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Congenial in vitro γ- ray induced Mutagenesis underlying the diverse array of petal colours in chrysanthemum (Dendranthemum grandiflorum *kitam*) cv. "Candid"

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Abstract
Chrysanthemum (Dendranthemum grandiflorum kitam.) is a leading flower with applied value worldwide. The flower color of ancestral species is limited to yellow, pink, and white, and is derived from carotenoids, anthocyanins, and the absence of both pigments, respectively. A wide range of flower colors, including purplish-red, orange, red, and dark red, has been developed by increasing the range of pigment content or the combination of both pigments. Recently, green-flowered cultivars containing chlorophylls in their ray petals have been produced, and have gained popularity. In addition, blue/violet flowers have been developed using a transgenic approach. Flower color is an important trait that influences the commercial value of chrysanthemum cultivars. Developing new chrysanthemum cultivars with novel characteristics such as new flower colors in a time- and cost-efficient manner is the ultimate goal for breeders. Understanding the molecular mechanisms that regulate flower pigmentation may provide important implications for the rationale manipulation of flower color. To generate diverse array of flower colour mutants in chrysanthemum cv. “Candid” through mutagenesis, in vitro grown micro shoots were exposed to 10, 20, 30 and 40 Gy gamma irradiation at 100 Gy per minute and were evaluated for different parameters. The rhizogenesis parameters decreased with the increase in irradiation dose from 0 Gy to 40 Gy, while as, 10 Gy dose proved to record minimum decline as compared to the control. Survival, leaf size and number of leaves plant-1 a□ er 8th week interval also decreased with the increasing trend of gamma irradiation dose but recorded minimum decline in plants developed from shoots irradiated with 10 Gy gamma irradiation dose with respect to the control. Apparently minimum delay in number of days to floral bud appearance took under 10 Gy as compared to control. Highest number of flower colour mutants were recorded under 10 Gy (light pink, orange pink,white and yellow). Amountable mutation frequency on the basis of flower colour was desirable in plants irradiated with least dose of 10 Gy

Keywords: Chrysanthemum, Mutagenesis, Gamma Irradiation, Mutants

“Objectives”

To generate diverse array of flower colour mutants through mutagenesis.

Technical Programme

I. Plant material

Explant: Shoot tips

II.Variety

Candid

III.Explant sterilization

Details of the Treatment	
T_1	**Mercuric chloride 0.1 % for 2 and 4 minutes.**
T_2	**Sodium hypochlorite 0.1 % for 2 and 4 minutes**
T_3	**Carbendazim 100 and 200 ppm for 30 minutes followed by Mercuric chloride 0.1 % for 2 and 4 minutes followed by 70 % ethyl alcohol dip for 10 seconds**
T_4	**Carbendazim 100 and 200 ppm for 30 minutes followed by Sodium hypochlorite 0.1 % for 2 and 4 minutes followed by 70 % ethyl alcohol dip for 10 seconds**

Omissions, incremental increase/decrease in the type/nature of sterilants/chemicals, time duration of application, strength of sterilant/chemicals combinations shall be tried in case aforementioned treatments are not found satisfactory.

IV. Basal culture media used

Murashige and Skoog medium (1962)

V. Growth regulators used for morphogenesis

i) Auxins

Indole Butyric Acid (IBA)	(0.5-5 mg l^{-1})
Naphthalene Acetic Acid (NAA)	(0.5-10 mg l^{-1})
2, 4-Dichlorophenoxyacetic acid (2, 4-D)	(0.5-5 mg l^{-1})
Indole Acetic Acid (IAA)	(0.5-5 mg l^{-1})

ii) Cytokinins

Benzyl Amino Purine (BAP)	(0.5-5 mg l^{-1})
Kinetin	(0.5-10 mg l^{-1})

iii) Gibberelic Acid (GA3) (0.2-0.5 mg l^{-1}).

Omissions, incremental increase/decrease in the constituents of basal media, concentration of growth regulator combinations/chemicals, shall be tried in case no satisfactory results are obtained with aforementioned concentration ranges.

VI. Hardening of rooted plantlets

The plantlets will be transferred to containers containing appropriate dosages of shoot and root promoting hormones along with controlled light and temperature regimes. Latter the semi-hardened plantlets shall be transferred to containers with different hardening media components like vermiculite, perlite, sterilized sand/soil etc.

“Effect of gamma-irradiation on *in-vitro* cultured explant of chrysanthemum”.

I. Varieties

a) Candid

II. Explant

Shoot tips (*in-vitro*).

III. Gamma irradiation doses

Explant were irradiated at a dose rate of 100 Gy per minute at 10, 20,30 and 40 Gy. Untreated explant were used as control.

Observations recorded:

- Number of irradiated shoots rooted (%)
- Number of roots per shoot
- Survival of plants (%)
- Leaf size (length/width)
- Leaf number
- Number of days to bud appearance
- xii) Plant height at flowering
- Flower colour
- Mutation frequency

- **Statistical analysis**

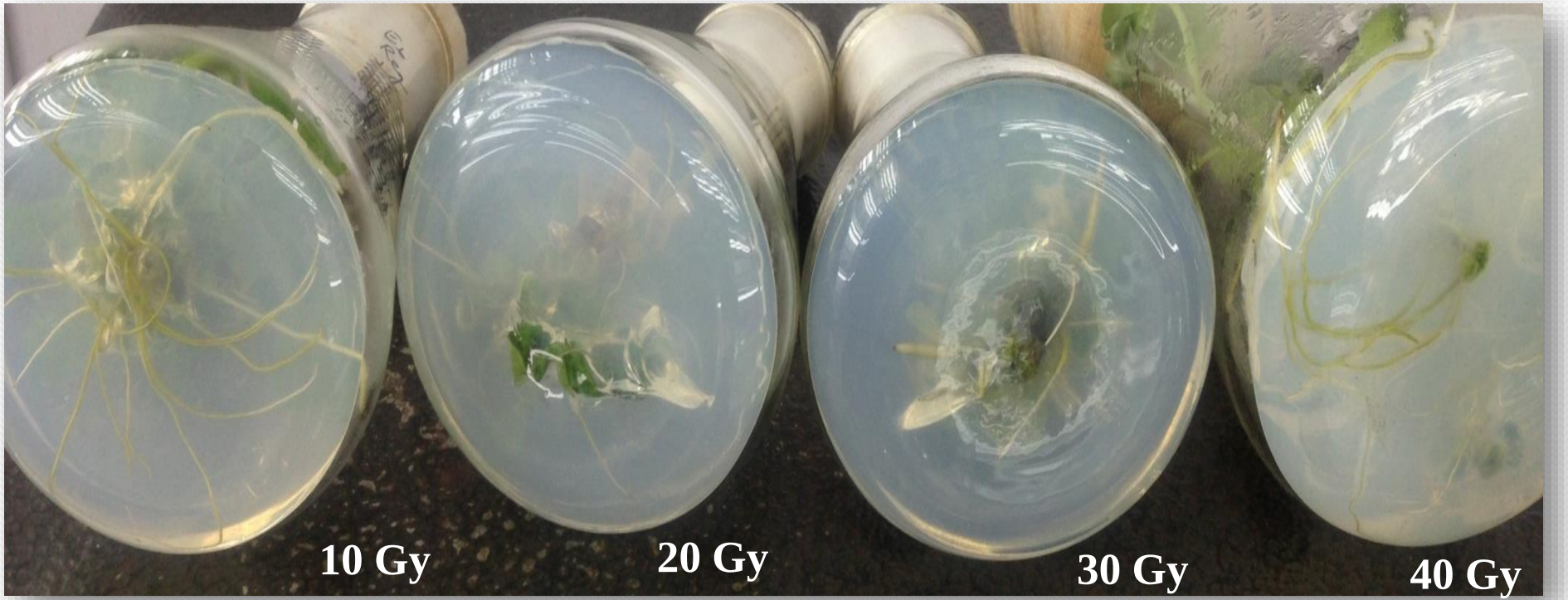
CRD with 3 replications

Table 1: Influence of ^{60}Co gamma irradiation on rhizogenesis in shoots of Chrysanthemum (*Dendranthemum morifolium*) cv. "Candid"

Dose	Rooting (%)	Root number shoot^{-1}
0 Gy	89.28 *(9.44)	15.75 -
10 Gy	**85.71** ***(9.26)** ****(1.90)**	**7.75** ****(50.79)**
20 Gy	79.76 (8.93) (5.40)	5.50 (65.07)
30 Gy	74.99 (8.66) (8.26)	3.00 (80.95)
40 Gy	73.80 (8.59) (9.00)	2.75 (82.53)
L.S.D $_{P\leq0.05}$	**0.18**	**0.90**

*Figures in the parenthesis are the square root transformed values of percentage data

**Figures in the parenthesis are the per cent decrease in vegetative parameters over control



Rooting

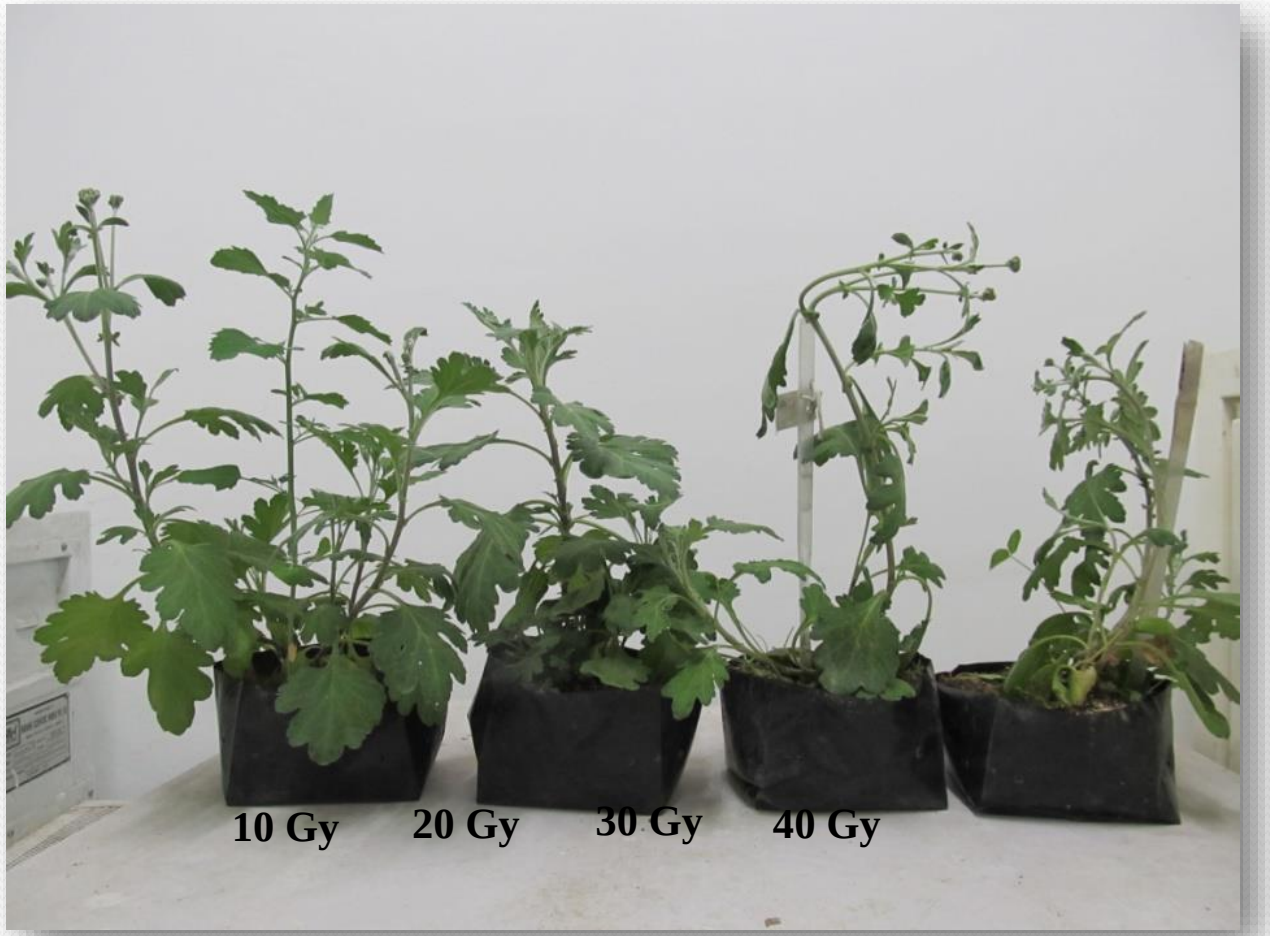

10 Gy
20 Gy
30 Gy
40 Gy

Table 2: Influence of ^{60}Co gamma irradiation on survival of rooted plantlets under polyhouse conditions in Chrysanthemum (*Dendranthemum morifolium*) cv. "Candid"

Survival (%)			
Dose	**Week 4th**	**Week 8th**	**Mean**
0 Gy	95.23 *(9.76)	95.23 *(9.76)	**95.23** ***(9.76)**
10 Gy	85.71 *(9.25) **(5.22)	82.14 *(9.06) **(7.17)	83.92 (9.16)
20 Gy	76.18 (8.72) (10.65)	67.85 (8.23) (15.67)	**70.01** **(8.48)**
30 Gy	61.90 (7.86) (19.46)	53.56 (7.31) (25.10)	**57.73** **(7.59)**
40 Gy	53.56 (7.31) (25.10)	40.47 (6.34) (35.04)	**40.01** **(6.83)**
Mean	**74.51** **(8.58)**	**67.85** **(8.14)**	

	LSD $_{P\leq0.05}$
Week	**0.17**
Dose	**0 .27**
Week*Dose	**0.39**

*Figures in the parenthesis are the square root transformed values of percentage data
**Figures in the parenthesis are the per cent decrease in vegetative parameters over control



survival

Table 3: Influence of ^{60}Co gamma irradiation on leaf number and leaf size in Chrysanthemum (*Dendranthemum morifolium*) cv. "Candid"

Dose	Leaf number plant^{-1}		Leaf size (length/width) (cm^2)	
	4th week	8th week	4th week	8th week
0 Gy	14.00 -	15.75 -	22.30 -	28.52 -
10 Gy	11.00 (21.42)	13.00 (17.46)	20.42 (8.43)	27.80 (2.52)
20 Gy	9.25 (33.92)	11.75 (25.39)	17.34 (22.24)	20.42 (28.40)
30 Gy	8.75 (37.50)	10.75 (31.74)	13.52 (39.37)	14.82 (48.03)
40 Gy	8.00 (42.85)	9.75 (38.09)	5.317 (76.18)	10.74 (62.34)
L.S.D $_{P\leq0.05}$	0.92	1.41	2.69	2.37

Figures in the parenthesis are the per cent decrease in vegetative parameters over control

Table 4: Influence of ^{60}Co gamma irradiation on plant height at flowering, number of days to bud appearance, flower colour and mutation frequency in Chrysanthemum (*Dendranthemum morifolium*) cv. "Candid"

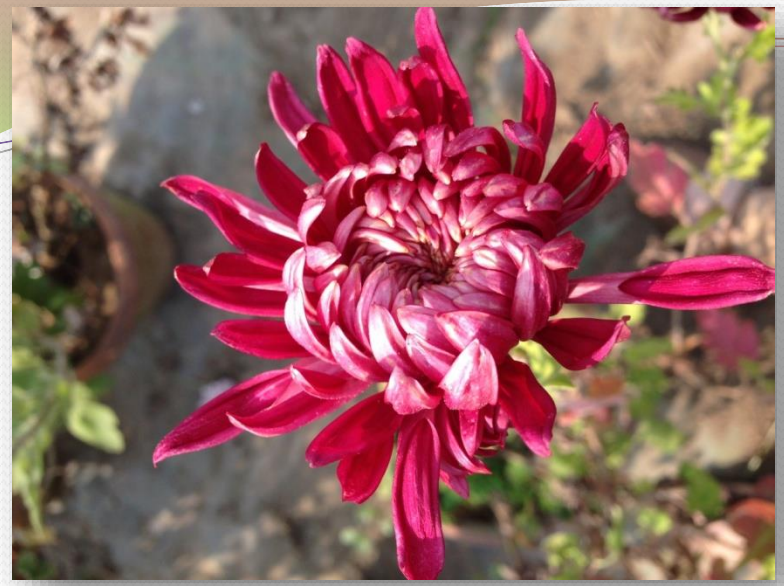
Dose	Number of days to bud appearance	Flower colour	Plant height at flowering(cm)	Mutation frequency (%)
0 Gy	23.50 -	Red	53.25 -	0
10 Gy	**27.25** **(15.95)**	**Light-pink (12/20)** **White (3/20)** **Orange pink(2/20)** **Light Yellow (1/20)**	**49.00** **(7.98)**	**90(60,10,15,5)**
20 Gy	37.00 (57.44)	Red bud distorted(12/20) Red floret distorted(8/20)	36.50 (31.45)	100(60,40)
30 Gy	39.25 (67.02)	Red bud distorted (20/20)	34.00 (36.15)	100
40 Gy	40.75 (73.40)	Bud aborted(17/20) Red bud distorted(3/20)	31.50 (40.84)	100(85, 15)
L.S.D $_{P\leq0.05}$	**2.58**		**2.03**	

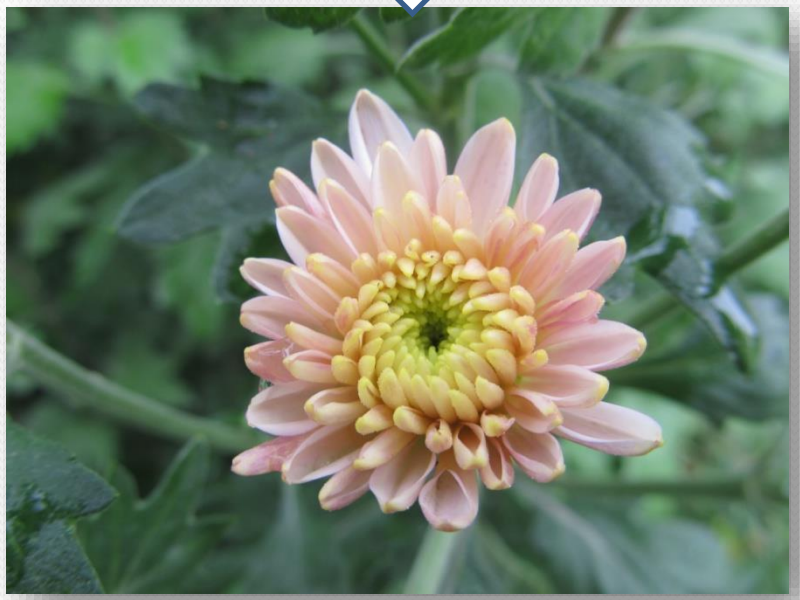
Figures in the parenthesis are the per cent decrease in vegetative parameters over control






Buds of mutants of 10 Gy





Mutants of 10 Gy

Mutants of 10 Gy

Red distorted bud of 20, 30 and 40 Gy

Red distorted florets of 20 Gy

Mutants of 20-40 Gy

Conclusions

- Irradiation dose of 40 Gy resulted in significant decrease in rooting percentage and subsequent decline in field survival, number of days to bud appearance and mutation frequency.

- Highest number of mutants in the form of flower colour (Light pink, White,orange pink and yellow) were recorded in shoots irradiated with 10 Gy.

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- Highest mutation frequency on the basis of colour was recorded with 10 Gy gamma irradiation treatment.

- LD_{50} value was around 28.68 Gy.


Thank you