EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 5
118-121
Year: 2024

RUBRUM: A NOVEL CHLOROPHYLL CHIMERA IN COCKSCOMB (*CELOSIA CRISTATA* L.)

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Article Received on 29/02/2024 **Article Revised on 20/03/2024** **Article Accepted on 10/04/2024**

ABSTRACT
We scored a novel chimera, *rubrum*, with red pigmentation over *albino* background in the leaf lamina of cockscomb. *Rubrum* was induced by EMS treatment to the seeds soaked in distilled water for 3h. In addition to *rubrum*, other chlorophyll chimera viz., *albino*, *xantha*, *chlorina* and *viridis* were also induced in the present study. The frequency of chlorophyll chimera was higher with EMS compared to gamma ray treatment. Notably, the frequency of chimera was much higher in M_1 generation (conventional mutagenesis) compared to RM_1 generation (in vitro mutagenesis).

KEYWORDS: Chimera; EMS; floriculture; gamma ray; ornamental plants.

INTRODUCTION

Screening the mutagenized population in M_1 generation reveals effectiveness of mutagen treatment. The mutagen effects are manifested in various form such as reduced germination & fertility, seedling injury, chlorophyll chimera, mortality, and morphological & chromosomal abnormalities. Among these, chlorophyll chimera are most obvious because of their distinct appearance. These are chlorophyll-deficient (complete or partial) and variously coloured sectors and streaks on leaf and stem of the seedlings and young plants. In extreme cases, the chlorophyll deficiency may affect the complete plant and moreover, the trait can be inheritable. Such plants have served as excellent system to study the process of chlorophyll metabolism and photosynthesis and to identify and isolate the genes involved in these processes.[1-2]

Subsequent to the mutagen treatment, individual cells of the plant propagule vary genetically (with respect to the mutation they carry) within themselves. This variation among the cells is responsible for chimerism, which is most apparent in form of chlorophyll chimera. In a chlorophyll chimera, genetically different cells exist side-by-side in the tissues of same individual.[3] The significance of chlorophyll chimeras in induced mutagenesis studies is that they testify the success of mutagen treatment. Chlorophyll metabolism being a complex process, involving several enzymes, is highly susceptible to the adverse effects mutagen treatment.[4] Similarly, biogenesis of chloroplast—organelle containing chlorophyll—is also an intricate phenomenon involving action of several genes is also equally vulnerable to the adverse effects of mutagen.[5] Moreover, chlorophyll is perceptible to the naked eye. Therefore, the chlorophyll-deficient sectors are easy to score and thus chlorophyll chimera is a convenient tool to assess the effectiveness of mutagen treatment. Whatsoever, these chimeras, in later phases of plant growth, mostly assume wild type phenotype or are eliminated from the population.

We mutagenized the seed of cockscomb by two modes viz., conventional and in vitro mutagenesis, and screened the population for chlorophyll chimeras to assess the effectiveness of the mutagen treatment. Present communication reports the scoring of a unique chlorophyll chimera, which we designated as '*rubrum*' due to its reddish hue. As per our knowledge and understanding such a chlorophyll chimera has not been reported earlier in cockscomb.

MATERIALS AND METHODS

Earlier we have reported the induction of mutations by EMS and gamma ray in cockscomb (*Celosia cristata* L.).[6-7] In short, we employed mutagen treatment in two modes viz., conventional mutagenesis and in vitro mutagenesis. For conventional mutagenesis, the seeds of *Celosia cristata* L. (cockscomb) of a probable land race were mutagenized with 0.15, 0.20 and 0.25% (w/v) aqueous EMS and 200, 250 and 300Gy dose of GR. The dry, 3h pre-soaked in water (PSW) and 6h PSW seeds were treated with EMS. The treated seeds, along with the untreated control, were sown in the experimental plots to

raise the M_1 generation. All the recommended agronomical practices were carried-out during the season.

We have earlier described the mutagen treatment and recurrent regeneration of the plants from the mutagenized seeds for in vitro mutagenesis.[6] In short, the shoot-tip explant from 12d-old EMS treated seedling was cultured over MS medium containing 8.8µM BAP for one generation and subsequently over MS medium containing 0.5µM NAA and 6.6µM BAP for next four generations. Similarly, the shoot-tip explant from 12d-old GR treated and control seedling was cultured over MS medium containing 0.5µM NAA and 6.6µM BAP for five generations. The regenerated shoots were rooted over $1/8^{th}$ strength basal MS medium and acclimatized. Subsequently, the regenerated plants from treated and untreated seedlings were grown in the field to raise the RM_1 generation.

The mutagenized population was screened for chlorophyll chimeras based on the variation leaf in colour, on daily basis.

RESULTS AND DISCUSSION

Several chlorophyll chimeras were scored in the mutagenized population. The spectrum of chlorophyll chimera included white coloured *albino*, golden yellow *xantha*, yellow-green *chlorina*, pale green *viridis* and a novel type with red patches over the white background of leaf lamina. We designated this novel chimera as '*rubrum*' due to its characteristic red hue (Fig. 1a–e). Several multiple chlorophyll chimera were scored in the present investigation, which has increased the overall frequency of chlorophyll chimera. *Rubrum* was induced by 0.20 and 0.25% EMS treatment to the 3h pre-soaked in water (PSW) seeds in conventional mutagenesis mode. The frequency of *rubrum* was 1.4 and 5.6% in response to the 0.20 and 0.25% EMS treatment, respectively (Table 1). In addition to chlorophyll, cockscomb also synthesises water soluble betalain pigments responsible for vibrantly coloured inflorescence and leaf variegation in cockscomb. However, in our study the leaves in control were always non-variegated. Therefore, presumably the *rubrum* chimera is the result of betalain synthesis in the chlorophyll deficient cells.

Fig. 1 Spectrum of chlorophyll chimera induced in cockscomb.

a. *Albino*

b. *Xantha*

c. *Chlorina*

d. Multiple chimera (i. *Viridis*, ii. *Xantha*)

e. *Rubrum*

Table 1: Induction of chlorophyll chimera by mutagen treatment in M_1 generation.

Treatment	Frequency (%)					
	Albino	***Xantha***	***Chlorina***	***Viridis***	***Rubrum***	**Total**
Ethyl methanesulphonate (%)						
0h PSW						
Control	0.0	0.0	0.0	0.0	0.0	0.00
0.15	2.9	0.7	2.2	3.6	0.0	9.55
0.20	4.2	0.0	6.3	8.3	0.0	19.00
0.25	9.0	0.0	10.4	16.4	0.0	36.05
3h PSW						
Control	0.0	0.0	0.0	0.0	0.0	0.00
0.15	0.0	0.0	0.0	0.0	0.0	0.00
0.20	0.0	0.0	2.7	4.1	1.4	8.40
0.25	0.0	0.0	2.4	7.9	5.6	16.15
6h PSW						
Control	0.0	0.0	0.0	0.0	0.0	0.00
0.15	3.1	0.0	3.1	11.6	0.0	17.95
0.20	2.4	0.0	0.0	16.7	0.0	19.30
0.25	3.7	0.0	6.5	22.2	0.0	32.65
Gamma ray (Gy)						
Control	0.0	0.0	0.0	0.0	0.0	0.00
200	0.0	0.0	4.1	3.1	0.0	7.20
250	0.0	0.0	1.6	3.1	0.0	4.70
300	0.0	0.0	10.2	5.8	0.0	16.00

EMS induced chlorophyll chimera at higher frequency as compared to GR treatment in M_1 generation (conventional mutagenesis). Moreover, the frequency of chlorophyll chimera increased with an increase in EMS concentration. However, the spectrum of chlorophyll chimera was wider with 0.15% EMS treatment as compared to the higher concentrations of EMS. Chimerism occurs because the mutagenized cells of embryo vary within themselves with respect to the mutations they harbour.[3] Thus, cells constituting the chlorophyll chimera have compromised chlorophyll synthesis.[8] Pre-soaking of seeds in water modified the effect of EMS treatment. While 3h PSW treatment reduced the frequency of chlorophyll chimera; the 6h PSW treatment induced them with the frequency comparable to that induced by dry seed treatment. Moreover, the spectrum of chlorophyll chimera was also narrower in 3h PSW treatment (Table 1). Pre-soaking of seeds activates the cell metabolism. Therefore, they are capable to repair the damage caused by mutagen, thereby decreasing its effectiveness. Thus, frequency and spectrum of chlorophyll chimera with EMS treatment to 3h PSW seeds might have decreased in the present investigation. In contrast, the pre-soaking also increases the permeability of cell membrane thereby increasing mutagen availability in the cell. Under such situations two opposing forces are active in cell, i) active repair mechanism nullifying the mutagen action and ii) increase in mutagenic lesions due to higher mutagen content in the cell. Therefore, outcome of mutagen treatment will be the resultant of these opposing forces.[9-10] The increase in frequency and spectrum of chimera in present investigation in 6h PSW treatment might be because of increased availability of EMS in the cell to induce mutation.

Table 2: Induction of chlorophyll chimera by mutagen treatment in RM_1 generation.

Treatment	Frequency (%)					
	Albino	***Xantha***	***Chlorina***	***Viridis***	***Rubrum***	**Total**
Ethyl methanesulphonate (%)						
Control	0.0	0.0	0.0	0.0	0.0	0.00
0.15	0.0	0.0	0.0	0.0	0.0	0.00
0.20	0.0	0.0	0.0	0.0	0.0	0.00
0.25	0.0	0.0	0.0	0.0	0.0	0.00
Gamma ray (Gy)						
Control	0.0	0.0	0.0	0.0	0.0	0.00
200	0.0	0.0	0.0	0.0	0.0	0.00
250	0.0	6.7	0.0	0.0	0.0	6.70
300	0.0	0.0	0.0	0.0	0.0	0.00

The frequency of chlorophyll chimera due to GR treatment was lower and their spectrum was also narrower than EMS (Table 1). EMS induces point mutations; whereas GR pre-dominantly induce chromosomal breaks which have drastic consequences. Thus, the effect of EMS on cell is milder compared to GR. Mere mutagen action is insufficient to produce its effect in the plant. For the mutagen action to be manifested in terms of altered phenotype, the lesion induced by mutagen must skip the repair process and also the cell in which mutation has occurred should not be eliminated during subsequent growth. Therefore, the lower frequency of chlorophyll chimera in response to GR treatment might be because of i) the drastic effect of GR, which reduced the cell viability, ii) the lesion induced by GR was repaired and iii) the diplontic selection.[11,12]

The frequency and spectrum of chlorophyll chimera was greatly reduced in RM_1 generation (in vitro mutagenesis) with only *xantha* (6.7%) being induced by the treatment of 250Gy of GR. However, rest of the doses of GR and EMS failed to induce chlorophyll chimera (Table 2). While the explants derived from EMS-treated seedling were cultured over media containing 8.8µM BAP; those harvested from GR-treated seedling were cultured over media containing 0.5µM NAA and 6.6µM BAP.[6] Thus, the difference in plant growth regulators in media might have affected the cells of EMS- and GR-treated explants differently. The media used to culture EMS-treated explant presumably favoured diplontic selection resulting in no induction of chlorophyll chimera. The diplontic selection, on the other hand, was probably suppressed by the media over which GR-treated explants were cultured thereby inducing the chlorophyll chimera. These findings are in tune with our earlier report wherein we have found contrasting outcome of GR and EMS treatment in in vitro mutagenesis studies.[7]

Thus, the high frequency of chlorophyll chimera along with *rubrum* makes cockscomb an ideal material to study chlorophyll and betalain metabolism and chloroplast biogenesis.

FUNDING

RSB thank Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur for providing partial financial support to this study vide Grant No. Dev/RTMNURP/AH/VVJ/2116 dtd. 18th January, 2018.

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