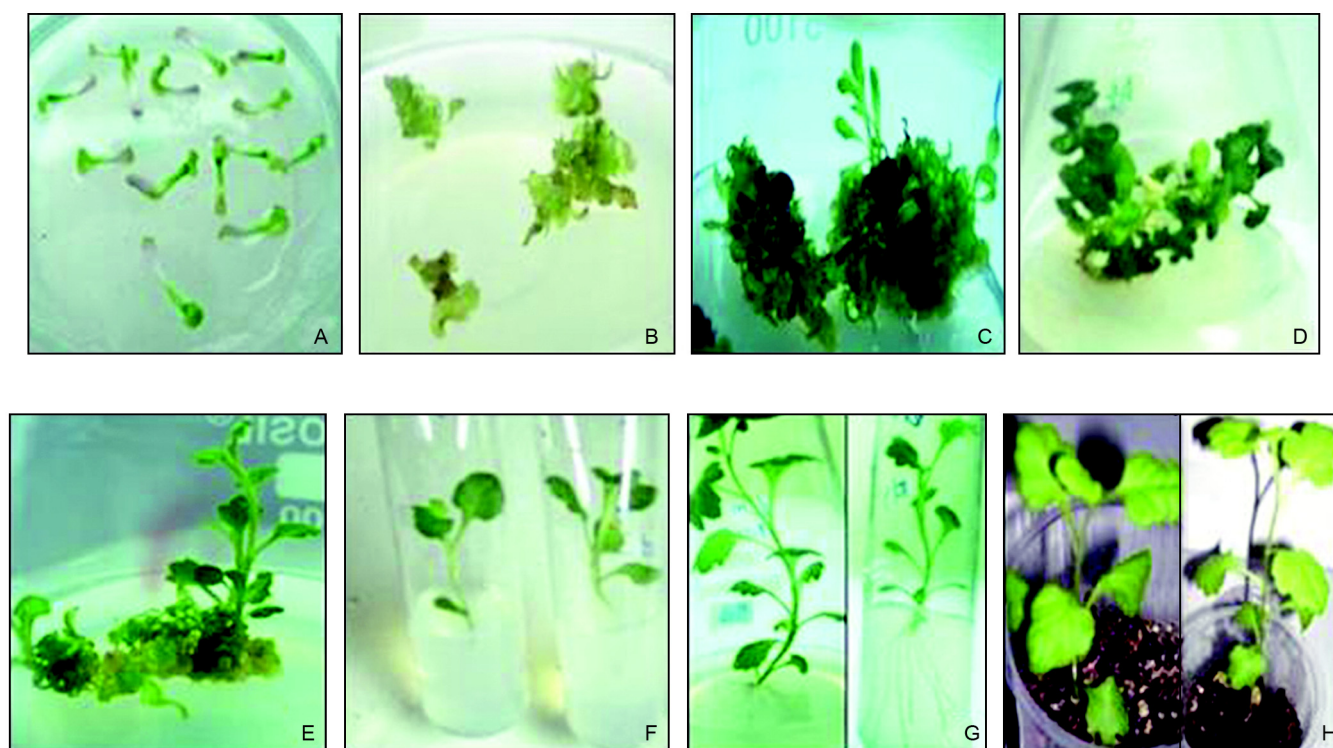


Fig 1 Different stages of chrysanthemum plant developed through tissue culture (A) Ray florets (initial culture); (B) callus initiation; (C) callus differentiation; (D) shoot development; (E) and (F) shoot elongation; (G) root formation; (H) transplant in soil nite

Callus growth and development are influenced by a complex relationship between the genotypes and the constituents of the protocol. The estimates of significance for the effects of cultivars and protocols on callus weight, are presented in Table 2. The effect of plant genotype and media modifications on culture behaviour has already been studied in detail (Tanaka *et al.* 2000, Barakat 2008).

The highest percentage of embryogenic callus was obtained in Purnima among the four protocols used and it was significantly different from all other cultivars (Fig 1) (Table 2). Somatic embryogenesis of chrysanthemum was induced by high concentrations of both BAP (6-benzyl amino purine) and NAA (naphthalene acetic acid) in protocols A and B. These results correlate with previous study (Tanaka *et*

Table 2 Means of callus initiation, callus weight, embryogenic callus, shoot formation, number of shoots and shoot length as influenced by different chrysanthemum cultivars, media used and its interactions

Cultivar	Medium (A)	Medium (B)	Medium (C)	Medium (D)	Cultivar mean (%)
Callus initiation (%)					
Pooja	59.76	51.61	24.90	0.20	34.11
Purnima	80.16	63.21	7.20	23.10	43.41
Otome Zakura	53.30	48.30	20.33	21.00	35.73
Flirt	76.90	59.20	42.41	10.30	47.20
Little Darling	58.60	68.32	38.10	9.33	43.50
Medium mean	65.74	58.12	26.58	12.78	
Callus weight (g/explant)					
Pooja	0.32	0.12	0.08	0.30	0.20
Purnima	0.88	0.71	0.19	0.06	0.46
Otome Zakura	0.69	0.36	0.01	0.05	0.27
Flirt	0.53	0.41	0.17	0.08	0.30
Little Darling	0.32	0.21	0.16	0.04	0.18

Continued

Table 2 Concluded

Cultivar	Medium (A)	Medium (B)	Medium (C)	Medium (D)	Cultivar mean (%)
Medium mean	0.54	0.36	0.12	0.10	
Embryogenic callus (%)					
Pooja	9.32	20.11	0.00	0.16	7.39
Purnima	33.50	29.10	0.00	0.00	15.65
Otome Zakura	27.30	21.10	0.12	0.00	12.13
Flirt	17.31	8.33	0.00	0.00	6.40
Little Darling	21.20	7.07	0.13	0.00	7.10
Medium mean	21.72	17.14	0.05	0.03	
Shoot formation (%)					
Pooja	2.39	2.11	0.00	0.12	1.15
Purnima	32.33	26.50	0.00	0.00	14.70
Otome Zakura	17.44	0.00	1.07	0.00	4.62
Flirt	12.32	6.10	0.00	0.00	4.60
Little Darling	9.31	5.21	1.12	0.00	3.90
Medium mean	14.76	7.98	0.43	0.02	
Number of shoots/explant					
Pooja	0.35	0.11	0.0	0.00	0.11
Purnima	3.11	2.13	1.10	1.08	1.85
Otome Zakura	1.40	1/02	0.00	0.00	0.60
Flirt	1.18	1.07	0.00	0.00	0.56
Little Darling	0.00	0.04	0.00	0.00	0.01
Medium mean	1.20	0.87	0.32	0.21	
Shoot length (cm)/explant					
Pooja	0.00	0.00	0.00	0.00	0.00
Purnima	1.54	0.78	0.00	0.00	0.58
Otome Zakura	0.88	0.21	0.00	0.00	0.27
Flirt	0.41	0.19	0.00	0.00	0.15
Little Darling	1.31	0.00	0.00	0.00	0.32
Medium mean	0.82	0.23	0.00	0.00	

Table 3 Means of number of roots (%)/explant as influenced by different chrysanthemum cultivars, media used and its interactions

Cultivar	Medium (A)	Medium (B)	Medium (C)	Medium (D)	Cultivar mean (%)
Pooja	0.00	0.00	0.00	0.00	0.00
Purnima	0.82	0.34	0.00	0.00	0.29
Otome Zakura	0.41	0.21	0.00	0.00	0.15
Flirt	0.04	0.00	0.00	0.00	0.01
Little Darling	0.08	0.00	0.00	0.00	0.02
Medium mean	0.27	0.11	0.00	0.00	

al. 2000). Purnima produced the highest value of shoot formation among the different protocols. The protocol A was significantly better than all other protocols across the cultivars (Table 2). Purnima produced the highest shoot number among the different protocols (Table 2). The protocol A showed the highest potential for shoot number (1.2 shoot/explant) and it

was significantly higher than the other protocols (Fig 1). The highest value of shoot length among different protocols has been recorded in Purnima (Table 2). Purnima cultivar produced the highest value of root formation (Table 3).

SUMMARY

In the present investigation five different cultivars of *Chrysanthemum morifolium* were screened using the ray floret explants in order to determine the capability for plant regeneration on four media protocols and consequently to find out the best genotype source linked with the optimum medium conditions for the high potentiality of shoot formation. The results indicated that all *in vitro* culture traits were significantly influenced by the differences in cultivars, medium protocols and their interaction. The percentage of explants which developed in to calli ranged from 34.1% to 47.2% among five different cultivars across the four protocols with an average of 40.80%. The highest percentage of embryogenic callus, shoot formation and mean value of shoot length was produced

by cultivar Purnima when calli were differentiated on protocol A. The protocol A showed the greatest potential for shoot length across the cultivars and it was significantly superior to all other protocols except the protocol B. Results indicated that the protocol A, followed by B appear to be the best protocols for plant regeneration and it can be used by the breeders in chimera management to establish the new cultivar in a better way.

REFERENCES

- Barakat M N. 2008. Application of In Vitro Culture for Resistance to Desertification. *Meteorology, Environment and Arid Land Agriculture* **19**: 3–18.
- Chakraborty D, Mandal A K A and Datta S K. 2002. Retrieval of new coloured Chrysanthemum through organogenesis from sectorial chimera. *Current Science* **78(9)**: 1060–1.
- Dao T B, Nguyen P D, Do Q M, Vu T H, Le T L, Nguyen T K L, Nguyen H D and Nguyen X L. 2006. In vitro mutagenesis of Chrysanthemum for breeding. *Plant Mutation Report* **1**: 26–7.
- Dwivedi A K, Banerji B K, Chakrabarty D, Mandal A K A and Datta S K. 2000. Gamma ray induced new flower colour chimera and its management through tissue culture. *Indian Journal of Agricultural Science* **70**: 853–5.
- Murashige T and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiology Plant* **15**: 473–97.
- Misra P, Datta S K and Chakraborty D. 2004. Mutation in flower colour and shape of chrysanthemum morifolium induced by α radiation. *Biologia Plantarum*. **47 (1)**: 153–6.
- Tanaka K, Kanno Y, Kudo S and Suzuki M. 2000. Somatic embryogenesis and plant regeneration in chrysanthemum (*Dendranthema grandiflorum* (Ramat.)). *Plant Cell Report* **19**: 946–53.