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Full Length Research Paper

In vitro culture and plant regeneration derived from ray florets of *Chrysanthemum morifolium*

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Nine cultivars of *Chrysanthemum morifolium* were screened using the ray floret explants to determine the capability for plant regeneration on four media protocols and subsequently 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 highly significantly influenced by the differences in genotypes, medium protocols and their interaction. The percentage of explants which developed calli ranged from 73.83% "Ping Pong" to 25.67% "Palisade White" among the cultivars across the four medium protocols with an average of 48.28%. The highest percentage of embryogenic callus, shoot formation and mean value of shoot length was produced by cultivar "Delistar White" when calli were differentiated on medium protocol B. The medium protocol B showed the greatest potential for shoot length across the cultivars and it was significantly superior to all other medium protocols except the medium protocol A. The present study indicated that the medium protocol "B" and then "A" appear to be the best protocols for plant regeneration. The cultivar "Delistar White" with the medium protocols B and then A, could be successfully utilized for further *in vitro* mutagenesis investigations.

Key words: *Chrysanthemum morifolium, in vitro* culture, plant regeneration.

INTRODUCTION

Chrysanthemum (*Chrysanthemum morifolium* Ramat), currently classified as *Dendranthema x grandiflora* (Anderson, 1987) belonging to the Asteraceae family, was initially classified as a Compositae (Salinger, 1991). It is also known as florist's chrysanthemum or Higo-giku (in Japanese). The name originally came from the Greek word *krus anthemon*, meaning gold flower which originnated in China (where it has been cultivated for over 3000 years). This culturally rich flower is globally the second economically most important floricultural crop following rose and it is one of the most important ornamental species. Its popularity as cut flower had led to the introduction of thousands of new cultivars of large flower diversity (Cockshull, 1985).

For modern and industrialized horticulture, the cut flower industry, perhaps different from any other industry, is always in demand and in need of new varieties to routinely attend the continuous flower consumer demands. Consumer preferences change and show new and sometimes uncommon features. Therefore, the priority of the flower and ornamental plant biotechnology segments should be the generation of novel plant and flower types (Hutchinson et al., 1982). Chrysanthemum *in vitro* culture was extremely useful for producing a huge number of explants in a short time as stated by Dao et al. (2006). Tissue culture studies in chrysanthemum are being done as a tool for mutation induction and as a means of micropropagation. However, the ability to regenerate plants from a single cell of florets is a useful approach to establish a mutant in pure form and facilitate the production of a wide range of new flower cultivars as stated by Mandal et al. (2000).

The aim of the present study was to determine the capability of nine chrysanthemum cultivars for plant regeneration on four medium protocols and subsequently, to find the best genotype source linked with the optimum

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Table 1. *Chrysanthemum morifolium* cultivars* used in the *in vitro* regeneration experiment and their characteristics.

S/N	Cultivar*	Colour*	Form[1]	Response time[2]	Vigour[3]	Ø[4]
1	Delistar white	White	Spider	7	5	100/20
2	Delistar yellow	Yellow	Spider	7	5	100/20
3	Palisade white	White	Decorative	8.5	5.5	160/20
4	Palisade yellow	Yellow	Decorative	8.5	5.5	160/20
5	Ping Pong	White	Pompon	8	4.5	70/15
6	Ping Pong Golden	Yellow	Pompon	8	4	70/15
7	Rodet	Orange	Decorative	11	5.5	180/20
8	Cassandra	Purple	Decorative	10.5	4	150/20
9	SunnyCassandra	Bronze	Decorative	10.5	4	150/20

Form[1] = The most important characteristics of each variety are given under each form; Response time[2] = indicates the number of weeks between the beginning of the short day period and the flowering date; Vigour[3] = group is a scale of '7' which is used for the most vigorous varieties and the lower numbers for the less vigorous ones; Ø[4] = Diameter of flower (ray florets) mm / diameter of flower centre (disc florets) mm.

Table 2. Composition of the four medium protocols used for callus initiation, plant regeneration and root induction in chrysanthemum cultivars.

Protocol components	Callus Induction	Shoot differentiation	Root Induction	Callus Induction	Shoot differentiation	Root induction
	Protocol (A)			**Protocol (B)**		
MS basic medium	1X	1X	1/2 X	1X	1X	1/2 X
BAP	0.5 mg/l	0.5 mg/l	-	1.0 mg/l	1.0 mg/l	-
NAA	0.2 mg/l	0.2 mg/l	0.02 mg/l	0.5 mg/l	0.5 mg/l	0.1 mg/l
Sucrose	30 g/l	30 g/l	15 g/l	30 g/l	30 g/l	15 g/l
Agar	5.5 g/l	5.5 g/l	5.5 g/l	5.5 g/l	5.5 g/l	5.5 g/l
	Protocol (C)			**Protocol (D)**		
MS basic medium	1X	1X	1/2 X	1X	1X	1/2 X
IAA	0.5 mg/l	0.5 mg/l	_	-	-	-
BAP	0.2 mg/l	0.2 mg/l	0.02 mg/l	-	-	-
NAA	-	-	-	0.2 mg/l	0.2 mg/l	-
Kinetin	0.1 mg/l	-	-	2.0 mg/l	2.0 mg/l	-
Sucrose	30 g/l	30 g/l	15 g/l	30 g/l	30 g/l	15 g/l
Agar	5.5 g/l	5.5 g/l	5.5 g/l	5.5 g/l	5.5 g/l	5.5 g/l

medium conditions for the high potentiality of shoot formation.

MATERIALS AND METHODS

Plant material and *in vitro* culture response

Flower heads of nine *C. morifolium* cultivars were used in this study. A list of these cultivars and their characteristics is given in Table 1. Ray florets were collected from the inner whorl of a half bloom flower head of the *C. morifolium* cultivars, after approximately 110 days of planting the cuttings in the greenhouse. Ray florets of chrysanthemum explants were surface sterilized by immersing in 70% ethanol containing one drop of Tween 20, for 2 min, followed by immersing in 0.1% mercuric chloride for 4 min, then, washed with six changes of sterile distilled water.

The ray florets were, aseptically, placed in Petri dishes containing 25 ml of culture medium. Full details of the four medium protocols used for callus induction, differentiation and root induction are given in Table 2. Each dish with five explants was considered as a replication. Cultures were incubated in a growth chamber at 25 ± 2°C under 16 h illuminations (200 Lux, daylight florescent tubes). After four weeks of incubation, callus induction response was recorded and the following determinations were made for each Petri dish: Callus induction, Callus weight and the percentage of somatic embryogenesis derived from the ray floret base explants.

Calli with somatic embryogenesis, derived from the ray floret explants, were transferred to jars each containing 30 ml of the callus differentiation sequence media (Table 2) and the following determinations were made for each jar after 6 weeks of incubation: Shoot formation, number of shoots per explant and shoot length derived from the ray floret base explants. Regenerated shoots were

Table 3. Analysis of variance for callus induction (%), callus weight (gm/explant) and somatic embryogenesis (%) as affected by chrysanthemum cultivars, media protocols and their interactions.

S.O.V.	D.F.	M.S.		
		Callus Induction (%)[a]	Callus Weight (gm)	Somatic embryogenesis (%)[a]
Blocks	9	875.20 $^{N.S.}$	0.004 $^{N.S.}$	150.130 $^{N.S.}$
Cultivars (A)	8	8615.03 **	0.1527 **	1589.31 **
Media (B)	3	65143.58 **	1.1374 **	3645.60 **
A x B	24	2988.34 **	0.0496 **	397.69 **
Error	315	345.83	0.009	105.93

[a] Data were subjected to arcsine transformation; **highly significant at 0.01 probability level; $^{N.S.}$ not significant at 0.05 probability level.

transferred to jars with half strength Murashige and Skoog (1962) medium with different growth regulators for root induction.

Statistical analysis

Data were statistically analyzed as a 2- factor experiment (cultivars and medium protocols) in a randomized complete block design (RCBD) with ten replicates. Data with percentage were subjected to arcsine transformation prior to statistical analysis (Steel and Torrie, 1980). Comparisons among means were made using the least significant differences test (LSD). The data were analyzed, using statistical analysis system (SAS) programme, version 6 (1985).

RESULTS AND DISCUSSION

In vitro culture and plant regeneration of chrysanthemum cultivars

Establishing reliable *in vitro* plant regeneration is a pre-requisite step before conducting any *in vitro* selection experiments. If a particular plant species shows no competence for *in vitro* regeneration, the chances are that regeneration from useful variant cell lines in the same species may also be unsuccessful. Once a tissue culture system capable of plant recovery has been established, the next step is to apply the developed system to *in vitro* mutagenesis studies. Having this as a principle, the present work was initiated with the aim of finding a tissue culture system competent to regenerate plants from cultured tissue of chrysanthemum.

Response of explants to callus induction

Data on production of callus, derived from ray floret explants of nine cultivars of chrysanthemum (*C. morifolium),* were recorded after four weeks of incubation. These explants were incubated on media previously developed and successfully employed by other investigators for chrysanthemum. The analysis of variance, presented in Table 3 indicates that callus induction was highly significantly influenced by differences within chrysanthemum cultivars and medium protocols. The two - way- interaction was, also, highly significant.

Callus formation varied widely among chrysanthemum cultivars. The percentage of explants that developed calli ranged from 73.84% (Ping Pong) to 25.67% (Palisade White) among the cultivars across the four medium protocols with an average of 48.28% (Table 4).

The response of callus induction varied according to the medium. Table 4 indicates that the medium protocols A and B gave the highest average of callus induction, 68.83 and 74.09% respectively, across cultivars with significant differences from the other protocols.

The interaction was highly significant between cultivars and medium protocols. The cultivars “Delistar White” and "Cassandra" gave the highest callus induction (90%) with medium protocol B, while cultivar "Delistar White" had significantly the lower callus induction percentage (7.97 and 21.92%) when its explants were cultured on medium protocols C and D, respectively. On the other hand, cultivars "Cassandra" and "Sunny Cassandra" had the significantly lowest callus induction percentage (5.31%) and (2.66%), respectively, when its explants were cultured on medium protocol D (Table 4).

The results in Table 4 shows that calli were not observed with the cultivar Delistar yellow on the medium protocol C as well as the cultivar Palisade white on the medium protocols C and D. The callus was light green in colour and began to develop darker green regions within the callus for many genotypes on different media.

Callus weight

Callus growth and development are influenced by a complex relationship between the genotypes and the constituents of the medium protocol. The estimates of significance for the effects of cultivars, medium protocols and their interaction, on callus weight, are presented in Table 5. The analysis of variance indicated that the callus weight was highly significantly influenced by cultivars,

Table 4. Means of callus induction (%) as influenced by chrysanthemum cultivars, media and their interactions.

Cultivar	Callus induction (%)				
	Medium protocol				Cultivar mean (%)
	A	B	C	D	
Delistar Yellow	58.60	56.18	0.0	23.08	34.47
Delistar White	84.69	90.00	7.97	21.92	51.15
Palisade White	45.12	57.57	0.0	0.0	25.67
Palisade Yellow	44.08	54.00	33.47	22.16	38.43
Sunny Ping Pong	83.42	82.03	9.24	42.34	54.26
Ping Pong	84.69	78.11	57.92	74.66	73.84
Rodet		82.03		14.55	52.38
Cassandra	80.76	90.00	70.50	5.31	61.64
Sunny Cassandra	76.72	76.84	14.55	2.66	42.69
Medium mean (%)	68.83	74.09	27.25	22.96	48.28

L. S. D. $_{(0.\ 05)}$ for cultivar means = 8.182; L. S. D. $_{(0.\ 05)}$ for medium means = 5.454; L. S. D. $_{(0.\ 05)}$ for cultivar x medium protocol interaction = 16.38.

Table 5. Means of callus weight (g /explant) as influenced by chrysanthemum cultivars, media and their interaction.

Cultivar	Callus weight (g/explant)				
	Medium protocol				Cultivar mean (g/explant)
	A	B	C	D	
Delistar Yellow	0.162	0.110	0.0	0.059	0.083
Delistar white	0.168	0.233	0.006	0.101	0.127
Palisade White	0.075	0.333	0.0	0.0	0.102
Palisade Yellow	0.150	0.215	0.051	0.0	0.108
Sunny Ping Pong	0.226	0.339	0.011	0.049	0.156
Ping Pong	0.261	0.393	0.185	0.106	0.236
Rodet	0.084	0.139	0.105	0.019	0.087
Cassandra	0.323	0.458	0.216	0.003	0.249
Sunny Cassandra	0.087	0.374	0.024	0.021	0.127
Medium mean (g/explant)	0.171	0.288	0.066	0.042	

L. S. D. $_{(0.\ 05)}$ for cultivar means = 0.0415; L. S. D. $_{(0.\ 05)}$ for medium means = 0. 0277; L. S. D. $_{(0.\ 05)}$ for cultivar x medium protocol interaction = 0.083.

medium protocols and their interaction. The growth rate of callus was dependent on the cultivar and the medium employed. The cultivar “Cassandra” produced the highest callus weight (0.249 gm/explant) across medium protocols (Table 5) and it was significantly different from all other cultivars except Ping Pong cultivar. On the other hand, the cultivars “Delistar Yellow” and "Rodet" had the significantly lowest callus weight (0.082 and 0.087 gm/ explants, respectively). Among medium protocols, B medium protocol gave the highest callus weight (0.288 gm/explant) and was significantly different from all other medium protocols (Table 5). The cultivar “Cassandra”, significantly, gave the highest response to callus weight for all medium protocols.

Finally, it can be suggested that before utilizing tissue culture techniques as tools in crop improvement, it is necessary to determine the factors influencing callus formation, its quality during induction and maintenance and subsequently shoot regeneration from callus. The previous results provide an indication of the relative importance of genotype, medium protocols and explant effect in culture response. These results showed that the growth rate of the callus was dependent on the genotypes and the culture medium employed. The efficiency of callus induction and callus growth rate have been reported to be in part genotype dependent (Ohishi and Sakurai, 1988; Kaul et al., 1990; Tanaka et al., 2000; Mandal and Datta, 2005; Barakat, 2008). They compared

Table 6. Means of embryogenic callus (%) as influenced by chrysanthemum cultivars, media and their interaction.

Cultivar	Embryogenic callus (%)				
	Medium protocol				Cultivar mean (%)
	A	B	C	D	
Delistar Yellow	9.51	20.17	0.0	0.0	7.42
Delistar White	32.66	36.47	0.0	9.58	19.68
Palisade White	0.0	0.0	0.0	0.0	0.00
Palisade Yellow	0.0	6.17	0.0	0.0	1.54
Sunny Ping Pong	0.0	0.0	0.0	0.0	0.00
Ping Pong	11.32	9.24	5.66	0.0	6.55
Rodet	12.16	15.47	0.0	0.0	6.91
Cassandra	11.82	28.63	7.97	0.0	12.10
Sunny Cassandra	8.66	10.73	0.0	0.0	4.85
Medium mean (%)	9.57	14.10	1.51	1.07	

L. S. D. $_{(0.05)}$ for cultivar means = 4.528; L. S. D. $_{(0.05)}$ for medium means = 3.019; L. S. D. $_{(0.05)}$ for cultivar x medium protocol interaction = 9.0.

the effect of plant genotype and media modifications on culture behavior. Their results provide an indication of the relative importance of genotype and media effects on culture responses.

Somatic embryogenesis

Statistical analysis of embryogenic callus was highly significant influenced by differences between cultivars, medium protocols and their interaction (Table 3). The highest percentage of embryogenic callus, across the four medium protocols resulted from "Delistar White" cultivar (19.68%) and it was significantly different from all other cultivars (Table 6). On the other hand, no embryo-genic callus was observed with any medium protocol for the “Palisade White” cultivar (Table 6). Medium protocol B produced the highest percentage of embryogenic callus (14.10%) across the chrysanthemum cultivars and was significantly different from all other medium protocols (Table 6).The interaction between cultivars x medium protocols was highly significant. The variation ranged from 36.47% for cultivar "Delistar White" with medium protocol B to 0.0% for the cultivar “Palisade white” with all medium protocols (Table 6). These results indicate that somatic embryogenesis of chrysanthemum was induced by high concentrations of both BAP (6-benzyl amino purine) and NAA (naphthalene acetic acid) in medium protocols A and B. These results agree with previous studies (May and Trigiano, 1991; Pavingerona et al., 1994; Urban et al., 1994, Tanaka et al., 2000; Mandal and Datta, 2005). They reported that successful somatic embryogenesis and subsequently plant regeneration of chrysanthemum were obtained from different callus cultures. It seemed that both BAP (1.0 mg/L) and NAA (0.5 mg/L) concentrations were more effective for the somatic emberyogenesis of chrysanthemum.

Morphogenetic response

Shoot formation

The ability to regenerate large number of shoots from cultured tissues is important for the success of most of biotechnological techniques such as *in vitro* mutagenesis. In the present investigation, the callus derived from chrysanthemum ray floret explants, which was induced on the induction media (Table 2), was sub-cultured on a wide range of differentiation media. When calli were placed on differentiation media, some calli differentiated into shoots and some did not differentiate. Shoots were subcultured on rooting medium. Plants were established *ex-vitro* and then established into soil till flowering.

Analysis of variance for the formation of shoots derived from the induced callus in chrysanthemum indicated that the effects of cultivars and medium protocols and their interaction were highly significant (Table 7).

The results in Table 8 showed that the cultivar "Delistar White" produced the highest mean value of shoot formation (21.90%) across medium protocols. The medium protocol B (15.68%) was significantly better than all other medium protocols across the cultivars. The results also revealed that there was a significant interaction between cultivars and medium protocols. For instance, Delistar White and Cassandra cultivars gave the highest percentage of shoot formation (41.43 and 33.58%, respectively) when calli differentiated on medium protocol B (Figure 1). However, the same protocol resulted in low percentage of shoot formation for some other cultivars such as Palisade

Table 7. Analysis of variance for the effect of chrysanthemum shoot formation (%), number of shoots per explant and shoot length (cm) as influenced by cultivars, media and their interaction.

S.O.V.	D.F.	M.S.		
		Shoot formation (%)[a]	Number of shoots per explant	Shoot Length (cm)
Cultivars (A)	8	2125.32 **	2.619 **	3.279 **
Media (B)	3	4395.65 **	5.129 **	8.027 **
A x B	24	507.58 **	0.746 **	0.8868 **
Error	315	126.11	0.1247	0.269

[a]Data were subjected to arcsine transformation; ** highly significant at 0.01 probability level.

Table 8. Means of shoot formation (%) as influenced by chrysanthemum cultivars, media and their interaction.

Cultivar	Shoot formation (%)				
	Medium protocol				Cultivar mean (%)
	A	B	C	D	
Delistar Yellow	9.51	20.17	0.0	0.0	7.42
Delistar White	36.58	41.43	0.0	9.58	21.90
Palisade White	0.0	0.0	0.0	0.0	0.00
Palisade Yellow	0.0	6.16	0.0	0.0	1.54
Sunny Ping Pong	0.0	0.0	0.0	0.0	0.00
Ping Pong	11.31	9.58	5.66	0.0	6.64
Rodet	12.16	16.74	0.0	0.0	7.23
Cassandra	15.85	33.58	12.92	0.0	15.59
Sunny Cassandra	8.66	13.50	0.0	0.0	5.54
Medium mean (%)	10.45	15.68	2.06	1.06	

L. S. D. $_{(0.\ 05)}$ for cultivar means = 4.94; L. S. D. $_{(0.\ 05)}$ for medium means = 3.29; L. S. D. $_{(0.\ 05)}$ for cultivar x medium protocol interaction = 9.89.

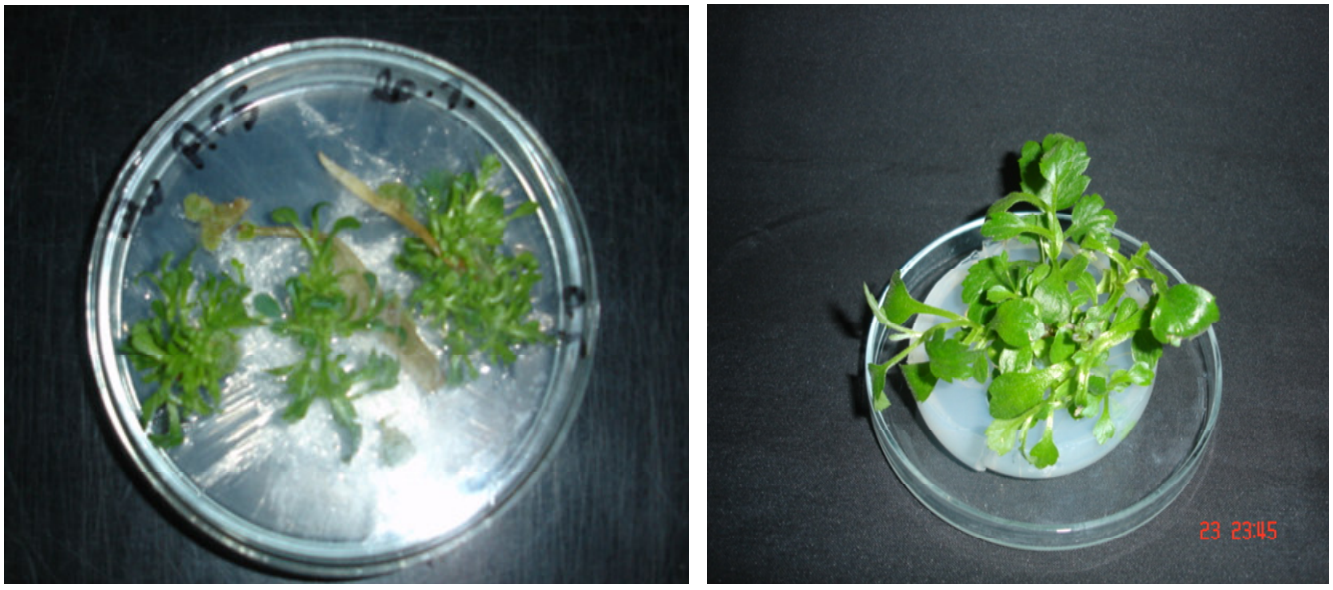

Figure 1. Shoot formation derived from ray floret explant.

Table 9. Means of shoot number per chrysanthemum explant as influenced by cultivars, media and their interaction.

Cultivar	Number of shoots				
	Medium protocol				Cultivar mean Shoot No.
	A	B	C	D	
Delistar Yellow	0.30	0.60	0.0	0.0	0.23
Delistar White	1.40	1.70	0.0	0.20	0.83
Palisade White	0.0	0.0	0.0	0.0	0.00
Palisade Yellow	0.0	0.20	0.0	0.0	0.05
Sunny Ping Pong	0.0	0.0	0.0	0.0	0.00
Ping Pong	0.40	0.30	0.20	0.0	0.23
Rodet	0.50	0.70	0.0	0.0	0.30
Cassandra	0.40	0.80	0.30	0.0	0.38
Sunny Cassandra	0.30	0.30	0.0	0.0	0.15
Medium mean shoot no.	0.37	0.51	0.06	0.02	

L. S. D. $_{(0.05)}$ for cultivar means = 0.155; L. S. D. $_{(0.05)}$ for medium means = 0.104; L. S. D. $_{(0.05)}$ for cultivar x medium protocol interaction = 0.317

Yellow, Ping Pong and Sunny Cassandra (6.16, 9.58 and 13.80%, respectively) and gave no shoot formation at all by the cultivars Palisade White and Sunny Ping Pong.

In addition, the ability of fresh callus to differentiate into plantlets depends on the hormone level of the initial callus induction medium, as well as on the cultivar or the donor plant. The cultivar factors, which are considered important for eliciting success in morphogenesis, have been listed by Thorpe (1980). These factors included: (a) selection of the organ to be used as the tissue culture source, (b) the appropriate physiological and ontogenetic age of the organ, (c) the suitable season in which explant is obtained, (d) the size of the explant and (e) the overall quality of the plant from which explants are derived.

Results from the present investigation showed that there was significant genotype x medium interaction for morphogenetic response. The probable reasons for differences in morphogenetic response *in vitro* may be attributed to (a) genetic differences among the genotypes used (b) differences in the growth regulators, or (c) differences in the growth conditions and age of the source of explants.

Prasad et al. (1983) reported that the rate of shoot multiplication is genotypic dependent in *Dendranthema grandiflorum*. Bhattacharya et al. (1990) studied the influence of different growth regulators on the *in vitro* morphogenesis of chrysanthemum. They reported that a combination of 0.1 mg/l IAA and 0.2 mg/l BAP was most appropriate for callus formation and for the regeneration of shoots from callus. Rademaker and de Jong (1990) reported that cultivar and explant type had a greater effect on regeneration than the type of medium.

Number of shoots per explant

Statistical analysis of the shoot number per explant derived from callus of chrysanthemum ray floret explant (Table 7) revealed highly significant differences among cultivars, medium protocols and their interaction. Results in Table 9 shows that the cultivar "Delistar White" produced the highest mean value of shoot number (0.83shoot/explant) across medium protocols which were significantly different from all other cultivars. The medium protocol B showed the highest potential for shoot number (0.51shoot/explant) and it was significantly higher than the other protocols (Table 9). The cultivar x medium protocol interaction was highly significant. Higher mean values for the number of shoots in Delistar White, Rodet and Cassandra (1.7, 0.7 and 0.8% respectively) were gotten in the medium protocol B. However, lower mean values for the number of shoots in the same cultivars were obtained in the medium protocols C and D (Table 9).

Shoot length

The analysis of variance, presented in Table 7 indicates that shoot length was highly significantly influenced by chrysanthemum cultivars, medium protocols and their interaction.

Results in Table 10 shows that the cultivar "Delistar White" produced the highest mean value of shoot length (0.78 cm) across medium protocols. The medium protocol B showed the greatest potential for shoot length

Table 10. Means of shoot length (cm) as influenced by chrysanthemum cultivars, media and their interaction.

Cultivar	Shoot length (cm)				
	Medium protocol				Cultivar mean (cm)
	A	B	C	D	
Delistar Yellow	0.33	0.28	0.0	0.0	0.28
Delistar White	1.54	0.78	0.0	0.33	0.78
Palisade White	0.0	0.00	0.0	0.0	0.00
Palisade Yellow	0.0	0.09	0.0	0.0	0.09
Sunny Ping Pong	0.0	0.00	0.0	0.0	0.00
Ping Pong	0.52	0.32	0.35	0.0	0.32
Rodet	0.89	0.52	0.0	0.0	0.52
Cassandra	0.92	0.69	0.48	0.0	0.69
Sunny Cassandra	0.40	0.20	0.0	0.0	0.20
Medium mean (cm)	0.51		0.09	0.04	

L. S. D. $_{(0.05)}$ for cultivar means = 0.228; L. S. D. $_{(0.05)}$ for medium means = 0.152.

(0.63 cm) across cultivars and it was significantly superior to all other medium protocols except the medium protocol A. The data, also, revealed that cultivars x medium protocols interaction was highly significant. In "Delistar White" cultivar, protocols A and B were significantly superior to C and D protocols in shoot length. On the other hand, in "Palisade White" and "Sunny Ping Pong" cultivars, none of the applied protocols showed response in shoot length in comparison to the other protocols. Moreover, protocol B showed significant increase in shoot length in "Cassandra", "Rodet and "Delistar Yellow" cultivars (1.35, 1.18 and 0.77, respectively) compared to other protocols (Table 10). The results of the present investigation indicated that the cultivar "Delistar White" with the medium protocols A and B could be successfully utilized for further *in vitro* mutagenesis to select several unique traits relevant to chrysanthemum.

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