Biological Forum – An International Journal **14(3): 1074-1078(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Effect of Micro-wave Radiation on *in vitro* Plant Regeneration in Chrysanthemum (*Chrysanthemum morifolium*)

***M. Kiranmayi*[1*], *K. Ravindra Kumar*[2], *G. Ramanandam*[3] *and M. Paratpara Rao*[4]**

[1]*M.Sc. Scholar, Department of Floriculture and Landscape Architecture, College of Horticulture, Dr. YSRHU, VR Gudem (Andhra Pradesh), India.*
[2]*Senior Scientist, Department of Floriculture and Landscape Architecture, Horticultural Research Station, Kovvur, (Andhra Pradesh), India.*
[3]*Principal Scientist, Horticulture, Horticultural Research Station, Kovvur (Andhra Pradesh), India.*
[44]*Associate Professor, Department of Genetics and Plant Breeding, College of Horticulture, Dr. YSRHU, VR Gudem (Andhra Pradesh), India.*

(Corresponding author: M. Kiranmayi)*
(Received 29 June 2022, Accepted 08 August, 2022)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The present investigation on effect of micro-wave irradiation on chrysanthemum *in vitro* regeneration was conducted at Horticultural Research station, Kovvur, Andhra Pradesh during the year 2021-22. The mutagenic capacity of this electromagnetic radiation still in ambiguous but usage of this micro-wave radiation for crop improvement could be highly useful. The present experiment was conducted in Factorial completely randomized design (CRD). *In vitro* leaves of chrysanthemum cultivars *viz.* Marigold (Yellow), New Man (White) and Journey Dark (Purple) were used as explants for micro-wave irradiation and culture initiation. Leaf explants were subjected to different micro wave irradiation periods (0 seconds, 8s, 16s, 32s and 48s). The treated *in vitro* leaf of all cultivars was cultured on MS medium supplemented with BAP 4.0 mg L^{-1} + NAA 1.0 mgL^{-1} for shoot bud morphogenesis. Observations revealed that, percent explant survival was gradually decreased with the increase of irradiation time from zero to 48 seconds in different cycles. Similar trend was found in parameters like per cent callus induction, number of regenerated shoots per explant, culture establishment index.

Keywords: *in vitro*, leaf, micro-wave, electromagnetic, physical mutagen, radiation, mutation.

INTRODUCTION

Chrysanthemum (*Chrysanthemum morifolium*) is one of the important cut flower and pot plants and are commonly known as 'Autumn Queen' or 'Queen of East'. It is a member of the Asteraceae family and native to Northern hemisphere, chiefly Europe and Asia Anderson (1987). It is one of the important cut flower crops in the international market and ranks 3rd in the global cut flower trade after rose and carnation Datta and Gupta (2012).

Mutation breeding is one of the most important and relatively easy breeding methods for creating genetic variability in vegetatively propagated crops especially in ornamentals. Floriculture industry constantly required variability in flower form, shape, size, colour etc. for constant demand in the flower market or nursery. Chrysanthemum is one of the most popular flower crops in Andhra Pradesh and other states of India. Several studies were conducted on chrysanthemum mutation breeding with physical and chemical mutagenic agents (Teixeira da Silva and Kulus 2014; Oladosu *et al*., 2016) for crop improvement. However, on the other hand, very little information is available on the use of micro-wave irradiation in crop improvement of horticultural crops especially ornamental plants. Micro-waves are part of electromagnetic radiation and widely used in industrial and commercial applications. These are also useful in sterilization (Tisserat *et al*., 1992), rapid drying Diprose (2001), rewarming of cryopreserved explants (Halmagyi *et al*., 2017) and mutagenic studies (Miler and Kulus 2018). This radiation is mainly used in the laboratory for dielectric heating Diprose (2001). Micro-wave ovens used as home appliances 2.45 GHz frequency are common for heating. This low cost and easy accessible radiation could be an alternate source for changing genetic variability in plants to the harmful chemical mutagens or less available physical mutagens in crop improvement programmes.

Robust *in vitro* protocols were standardized at Dr. YSRHU-HRS, Kovvur for chrysanthemum plant regeneration in different genotypes by employing different explants like nodal segments, shoot tips, leaves and ray florets. Chrysanthemum was selected for micro-wave induced mutation breeding, owing to ease in inducing variation and amenability to *in vitro* experiments. Different cultivars may act differently in terms of mutation response. Due to presence of a high portion of dominant alleles for flower colour, pink or purple genotypes undergo mutations more frequently,

while yellow-coloured genotypes were most stable and mutate the less frequently (Kulus, 2017). To clarify the ability of micro-wave radiation for inducing the mutants, the present experiment is conducted on three chrysanthemum cultivars *viz.* yellow flowered 'Marigold', white flowered 'New Man' and pink flowered 'Journey Dark'.

MATERIAL AND METHODS

The experiment was conducted at Dr. YSRHU, Horticultural Research Station, Kovvur during the year 2021-22. The experiment was conducted in factorial completely randomized block design (FCRD). *In vitro* leaves of chrysanthemum cultivars 'Marigold', 'New Man' and 'Journey Dark' were used as explants. The uniform size explants were collected from *in vitro* grown cultures from the healthy shoots. Micro-wave radiation was carried out at the IFB microwave oven with the power of 800 Wcm^{-2} and the frequency of 2.45 GHz. Explants were irradiated with Micro-wave radiation for eight seconds in repeated cycles, as following: 1 × 8s, 2 × 8s (16s), 3 × 8s (24s) and 4 × 8s (32s). The excised leaves were cultured on MS media supplemented with BAP 4.0 mg L^{-1} + NAA 1.0mg L^{-1} for regeneration. Culture bottle containing leaves were treated with the different cycles of irradiation times. In between successive irradiations, the bottle with explants were cooled in ice water for 5minutes to maintain constant room temperature. Data recorded 30 days after culture initiation of percent ex plant survival, callus induction, regeneration and culture establishment index.

RESULTS AND DISCUSSIONS

Effect of microwave irradiation on different cultivars

A. Explant survival (%)

The data depicted in Table 1. revealed that, the explant survival exhibited significant differences among the cultivar, irradiation time and their interactions. Among the three cultivars, the maximum explant survival (75.00 %) was noticed in cv. Marigold (C_1) which was significantly superior to cv. Journey Dark (C_3) (69.40%) and the lowest survival was recorded in cv. New Man (C_2) (60.68%). The maximum explant survival (92.56%) was obtained in without irradiation (E_1) followed by 8s irradiation (one cycles) (E_2) (81.00%). The minimum explant survival was noticed in 8s irradiation (4 cycles) (E_5) (41.33%).

Cultivars × micro-wave irradiation interaction results revealed that, the maximum explant survival (93.33%) was observed in cv. New Man exposed to no irradiation (C_2E_1) which was on par with cv. Journey Dark + without irradiation (C_3E_1) (92.67%) and cv. Marigold + without irradiation (C_1E_1) (91.67%). The minimum explant survival was recorded in cv. New Man + (4 cycles) (C_2E_5) (31.1%) which was on par with cv. Journey Dark + 8s irradiation (1 cycle) (C_3E_2) (36.33%).

Micro-waves are also part of electromagnetic radiation with a range of 300 MHz to 300 GHz and the wavelengths of 1 m - 1mm (Halmagyi *et al*., 2017). When the electromagnetic microwave radiation from oscillating electric fields is absorbed in tissues, it provokes a rotation of water molecules, which leads to heating Khalafallah and Sallam (2009). It is known that this increase in temperature may lead to reduction of explant survival, regeneration and proliferation Diprose (2001). There are very few research findings on the change of gene expression and mutations induction after the microwave irradiation in *Vigna aconitifolia* (Jacq.), Marechal (Jangid *et al*., 2010) and chrysanthemum Miler and Kulus (2018).

B. Callus induction (%)

The data presented in Table 2 revealed that, among the cultivars, Marigold (C_1) recorded maximum per cent callus induction (71.20%) which was significantly superior to cv. Journey dark(C_3) (60.07) and minimum callus induction was recorded in cv.New Man (C_2) (55.13%). An irradiation of 0 s (E_1) recorded maximum percent of callus induction (86.67%) followed by 8 s irradiation (1 cycle) (E_2) (76.78). However, minimum per cent callus induction was recorded in 8 s irradiation (4 cycles) (E_5)(33.89%).

Table 1: Influence of cultivar and irradiation time on *in vitro* leaf explant survival (%) in chrysanthemum at 30 DAI.

Explant survival (%)						
Cultivars (C)	**Irradiation time (E)**					**Mean**
	E_1- 0 s	E_2 - 8 s (1 cycle)	E_3 - 8 s (2 cycles)	E_4 - 8 s (3 cycles)	E_5 - 8 s (4 cycles)	
Marigold (C_1)	91.67 (73.37) *	84.00 (66.60)	78.67 (62.53)	64.00 (53.22)	56.67 (48.83)	**75.00 (60.91)**
New Man (C_2)	93.33 (74.97)	71.33 (57.71)	60.00 (50.77)	47.67 (43.64)	31.00 (33.80)	**60.68 (52.18)**
Journey Dark (C_3)	92.67 (74.60)	87.67 (69.48)	72.67 (58.52)	57.67 (49.44)	36.33 (36.96)	**69.40 (57.80)**
Mean	**92.56 (74.31)**	**81.00 (64.60)**	**70.44 (57.27)**	**56.44 (48.77)**	**41.33 (39.86)**	***68.46 (56.96)***
Factors	SE(m)			CD at 5%		
Cultivar (C)	1.06			3.08		
Irradiation time(E)	1.37			3.98		
C x E	2.37			6.89		

*Figures in parenthesis are arc sine transformed values

Table 2: Influence of cultivar and irradiation time on callus induction (%) from *in vitro* leaf explant in chrysanthemum at 30 DAI.

Callus induction (%)						
Cultivars (C)	**Irradiation time (E)**					**Mean**
	E_1- 0 s	E_2 - 8 s (1 cycle)	E_3 - 8 s (2 cycles)	E_4 - 8 s (3 cycles)	E_5 - 8 s (4 cycles)	
Marigold (C_1)	90.33 (71.89)*	80.33 (63.66)	75.00 (60.05)	60.33 (51.01)	50.00 (44.98)	**71.20 (58.32)**
New Man (C_2)	85.00 (67.38)	70.00 (56.77)	55.33 (48.05)	40.33 (39.38)	25.00 (29.91)	**55.13 (48.30)**
Journey Dark (C_3)	84.67 (67.07)	80.00 (63.55)	61.33 (51.61)	47.67 (43.64)	26.67 (30.93)	**60.07 (51.36)**
Mean	**86.67 (68.78)**	**76.78 (61.33)**	**63.89 (53.24)**	**49.44 (44.68)**	**33.89 (35.28)**	***62.13 (52.66)***
Factors	SE(m)			CD at 5%		
Cultivar (C)	0.92			2.68		
Irradiation time (E)	1.19			3.46		
C x E	2.06			NS		

*Figures in parenthesis are arc sine transformed values

C. Regeneration (%)

The percent regeneration varied significantly with different cultivars and irradiation frequencies. Maximum per cent regeneration was recorded in cv. Marigold (66.80%) which was statistically superior with cv. Journey Dark (C_3) (52.27%). However, minimum per cent regeneration was observed in New Man (C_2) (46.33%) (Table 3). Among the irradiation cycles, maximum per cent regeneration (82.44%) was noticed in 0s (without irradiation) (E_1) followed by (70.89%) in 8s irradiation (1 cycle) (E_2) and lowest percent regeneration was observed in 8 s irradiation + 4 cycles (E_5) (27.33%).

Among the interaction effects, Marigold + without irradiation (C_1E_1) recorded maximum per cent regeneration (85.67%) which was on par with cv. New Man + without irradiation (C_2E_1) (81.33%) followed by cv. Journey Dark + without irradiation (C_3E_1) (80.33%) and minimum per cent regeneration (15.00 %) was observed in cv. New Man + 8s irradiation (4 cycles) (C_2E_5) and it was statistically on par with cv. Journey Dark + 8s irradiation (4 cycles) (C_3E_5) (22.00%).

D. Number of regenerated shoots

Number of regenerated shoots was decreased significantly with increasing irradiation exposure. Significant differences were observed among the cultivars (C) and irradiation time (E) with respect to number of regenerated shoots per explant in *in vitro* conditions (Table 4). Among the cultivars tested, maximum number of regenerated shoots per explant were observed in cv. Marigold (C_1) (5.69) and statistically superior over cv. Journey Dark (C_3) (5.26) and minimum number of regenerated shoots in cv. New Man (C_2) (4.34). Number of regenerated shoots were maximum in 8s irradiation + 1 cycle (E_2) (6.01) followed by in 8s irradiation + 2 cycles (E_3) (2.88). The minimum number of shoots multiplied per explant was noticed in 8 s irradiation + 4 cycles (E_5) (0.56).

The effect of physical mutagens on different chrysanthemum explant types and their *in vitro* morphogenetic response was also reported by (Zalewska *et al.*, 2010). They had reported the reduction of adventitious shoots on nodal explants of 'Satinbleu' and completely inhibited the morphogenesis in leaf explants of 'Albugo' and 'Satinbleu'. On the other hand, (Miler and Kulus 2018) observed very less influence of the microwave irradiation on the explant survival, callus formation and further regeneration of shoots which is in contrast to the present experimental results. This variation might be occurred due to the genotype variation, physiological status of mother plants and other external factors.

Table 3: Influence of cultivar and irradiation time on regeneration (%) from *in vitro* leaf explant in chrysanthemum at 30 DAI.

Regeneration (%)						
Cultivars (C)	**Irradiation time (E)**					**Mean**
	E_1- 0 s	E_2 - 8 s (1 cycle)	E_3 - 8 s (2 cycles)	E_4 - 8 s (3 cycles)	E_5 - 8 s (4 cycles)	
Marigold (C_1)	85.67 (67.86)*	78.00 (62.02)	69.67 (56.62)	55.67 (48.25)	45.00 (42.10)	**66.80 (55.37)**
New Man (C_2)	81.33 (64.43)	60.67 (51.21)	49.00 (44.41)	25.67 (30.26)	15.00 (22.59)	**46.33 (42.58)**
Journey Dark (C_3)	80.33 (63.75)	74.00 (59.36)	47.33 (43.45)	37.67 (37.84)	22.00 (30.82)	**52.27 (42.43)**
Mean	**82.44 (63.35)**	**70.89 (57.33)**	**55.33 (48.16)**	**39.67 (38.78)**	**27.33 (30.82)**	***55.10 (46.79)***
Factors	SE(m)			CD at 5%		
Cultivar (C)	1.40			4.03		
Irradiation time (E)	1.80			5.20		
C ×x E	3.12			9.01		

*Figures in parenthesis are arc sine transformed values

Table 4: Influence of cultivar and irradiation time on number of regenerated shoots per*in vitro* leaf explantin chrysanthemum at 30 DAI.

Number of regenerated shoots per explant						
Cultivars (C)	**Irradiation time (E)**					**Mean**
	E_1- 0 s	E_2 - 8 s (1 cycle)	E_3 - 8 s (2 cycles)	E_4 - 8 s (3 cycles)	E_5 - 8 s (4 cycles)	
Marigold (C_1)	15.61 (4.07)*	6.47 (2.72)	3.00 (1.99)	2.37 (1.81)	1.00 (1.38)	**5.69 (2.40)**
New Man (C_2)	14.17 (3.89)	4.45 (2.33)	2.27 (1.79)	0.83 (1.33)	0.00 (1.00)	**4.34 (2.07)**
Journey Dark (C_3)	13.00 (3.74)	7.10 (2.84)	3.37 (2.07)	2.17 (1.76)	0.67 (1.24)	**5.26 (2.33)**
Mean	**14.26 (3.90)**	**6.01 (2.63)**	**2.88 (1.95)**	**1.79 (1.63)**	**0.56 (1.21)**	***5.09 (6.80)***
Factors	SE(m)			CD at 5%		
Cultivar (C)	0.07			0.20		
Irradiation time (E)	0.09			0.26		
C x E	0.15			NS		

*Figures in parenthesis are square transformed values

E. Culture establishment index

The data revealed in Table 5 indicated that, there were significant differences for culture establishment index among the cultivar (C), irradiation time (E) and interaction between cultivars and irradiation time (C × E). The maximum culture establishment index was observed in cv. Marigold (C_1) (448.64) followed by cv. Journey Dark (C_3) (367.00) which was statistically significant with each other and minimum culture establishment index was recorded in cv. New Man (C_2) (313.70). An irradiation of 0 s (E_1) observed the maximum culture establishment index (1179.51) followed by 8 s irradiation + 1 cycle (E_2) (435.00). The minimum culture establishment index was recorded in 8 s irradiation + 4 cycles (E_5) (22.78).Among the interactions between cultivar and irradiation time (C × E), cv. Marigold + 0 s irradiation (C_1E_1) (1339.02) was found to be significantly superior over cv. New Man + without irradiation (C_2E_1) (1153.8) and cv. Journey Dark + without irradiation (C_3E_1) (1045.6). In all three cultivars, increasing the exposure of micro - wave irradiation significantly decreased the culture establishment index. No cultures were established when the explants of New Man exposed to 4 cycles of irradiation 8 seconds each (C_2E_5).

Table 5: Influence of cultivar and irradiation time on culture establishment index from *in vitro* leaf explant in chrysanthemum at 30 DAI.

Culture establishment index						
Cultivars (C)	**Irradiation time (E)**					**Mean**
	E_1- 0 s	E_2 - 8 s (1 cycle)	E_3 - 8 s (2 cycles)	E_4 - 8 s (3 cycles)	E_5 - 8 s (4 cycles)	
Marigold (C_1)	1339.02	506.27	212.67	136.9	48.33	**448.64**
New Man (C_2)	1153.83	274.67	115.17	24.83	0.00	**313.70**
Journey Dark (C_3)	1045.67	524.07	161.93	83.33	20.00	**367.00**
Mean	**1179.51**	**435.00**	**163.26**	**81.69**	**22.78**	***376.44***
Factors	SE(m)			CD at 5%		
Cultivar (C)	29.08			59.67		
Irradiation time (E)	37.54			77.03		
C × E	65.02			133.42		

CONCLUSION

Prolonged exposure of irradiation to explants resulted in gradual reduction of survival rate (%), callus induction (%), regeneration (%), number of regenerated shoots per explant and culture establishment index compared to lesser irradiation time (8 s irradiation up to 2 cycles). These experimental results confirmed the effect of micro-wave irradiation on damaging of plant tissues to a certain extent. Field evaluation of the regenerants which are exposed to micro-wave radiation will be helpful to confirmation of changes in genetic level.

FUTURE SCOPE

— In case of microwave radiation, similar studies can be conducted by using chrysanthemum petals and nodes as explants.

— Different biochemical and molecular markers can be screened to study the genetic variation of the mutated populations.

Acknowledgement. The authors are thankful to Dr. YSRHU-Horticultural Research Station, Kovvur, Andhra Pradesh, India for providing use necessary facilities to undertake the studies.

Conflict of Interest. None.

REFERENCES

Anderson, R. L. (1987). Reclassification of genus Chrysanthemum. *Horticultural Science*, *22*: 313.

Diprose, M. F. (2001). Some considerations when using a microwave oven as laboratory research tool. *Plant Soil Journal*, *229*: 271-280.

Datta, S. K., and Gupta, V. N. (2012). Year-round cultivation of garden chrysanthemum through Photoperiodic response. *Scientific Culture*, *8*: 71-77.

Haamagyi, A., Surducan, E., and Surducan, V. (2017). The effect of low and high-power micro wave irradiation on *in vitro* grown Sequoia plants and their recovery after cryostorage. *Journal of Biological Physics*, *43*(3): 367–379.

Jangid, R. K., Sharma, R., Sudarson, Y., Eapen, S., Singh, G., and Purohit, A. K. (2010). Micro wave treatment induced mutations and altered gene expression in *Vigna aconitifolia*. *Biologia Plantarum*, *54* (4): 703–706.

Khalafallah, A. A., and Sallam, M. S. (2009). Response of maize seedlings to microwaves at 945 MHZ. *Romanian Journal of Biophysics*, *19*(1): 49–62.

Kulus, D. (2017). Selected aspects of biology, breeding and reproduction of chrysanthemum. *ZPPNR 588*: 47–61.

Miler, N., and Kulus, D. (2018). Microwave treatment can induce chrysanthemum phenotypic and changes. *Scientia Horticulturae*, *227*: 223–233.

Oladosu, Y., Rafii, M. Y., Abdullah, N., Hussin, G., Ramli, A., Rahim, A. H., and Miah, G. (2016). Principle and application of plant mutagenesis in crop improvement: a review. *Biotechnology and Biotechnological Equipment*, *30*: 1-16.

Tisserat, B., Galletta, P. D., and Jones, D. (1992). Microwave sterilization of plant tissue culture media. *Journal of Horticultural Science*, *27* (4): 358–361.

Teixeira da Silva, J.A., and Kulus, D. (2014). Chrysanthemum biotechnology: discoveries from the recent literature. *Folia Horticulturae*, *26*(2): 67–77.

Zalewska, M., Miler, N., and Wenda-Piesik, A. (2010). Effect of *in vitro* topophysis on the growth, development, and rooting of chrysanthemum explants (*Chrysanthemum grandiflorum*). *Journal of Horticultural Science Biotechnology*, *85*(4): 362–366.

How to cite this article: M. Kiranmayi K., Ravindra Kumar, G. Ramanandam and M. Paratpara Rao (2022). Effect of Micro-wave Radiation on *in vitro* Plant Regeneration in Chrysanthemum (*Chrysanthemum morifolium*). *Biological Forum – An International Journal*, *14*(3): 1074-1078.