INDUCED COMB-COLOUR MUTANTS IN COCKSCOMB (*CELOSIA CRISTATA* L.)

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Abstract We have isolated twelve comb-colour mutants in cockscomb induced through conventional as-well-as in vitro mutagenesis. The spectrum of these mutants included the combs with rose-red, orange, vivid red, coral red, vermillion and carmine colour. These comb-colours were obviously distinct from that in control (magenta). The content of total betalain, amaranthin, betanin, betalamic acid and betaxanthin was reduced in the mutants. Notably, the relative contents of these pigments in the mutant also differed, which might be the reason for variation in comb-colour of these mutants. Therefore, the mutants isolated in the present investigation can, used for crop improvement and serve as a model to study betalain biosynthesis in cockscomb.

Keywords: EMS, gamma ray, in vitro mutagenesis, mutagen, ornamental

Running title: Colour mutants in cockscomb

Introduction

Celosia cristata (L.) or cockscomb is widely planted in the gardens of South America, Africa, and Asia (Bakar et al. 2014). The compact form and velvety texture of its inflorescence makes cockscomb a plant suitable for landscaping and roadside plantation (Meng et al. 2009). Cockscomb is the focal point of a garden due to the unique shape and fascinating hues of its inflorescence due to the presence of betalain pigments, which are restricted to the few members of Order Caryophyllales (Warhade and Badere 2018). Betalains have at least one heterocyclic nitrogen atom in their ring. However, they are not alkaloids *sensu stricto* as they are acidic in nature due to the presence of several carboxyl groups. Conjugated double bonds in the structure of betalains impart colouring properties to the molecules. Based on the colour, two major classes of betalains are identified viz., purple coloured betacyanins and yellow to orange coloured betaxanthins (Harris et al. 2012). The combination of these betacyanins and betaxanthins generates an array of hues in the plant part synthesising the betalains.

Mutation breeding is an effective method of crop improvement. It modifies one or two desirable traits, like flower colour, of the already established variety without altering the other traits (Ahloowalia et al. 2004). Coupling of induced mutagenesis with in vitro techniques has been suggested to increase the frequency of mutations. The technique of micropropagation supplements induced mutagenesis by amplifying the mutated sector thereby increasing the frequency of mutation (Maluszynski et al. 2001; Nasri et al. 2021).

We initiated a study to compare the effectiveness and efficiency of conventional mutagenesis vis-à-vis in vitro mutagenesis in cockscomb (Rinkey and Badere 2023). In this study, we treated the seed of cockscomb with either EMS or GR to raise the M_1 (conventional mutagenesis) and RM_1 (in vitro mutagenesis) population. This population was harvested on maturity and the seeds thus obtained were sown in next season to raise the M_2 and RM_2 generation. The M_2 and RM_2 populations were thoroughly screened to isolate the putative mutants for various traits. The seeds of these putative mutants were sown to raise the M_3 and RM_3 generation to study their inheritance thereby confirming the mutant. Among others, a few comb-colour mutants in cockscomb were isolated in this investigation. The present paper describes the morphological and phytochemical characterisation of the comb-colour mutants isolated during the investigation.

Materials and Methods

Isolation of comb colour mutants

Two modes of mutation induction viz. conventional and in vitro were employed in the present investigation. For this, the seeds of cockscomb treated with either EMS or gamma ray as described earlier (Rinkey and Badere 2023, 2024). Subsequently, the M_1 and RM_1 generations were raised and harvested plant-wise. In the subsequent season, the seeds harvested from M_1 and RM_1 plants were sown in a tray along with control to raise the M_2 and RM_2 generations on plant-to-row basis. Enough seedlings at 4 leaf stage were transplanted in the experimental plots to maintain 25 plants per concentration/dose. The population was thoroughly screened to isolate the putative mutants for various traits. In the next season the M_3 and RM_3 generations were also raised on plant-to-row basis from the seeds of putative mutants harvested in M_2/RM_2 generation. The inheritance of comb-colour, for which the putative mutants were isolated, was tested in this generation. Fifty seeds from each putative mutants were sown in the tray along with the control. Subsequently, the 30d-old seedlings were transplanted to the field and allowed to grow as earlier. The population was screened regularly to monitor the growth and development of plants. The putative mutant whose progenies exhibited the character for which it was isolated in the M_2/RM_2 generation was isolated as viable mutant. Such viable mutants were characterised for their morphological features.

Pigment analysis in the comb-colour mutants

The comb-colour mutants isolated in the present investigation were qualitatively and quantitatively analysed for their betalain pigments. The qualitative analysis was performed using thin layer chromatography, while the amaranthin, betanin, betalamic acid and betaxanthins were quantified spectrophotometrically as described by Warhade and Badere (2015). Later, the pigment contribution and pigment ratio were calculated according to the formulae given by Warhade and Badere (2015, 2018).

Statistical analysis

The data obtained during the present investigation were statistically analysed by computing the arithmetic mean, standard error and Student's t value using MS-Excel.

Results

Viable comb-colour mutants

The putative comb-colour mutants were induced with higher frequency in RM_2 population in response to the EMS treatment. In the same manner the frequency of putative comb-colour mutants, in response to GR treatment, was also higher in RM_2 population compared the M_2 population. Apart from this the pre-soaking of seeds prior to EMS treatment also reduced the frequency of putative comb-colour mutants (Rinkey and Badere 2023).

Seeds of putative comb-colour mutants were sown to raise the M_3/RM_3 generation. Eight plants from M_3 families and four plants from RM_3 families displayed the character for which they were selected. These twelve plants were designated as viable mutants and characterised. All but one viable comb-colour mutant was induced by EMS in M_3 generation. Moreover, most of the comb-colour mutants induced by EMS, in fact, were multiple mutants. The mutant E/0/0.15/47/8/5 with rose red colour had smaller leaves and larger comb. Contrastingly another rose red mutant, E/0/0.15/47/10/8, had marginally larger leaves and a greater number of branches compared to the control. The orange colour mutant, E/0/0.15/54/7/15, had slightly larger comb. Similarly, one of the coral red mutants, E/3/0.15/30/3/10, had shorter internode & inflorescence, and a vermillion mutant E/6/0.20/19/5/1 had comb with markedly longer diameter. In addition to these, EMS also induced vivid red mutant E/0/0.25/60/10/25, which was similar to control for other characters studied (Table 1). Among the comb colour mutants induced in RM_3 generation, R/E/0/15/2/2/15 (vivid red) was similar to the control. However, the other three mutants were multiple mutants. While comb of EMS-induced R/E/0/20/1/1/12 had carmine colour and smaller internodes, another one with vermillion coloured comb (R/E/0/25/11/1/8) had longer comb diameter. Similarly, gamma ray-induced R/G/30/4/3/14 was dwarf with carmine comb-colour (Table 1).

Phytochemical characterisation of the comb colour mutants

The TLC profile of betalain pigments in control and the comb-colour showed five bands having R_f values of 0.11 (magenta), 0.17 (magenta), 0.27 (reddish pink), 0.36 (light pink) and 0.49 (yellow). Notably, the profile of all the comb-colour mutants was alike control showing five bands of same R_f and colour. However, the intensity of bands with R_f value 0.11 and 0.17 increased in all the mutants in comparison to control except for orange, rose red and vivid red coloured mutants. Rather, the intensity of the band with R_f value 0.11 was decreased in the orange mutant (E/0/0.15/54/7/15) compared to that of control (Table 2).

Table 1. Morphological traits of the comb colour mutants

Generation	Mutant	Plant height (cm)	No. of branches	Length of internode (cm)	Diameter of stem (cm)	Leaf area (cm^2)	Length of comb (cm)	Diameter of comb (cm)	Circumfe-rence of comb (cm)	Length of fertile region (cm)	Colour of comb
M_3	Control*	29.0-80.0 (57.2)	7-16 (11)	1.7-6.9 (3.4)	0.34 - 1.75 (1.00)	15.8-36.2 (25.7)	8.2-19.5 (13.8)	1.0-8.8 (6.1)	7.5-14.0 (10.9)	6.5-14.0 (9.4)	Magenta
	E/0/0.15/47/8/5	75.0	13	4.2	0.92	10.8	13.8	11.4	17.9	7.0	Rose-red
	E/0/0.15/47/10/8	74.5	19	3.6	0.97	38.9	19.2	6.8	12.7	14.3	Rose-red
	E/0/0.15/54/7/15	70.0	8	4.0	0.90	24.5	16.0	6.9	15.0	11.0	Orange
	E/0/0.25/60/10/25	29.5	13	3.0	0.90	22.8	11.0	6.6	10.5	6.8	Vivid red
	E/3/0.15/30/3/10	65.5	15	1.5	0.53	17.8	8.0	6.5	12.0	10.0	Coral red
	E/3/0.25/23/1/5	44.0	14	2.5	0.55	16.3	10.0	5.5	8.7	7.0	Coral red
	E/6/0.20/19/5/1	76.0	11	6.0	1.08	22.1	12.0	10.0	14.0	8.0	Vermilion
	G/30/11/2/1	42.7	9	2.1	0.62	18.9	17.2	6.1	12.2	13.7	Vermilion
RM_3	Control*	23.0-80.0 (56.5)	7-16 (11)	1.7-6.9 (3.6)	0.34 - 1.25 (1.00)	10.8-36.2 (24.6)	7.2-19.5 (13.3)	1.8-8.0 (6.1)	2.5-14 (8.7)	6.5-14 (9.4)	Magenta
	R/E/0/15/2/2/15	60.2	10	5.2	0.72	13.7	12.7	7.2	12.2	7.1	Vivid red
	R/E/0/20/1/1/12	39.1	9	1.1	0.95	11.0	8.2	3.2	9.1	7.5	Carmine
	R/G/30/4/3/14	22.7	11	3.9	1.18	12.0	14.1	2.4	5.1	9.5	Carmine
	R/E/0/25/11/1/8	46.5	8	5.1	1.01	15.2	9.5	9.1	12.5	6.7	Vermilion

* The figures are the minimum and maximum value of the trait and the figure in parentheses is the mean value of the trait.

Subsequently, the content of betalain pigments viz. amaranthin, betanin, betalamic acid and betaxanthin was spectrophotometrically quantified in control and the mutants. The total betalain content in the mutants was either unaffected or reduced. Remarkably, the total betalain content in the mutants isolated from M_3 population was reduced except in the mutant E/3/0.15/30/3/10 with coral red colour. In contrast, the total betalain content was unaffected in the mutants isolated from RM_3 population with an exception of vermillion coloured mutant RE/0.25/11/1/8 where it was decreased (Table 3). The content of amaranthin and betanin was

Table 2. TLC profile of betalain pigments in the comb of comb-colour mutants

Mutant	Comb colour	R_f value and colour of the band				
		0.11	**0.17**	**0.27**	**0.36**	**0.49**
		Magenta	**Magenta**	**Reddish pink**	**Light pink**	**Yellow**
Control	Magenta	+	+	+	+	+
M_3 generation						
E/0/0.15/47/8/5	Rose-red	+	+	+	+	+
E/0/0.15/47/10/8	Rose-red	+	+	+	+	+
E/0/0.15/54/7/15	Orange	<	+	+	+	+
E/0/0.25/60/10/25	Vivid red	+	+	+	+	+
E/3/0.15/30/3/10	Coral red	++	++	+	+	+
E/3/0.25/23/1/5	Coral red	++	++	+	+	+
E/6/0.20/19/5/1	Vermilion	+++	++	+	+	+
G/30/11/2/1	Vermilion	+++	++	+	+	+
RM_3 generation						
RE/0.15/2/2/15	Vivid red	+	+	+	+	+
RE/0.2/1/1/12	Carmine	++	++	+	+	+
RG/30/4/3/14	Carmine	++	++	+	+	+
RE/0.25/11/1/8	Vermilion	+++	++	+	+	+

reduced in almost all the mutants compared to control. The amaranthin content was reduced, in comparison to control, in all the mutants induced through conventional mutagenesis. Similarly, all the mutants induced through in vitro mutagenesis had amaranthin content lower than control except the vivid red mutant RE/0.15/2/2/15 (Table 3). In the same way all the mutants induced through conventional mutagenesis, except rose red E/0/0.15/47/8/5 and coral red E/3/0.15/30/3/10, had lower betanin content compared to the control. In contrast, the betanin content was reduced only in vermillion mutant RE/0.25/11/1/8 among the mutants

induced by through vitro mutagenesis. A marked difference in the content of betalamic acid and betaxanthin was observed between the mutants induced through conventional and in vitro mutagenesis. Most of the mutants induced through conventional mutagenesis had content of betalamic acid and betaxanthin similar to control. Exceptionally the orange mutant

E/0/0.15/54/7/15 had reduced content of both these pigments. Similarly, betalamic acid and betaxanthin content were reduced in rose red mutant E/0/0.15/47/8/5 and vivid red mutant E/0/0.25/60/10/25, respectively. While the content of betaxanthins were reduced in all the mutants induced through in vitro mutagenesis; that of betalamic acid was increased in all but except vermillion mutant RE/0.25/11/1/8 (Table 3).

Table 3. Betalain content (mg/g FW) in the comb of comb-colour mutants

Mutant	Comb colour	Amaranthin	Betanin	Betalamic acid	Betaxanthin	Total Betalain content
Control	Magenta	1.07±0.05	0.76±0.14	0.27±0.03	0.0003±0.00	2.10±0.21
M_3 generation						
E/0/0.15/47/8/5	Rose-red	0.86±0.05*	0.59±0.03	0.18±0.01*	0.0002±0.00	1.50±0.04*
E/0/0.15/47/10/8	Rose-red	0.52±0.09*	0.33±0.02*	0.24±0.02	0.0002±0.00	0.93±0.01*
E/0/0.15/54/7/15	Orange	0.49±0.05*	0.33±0.02*	0.11±0.01*	0.0001±0.00*	0.83±0.04*
E/0/0.25/60/10/25	Vivid red	0.43±0.01*	0.31±0.02*	0.24±0.02	0.0001±0.00*	0.93±0.02*
E/3/0.15/30/3/10	Coral red	0.94±0.07*	0.72±0.07	0.21±0.04	0.0004±0.00	1.80±0.13
E/3/0.25/23/1/5	Coral red	0.55±0.07*	0.41±0.07*	0.28±0.04	0.0003±0.00	1.05±0.30*
E/6/0.2/19/5/1	Vermilion	0.46±0.04*	0.31±0.04*	0.26±0.06	0.0003±0.00	0.97±0.20*
G/30/11/2/1	Vermilion	0.53±0.06*	0.41±0.07*	0.31±0.02	0.0003±0.00	1.08±0.26*
RM_3 generation						
RE/0.15/2/2/15	Vivid red	1.15±0.09	0.79±0.06	0.45±0.07*	0.0001±0.00*	2.39±0.08
RE/0.2/1/1/12	Carmine	0.77±0.03*	0.56±0.11	0.50±0.08*	0.0002±0.00*	1.84±0.20
RG/30/4/3/14	Carmine	0.89±0.08*	0.70±0.16	0.41±0.08*	0.0002±0.00*	2.00±0.32
RE/0.25/11/1/8	Vermilion	0.30±0.05*	0.24±0.03*	0.22±0.02	0.0001±0.00*	0.76±0.08*

*The values are significantly different than control at $p = 0.05$ according to the Student's t-test

Every pigment, although, contributes to the colour of tissue; the resultant colour produced depends on the absolute content of a pigment and the relative content of each pigment in the tissue. To understand the role of latter, we used two metrics viz. the pigment contribution and pigment ratio. While pigment contribution conveys the proportion of a pigment among total betalain content in the tissue; the relative abundance of two pigments is understood from the pigment ratio. The pigment contribution of betalamic acid and betaxanthin was mostly affected

in the mutants. The pigment contribution of betalamic acid was increased in all the mutants induced through in vitro mutagenesis. Similarly, its pigment contribution was also increased in vermillion & vivid red along with coral red (E/3/0.25/23/1/5) & rose red (E/0/0.15/47/10/8) mutants induced through conventional mutagenesis. In the same manner the pigment contribution of betaxanthin was also increased in vermillion, coral red and rose red (E/0/0.15/47/10/8) mutants induced through conventional mutagenesis. In contrast in the vivid red mutant induced through in vitro mutagenesis the pigment contribution of betaxanthin was decreased. The pigment contribution of amaranthin was increased in the orange mutant, whereas it was decreased in carmine and vermillion mutants induced through in vitro mutagenesis (Table 4).

Mutant	Comb colour	Amaranthin	Betanin	Betalamic acid	Betaxanthin
Control	Magenta	0.51	0.36	0.13	0.00010
M_3 generation					
E/0/0.15/47/8/5	Rose-red	0.57	0.39	0.12	0.00010
E/0/0.15/47/10/8	Rose-red	0.56	0.40	0.26	0.00020
E/0/0.15/54/7/15	Orange	0.59	0.40	0.13	0.00010
E/0/0.25/60/10/25	Vivid red	0.46	0.17	0.26	0.00010
E/3/0.15/30/3/10	Coral red	0.52	0.40	0.12	0.00020
E/3/0.25/23/1/5	Coral red	0.52	0.42	0.27	0.00030
E/6/0.2/19/5/1	Vermilion	0.47	0.32	0.27	0.00030
G/30/11/2/1	Vermilion	0.49	0.38	0.29	0.00030
RM_3 generation					
RE/0.15/2/2/15	Vivid red	0.48	0.33	0.19	0.00004
RE/0.2/1/1/12	Carmine	0.42	0.30	0.27	0.00010
RG/30/4/3/14	Carmine	0.45	0.35	0.21	0.00010
RE/0.25/11/1/8	Vermilion	0.39	0.32	0.29	0.00010

Unlike pigment contribution, the pigment ratio was affected in all the mutants. The ratio of amaranthin : betanin was mostly reduced in the mutants. The same was true for the ratio of amaranthin : betalamic acid and amaranthin : betaxanthin. In the same way, the ratio of betanin : betalamic acid was decreased in all the mutants induced through in vitro mutagenesis. However, the ratio of betanin : betalamic acid was variedly affected in the mutants induced through conventional mutagenesis. The betanin : betalamic acid ratio was decreased in rose red (E/0/0.15/47/10/8), vivid red, coral red (E/3/0.25/23/1/5) and vermillion mutants. In contrast,

rose red (E/0/0.15/47/8/5), orange and coral red (E/3/0.15/30/3/10) had increased betanin : betalamic acid ratio. Notably, the betanin : betaxanthin ratio was decreased in coral red and vermillion mutants induced through conventional mutagenesis. Contrastingly, the betanin : betaxanthin ratio was increased in the orange and vivid red mutants. Similarly, the betalamic acid : betaxanthin ratio was increased in all the mutants induced through in vitro mutagenesis and the vivid red mutant (E/0/0.25/60/10/25). However, the coral red mutant (E/3/0.15/30/3/10) had strikingly decreased betalamic acid : betaxanthin ratio (Table 5).

Table 5. Pigment ratio in the comb of comb-colour mutants

Mutant	Comb colour	Am:Be	Am:Ba	Am:Bx	Be:Ba	Be:Bx	Ba:Bx
Control	Magenta	1.41	3.96	3566.67	2.81	2533.33	900.00
M_3 generation							
E/0/0.15/47/8/5	Rose-red	1.46	4.78	4300.00	3.28	2950.00	900.00
E/0/0.15/47/10/8	Rose-red	1.58	2.17	2600.00	1.38	1650.00	1200.00
E/0/0.15/54/7/15	Orange	1.48	4.45	4900.00	3.00	3300.00	1100.00
E/0/0.25/60/10/25	Vivid red	1.39	1.79	4300.00	1.29	3100.00	2400.00
E/3/0.15/30/3/10	Coral red	1.31	4.48	2350.00	3.43	1800.00	525.00
E/3/0.25/23/1/5	Coral red	1.34	1.96	1833.33	1.46	1366.67	933.33
E/6/0.2/19/5/1	Vermilion	1.48	1.77	1533.33	1.19	1033.33	866.67
G/30/11/2/1	Vermilion	1.29	1.71	1766.67	1.32	1366.67	1033.33
RM_3 generation							
RE/0.15/2/2/15	Vivid red	1.46	2.56	11500.00	1.76	7900.00	4500.00
RE/0.2/1/1/12	Carmine	1.38	1.54	3850.00	1.12	2800.00	2500.00
RG/30/4/3/14	Carmine	1.27	2.17	4450.00	1.71	3500.00	2050.00
RE/0.25/11/1/8	Vermilion	1.25	1.36	3000.00	1.09	2400.00	2200.00

Am: Amaranthin, Be: Betanin, Ba: Betalamic acid, Bx: Betaxanthin

Discussion

Isolation of flower/inflorescence colour mutants through conventional mutagenesis has been reported by Gnanamurthy and Dhanvel (2014) in *Vigna unguiculata* and Kumari and Kumar (2020) in gladiolous. Similarly, Latado et al. (2004) in *Chrysanthemum*, Bala and Singh (2015) in rose and Yoosumran et al. (2018) in *Dendranthemum grandiflora* have isolated colour mutants through in vitro mutagenesis. Anthocyanins impart colour to the flower/inflorescence in most Angiosperms (unlike cockscomb). Apart from this, other molecules such as flavonoids and carotenoids also play a role in imparting colour to the plant tissue. The alteration in the biosynthesis of these molecules might alter the colour of the tissue. Therefore, variation in the

content of betalain pigments, which was evident in the present investigation, might be responsible for the comb-colour variation in the mutants. Moreover, all the pigments were not affected in same proportion in these mutants, which is evident from the variation in their pigment contribution and pigment ratio. Thus, apart from absolute content of the pigment; the relative content of each pigment also has played a role in determining the comb-colour of the mutants in cockscomb.

The biosynthesis of betalain pigments in plants begins from L-DOPA, which is converted to various molecules classified as betalain in a step-wise manner with each step being catalysed by an enzyme. As mentioned earlier, change in comb-colour of the mutants clearly indicates alteration in the content of betalain pigments in the cells. This proposition is substantiated by the TLC profile and quantification of a few betalain pigments in mutants in the present investigation. Onozaki et al. (1999) and Kobayashi et al. (2001) have opined that the mutation in a single gene result in the accumulation of the intermediates, which changes the flower colour. The synthesis of betacyanins and betaxanthins diverge from betalamic acid during the biosynthesis of betalains. While spontaneous condensation of betalamic acid with amino acids/amines synthesises betaxanthins; betacyanins are synthesised by the condensation of betalamic acid with *cyclo*-DOPA, which itself is derived from L-DOPA (Harris et al. 2012). Thus, there are several candidate enzymes, whose activity might influence the colour of comb. Mutation in any such gene could be responsible for induction of comb-colour in the present investigation. It is noteworthy, that pigment contribution of betalamic acid and betaxanthin was increased in most comb-colour mutants. This indicates comparative increase in the biosynthesis of betalamic acid and betaxanthin among all the pigments. Betalamic acid is derived from 4,5-*seco*-DOPA, which is formed by cleavage of the ring of L-DOPA. Similarly, L-DOPA is also the precursor of *cyclo*-DOPA. As mentioned above condensation of betalamic acid with amino acids/amines and *cyclo*-DOPA forms betaxanthins and betacyanins, respectively. The increase in pigment contribution of betalamic acid indicates that channelizing the L-DOPA for biosynthesis of 4,5-*seco*-DOPA is favoured over the biosynthesis of *cyclo*-DOPA. Moreover, increase in the pigment contribution of betaxanthin in most mutants indicates that condensation of betalamic acid with amino acids/amines is preferred over its condensation with *cyclo*-DOPA, which presumably is in lesser amount in the mutants. Overall, this might have reduced the pigment ratio of betacyanins—amaranthine and betanin—in the mutants. Whatsoever, the absolute content of all the molecules quantified is decreased in the mutants. This might be due

to reduction in either the content of L-DOPA or its downstream metabolism. Therefore, it is likely that the gene involved in these reactions might be affected in the mutants.

Notwithstanding anything said above, apart from the pigments analysed in comb-colour mutants, there might be other pigments in the mutants which were affected and contributed to the change in comb-colour. This is reasonable from the fact that neither the absolute pigment content nor the relative pigment content of any two mutants with same comb-colour was similar, if not identical, in the present investigation.

Conclusion

The comb-colour mutants isolated in the present investigation can serve two purposes. First, these mutants can be utilised in crop improvement and second, they can be a system to study betalain biosynthesis in plants.

References

Ahloowalia BS, Maluszynski M and Nichterlein K (2004). Global impact of mutation-derived varieties. *Euphytica* **35(2)**: 187-204. Doi: http://dx.doi.org/10.1023/B:EUPH.0000014914.85465.4f

Bakar DA, Ahmed BA and Taha RM (2014). In vitro callus induction and plant regeneration of *Celosia argentea*— an important medicinal plant. *Brazilian Archives of Biology and Technology* **57**: 860-866. Doi: http://dx.doi.org/10.1590/S1516-8913201402611

Bala M and Singh KP (2015). In vitro mutagenesis in rose (*Rosa hybrida* L.) cv. Raktima for novel traits. *Indian Journal of Biotechnology* **14**: 525-531.

Cai Y, Sun M, Wu H, Huang R and Corke H (1998). Characterization and quantification of betacyanin pigments from diverse *Amaranthus* species. *Journal of Agric. and Food Chem.* **46**: 2063-2070. Doi: 10.1021/JF9709966

Gnanamurthy S and Dhanvel D (2014). Effect of EMS on induced morphological mutants and chromosomal variation in cowpea (*Vigna unguiculata* (L.) Walp). *International Letters of Natural Sciences* **22**: 33-43. Doi: https://doi.org/10.56431/p-i0xny2

Harris N, Javellana J, Davies K, Lewis D, Jameson P, Deroles S, Calcott K, Gould K and Schwinn K (2012). Betalain production is possible in anthocyanin-producing plant species given the presence of DOPA-dioxygenase and L-DOPA. *BMC Plant Biology* **12**: 34. Doi: 10.1186/1471-2229-12-34

Kobayashi N, Schmidt J, Wary V and Schliemann W (2001). Formation and occurrence of dopamine-derived betacyanins. *Phytochemistry* **56(5)**: 429-436. Doi: 10.1016/s0031-9422(00)00383-6

Kumari K and Kumar S (2020). Frequency and spectrum of flower colour mutations in gamma irradiated gladiolus varieties. *Journal of Pharmacognosy and Phytochemistry* **9(4)**: 227-233.

Latado RR, Adamas AH and Neto AT (2004). In vitro mutation of chrysanthemum (*Dendranthema grandiflora* Tzvelev.) with ethyl methanesulphonate (EMS) in immature floral pedicels. *PCTOC* **77**: 103-106. Doi: 10.1023/B:TICU.0000016481.18358.55

Maluszynski M, Nichterlein K, VanZanten L and Ahloowalia BS (2001). Officially released mutant varieties— the FAO/IAEA database. *PCTOC* **65**: 175–177. Doi: https://doi.org/10.1023/A:1010652523463

Meng LS, Ding W Q, Hu X and Wang CY (2009). Transformation of PttKN1 gene to cockscomb. *Acta Physiologiae Plantarum* **31**: 683-691. Doi: 10.1007/s11738-009-0278-3

Nasri F, Zakizadeh H, Vafee Y and Mozafari AA (2021). In vitro mutagenesis of *Chrysanthemum morifolium* using ethylmethanesulphonate (EMS) and mutations assessment by ISSR and IRAP markers. *PCTOC* **149**: 657-673. Doi: https://doi.org/10.1007/s11240-021-02163-7

Onozaki T, Mato M, Shibata M and Ikeda H (1999). Differences in flower colour and pigment composition among white carnation (*Dianthus caryophyllus* L.) cultivar. *Scientia Horticulturae* **82**: 103-111. Doi: 10.1016/s0304-4238(99)00040-0

Purshottam DK, Misra P and Srivastava RK (2020). A mutation study on *Gerbera jamesonii*: An important ornamental plant. *International Journal of Plant and Environment* **6(1)**: 91-93. Doi: https://doi.org/10.18811/ijpen.v6i01.10

Rinkey PK and Badere RS (2020). Mutagen treatment affects the regeneration efficiency of the shoot-tip explant in *Celosia cristata* L. (Cockscomb). *Indian J. Genet.* **80(3)**: 351-353. Doi: 10.31742/IJGPB.80.3.18

Rinkey PK and Badere RS (2023). In vitro intervention modifies the outcome of induced mutagenesis using gamma ray but not ethyl methanesulphonate in cockscomb (*Celosia cristata* L.). *Indian J. Genet.* **83(4)**: 614-617. Doi: https://doi.org/10.31742/ISGPB.83.4.19

Warhade MI and Badere RS (2015). Efficient micropropagation by multiple shoot induction through recurrent regeneration in *Celosia cristata* L. *Indian Journal of Biotechnology* **14**: 402-410.

Warhade MI and Badere RS (2018). *Fusarium oxysporum* cell elicitor enhances betalain content in the cell suspension culture of *Celosia cristata*. *Physiology and Molecular Biology of Plants* **24(2)**: 285-293. Doi: https://doi.org/10.1007/s12298-018-0511-x

Yoosumran V, Ruamrungsri S, Duangkongsan W and Kanjana S (2018). Induced mutation of *Dendranthemum grandiflora* through tissue culture by ethyl methanesulphonate (EMS). *International Journal of Agricultural Technology* **14(1)**: 73-82.