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In vitro propagation of chrysanthemum (*Chrysanthemum morifolium*) cultivars from ray floret explants

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Abstract
Eleven different cultivars of chrysanthemum were validated for *in vitro* propagation from ray floret explants. Analysis of variance revealed significant differences among total microbial contamination, explant survival, per cent callus induction and regeneration, number of days required for callus induction, number of shoots regenerated per explant and culture establishment index. The early callusing cultivars were found to be more successful compared to late callusing ones. The earliest callusing cultivars, New Man and Urban Red exhibited greatest culture establishment index values whereas, the late callusing cultivar, Candor Pink recorded the least culture establishment index.

Keywords: *in vitro* propagation, ray floret, chrysanthemum, culture establishment index

Introduction
Chrysanthemum is one of the most important cut flowers and potted plants in the world. The word chrysanthemum comes from Greek words '*Chrysos'* meaning golden and '*anthemon'* meaning flower. It belongs to the family *Asteraceae*. The inflorescence in chrysanthemum is called as 'head' and consists of hermaphrodite disc florets and pistillate ray florets. The crop is traditionally propagated by root suckers and terminal cuttings which have some limitations such as: low multiplication rate, restricted gene pool, longer ray florets that prevent timely pollination, self-incompatibility and high risk of virus transmission and other diseases. Novel biotechnological methods can be employed to overcome these problems. Tissue culture or *in vitro* propagation can facilitate for rapid multiplication and development of superior genotypes. Mutations in flower color or shape mostly appears as chimeras. Isolation of such mutated tissues is impossible through conventional methods of propagation. Shoot organogenesis from flower petals have overcome this problem and new mutants were developed through such protocols in chrysanthemum (Mandal and Datta, 2005) [6]. Ability of regenerating 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 cultivars (Mandal *et al,* 2000) [5].
The present investigation was carried out with an objective to validate *in vitro* regeneration capacity in different chrysanthemum cultivars.

Material and Methods
The present investigation was conducted at Tissue culture laboratory, Horticulture Research Station, Kovvur during August 2020-July 2021. Eleven cultivars of chrysanthemum *i.e.* Marigold, Blooming Beauty White, New Man, Aubisque Pink, Yellowish White Mutant, Pusa Sonia, Champagne Orange, Candor Pink, Arctic Queen, Journey Dark and Urban Red were used for study of *in vitro* propagation from ray floret explants. Ray floret explants pretreated with carbendazim 0.2% + mancozeb 0.2% + streptomycin 100 ppm + teepol 1% for 1 hour followed by surface sterilization with $HgCl_2$ @ 0.1% for 4 minutes were cultured on MS medium fortified with 4 mg L^{-1} BAP and 1 mg L^{-1} NAA. Completely randomized design was used for the present investigation.

Results and Discussion
The differences noticed among the cultivars in respect of total microbial contamination percentage were tested non-significant as presented in Table 1. The mortality due to microbial contamination could be maintained at the lowest level with non-significant differences among the cultivars because of execution of the best protocols for pre-treatment and surface sterilization of explants.

Significant differences were observed with respect to explants survival percentage among the cultivars (Table 1). Percentage of explants survival ranged from 25.77% to 86.11% with a mean of 47.92%. The highest explants survival (86.11%) was noticed in New Man followed by Urban Red (72.22%). The lowest explants survival was recorded in Candor Pink (25.77%) on par with Pusa Sonia (26.67%).

Table 1: Effect of chrysanthemum cultivars on percentage of total microbial contamination and explant survival

Cultivar	Total microbial contamination (%)	Explants survival (%)
T_1 – Marigold	38.60 (38.41)	33.33(35.22)
T_2 – Blooming Beauty White	25.27 (30.18)	55.56 (48.18)
T_3 - New man	33.33 (35.26)	86.11 (68.63)
T_4 – Aubisque Pink	37.40 (37.70)	44.44 (41.79)
T_5 - Yellowish white mutant	36.53 (37.19)	44.44 (41.79)
T_6 - Pusa Sonia	38.60 (38.41)	26.67 (31.07)
T_7 - Champagne Orange	33.27 (35.22)	44.44 (41.78)
T_8 - Candor Pink	44.93 (42.09)	25.77 (30.48)
T_9 - Arctic Queen	35.80 (36.75)	42.30 (40.55)
T_{10} - Journey Dark	45.20 (42.25)	51.85 (46.04)
T_{11} - Urban Red	22.60 (28.39)	72.22 (58.25)
Mean	35.59 (36.63)	47.92 (43.98)
SE(m)	-	1.73
CD at 5%	NS	5.10

Values in parenthesis were arc sine transformed values

Significant differences were observed with respect to number of days taken for callus induction among the cultivars (Table 2). Callus induction was at the earliest (17.85 days) in New Man which was on par with Urban Red (20.13 days) and Marigold (20.54 days). The cultivar Blooming Beauty White took the highest number of days for callus induction (30.67 days) on par with Aubisque Pink (29.36 days), Yellowish White Mutant (29.15 days) and Pusa Sonia (26.42 days).

The cultivars led to significant differences in per cent callus induction as evident from the data (Table 2). Percentage of callus induction ranged from 17.78% (Candor Pink) to 84.44% (New Man) with a mean of 39.39%. New Man was followed by Urban Red (55.56%).

The data presented in Table 2 indicated that there were significant differences among the cultivars for per cent regeneration. The highest per cent regeneration (71.11) was recorded in New Man followed by Urban Red (51.11%). The lowest per cent (2.22%) was exhibited by Aubisque Pink, Yellowish White Mutant and Candor Pink.

The results from the present experiment showed that percentage of regeneration was dependent on the genotypes (Table 2 and Figure 1). Relative importance of genotype and media effects on culture responses in chrysanthemum were reported by Barakat (2008) [1]. Capacity of shoot regeneration was found to be cultivar dependent. The results were in accordance with that of Takatsu *et al.* (1998) [7], Vilasini and Latipah (2000) [8] and Han *et al.* (2009) [3].

Table 2: Effect of chrysanthemum cultivars on per cent callus induction and regeneration

Cultivar	Per cent callus induction (%)	Number of days taken for callus induction	Per cent regeneration (%)
Marigold	24.44 (29.57)	20.54	17.78 (24.84)
Blooming Beauty White	28.89 (32.47)	30.67	28.89 (32.47)
New man	84.44 (66.84)	17.85	71.11 (57.49)
Aubisque Pink	44.44 (41.79)	29.36	2.22 (4.99)
Yellowish white mutant	31.11 (33.86)	29.15	2.22 (4.99)
Pusa Sonia	51.11 (45.62)	26.42	4.44 (9.97)
Champagne Orange	22.22 (28.06)	27.95	17.78 (24.84)
Candor Pink	17.78 (24.84)	31.24	2.22 (4.99)
Arctic Queen	28.89 (32.47)	25.84	4.44 (9.97)
Journey Dark	44.44 (41.79)	25.69	4.44 (9.97)
Urban Red	55.56 (48.18)	20.13	51.11 (45.62)
Mean	39.39 (38.68)	25.89	18.79 (20.92)
SE m±	1.44	1.52	3.82
CD at 5%	4.25	4.48	11.28

Values in parenthesis were arc sine transformed values

Number of shoots regenerated from each explant differed significantly among the cultivars as presented in the Table 3 and Figure 1. Among all the cultivars, the maximum number (9.5) of shoots regenerated was noticed in Urban Red followed by New Man (5.9). The lowest number (1) was recorded in Aubisque pink, Yellowish White Mutant, Pusa Sonia and Candor Pink.

The differential response in terms of shoot bud differentiation might be due to genotypical differences, as opined by Kazeroonian *et al.* (2018) [4] who stated that genotype was a key determinant of shoot organogenesis in chrysanthemum.

The differences observed among the cultivars with respect to culture establishment index were found significant (Table 3). The highest culture establishment index was exhibited by Urban Red (T_{11}) (486.67) followed by New Man (T_3) (419.78). However, the minimum culture establishment index (2.22) was recorded in Aubisque Pink (T_4) and Candor Pink (T_8).

These differences in morphogenetic response *in vitro* can be also attributed to genetic differences (Barakat *et al.*, 2010) [2].

Table 3: Number of shoots regenerated per explant and culture establishment index as influenced by chrysanthemum cultivars

Cultivar	Number of shoots regenerated from each explant	Culture establishment index
Marigold	4.80 (2.19)	84.44
Blooming Beauty White	5.20 (2.28)	149.78
New man	5.90 (2.43)	419.78
Aubisque Pink	1.00 (1.00)	2.22
Yellowish white mutant	1.00 (1.00)	4.44

Pusa Sonia	1.00 (1.00)	6.67
Champagne Orange	3.20 (1.79)	56.89
Candor Pink	1.00 (1.00)	2.22
Arctic Queen	4.10 (2.02)	18.89
Journey Dark	2.20 (1.48)	9.33
Urban Red	9.50 (3.08)	486.67
Mean	**3.17 (1.75)**	**112.85**
SE m±	0.04	13.27
CD at 5%	0.11	39.16

Values in parenthesis are square root transformed values

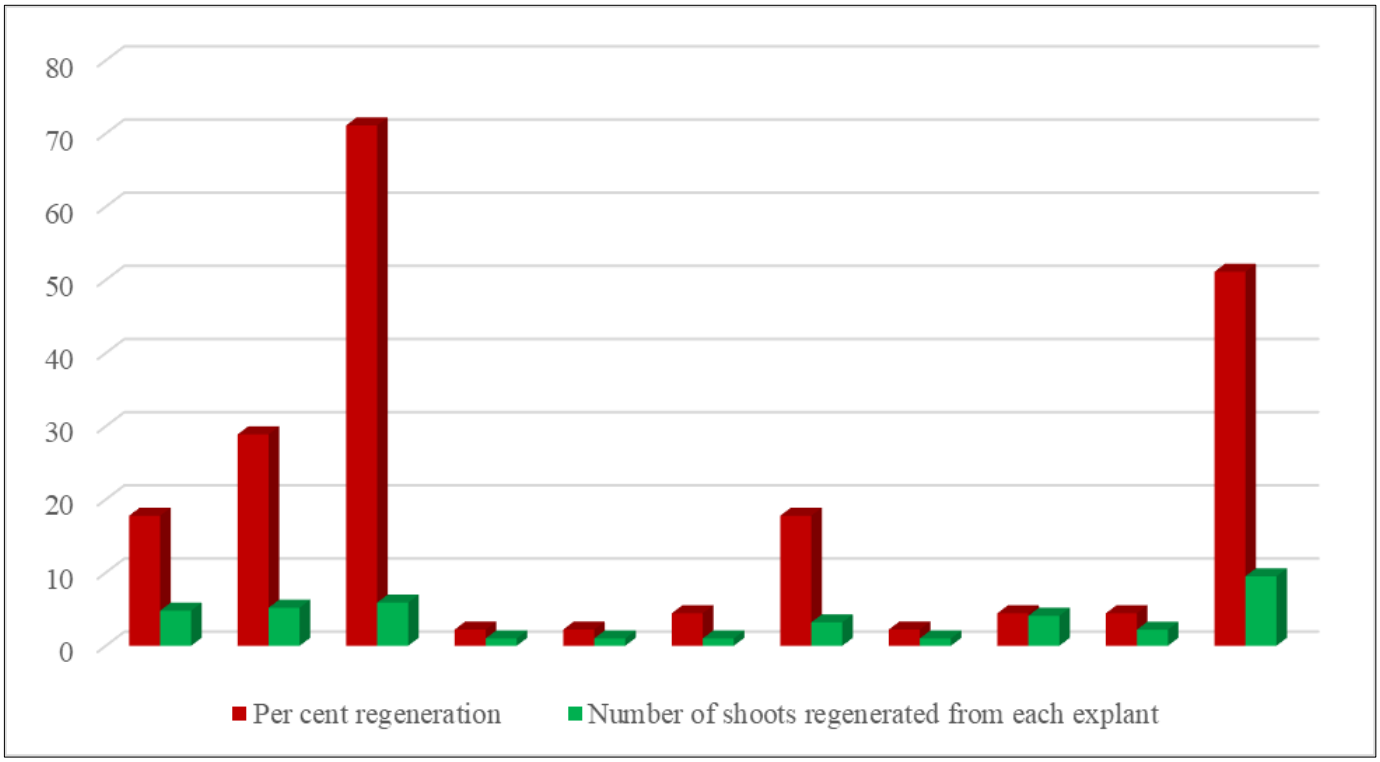

Fig 1: Per cent regeneration and number of shoots regenerated from each explant as influenced by chrysanthemum cultivars

Conclusion

An examination of results on culture establishment index in comparison with those on number of days taken for callus induction revealed that early callusing cultivars were more successful compared to late callusing ones. The earliest callusing cultivars, New Man and Urban Red exhibited greatest culture establishment index values whereas the late callusing cultivar, Candor Pink was showing the least culture establishment index.

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