Indian J. Genet., 80(3) 351-353 (2020)
DOI: 10.31742/IJGPB.80.3.18

Short Communication

Mutagen treatment affects the regeneration efficiency of the shoot-tip explant in *Celosia cristata* L. (Cockscomb)

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(Received: December 2019; Revised: June 2020; Accepted: July 2020)

Abstract

The shoot-tip explant harvested from ethyl methanesulphonate (EMS) and gamma ray (GR) mutagenized seedling was cultured over MS medium fortified with NAA and BAP for five generations to amplify the mutated sector. Mutagens reduced the regeneration efficiency of the explant and affected its plant growth regulator-dependence for multiple shoot induction. While the 12d-old shoot-tip from GR-treated seedling induced shoots with 0.5µM NAA+6.6µM BAP; that from EMS-treated seedling induced shoots with 8.8µM BAP. The present study establishes that the mutagens affect the regeneration process in the explant.

Key words: EMS, gamma ray, in vitro mutagenesis, plant growth regulator

There is demand for novel varieties of cockscomb (*Celosia cristate* L.), which is known for its vibrantly coloured inflorescence appearing like a crest of a fowl. Although, mutation breeding is an effective method to develop new varieties; the low frequency of mutations is an inherent predicament associated with them (Rampure et al. 2017; Roy et al. 2018). This hurdle can be overcome by augmenting induced mutations with in vitro techniques (Maluszynski et al. 1995). The endogenous plant growth regulator (PGR) concentration of the explant and the exogenous PGR concentration in the medium determines the fate of explant during in vitro manipulations (Madke et al. 2014). We have earlier standardised the conditions for regeneration in cockscomb (Warhade and Badere 2015). However, since the mutagen treatment disturbs the level of PGRs in the explant, which may alter its regeneration potential, the effect of mutagen treatment on the regeneration efficiency of the cockscomb explant was investigated.

The seed (probably a landrace) of cockscomb was obtained from Satpuda Botanical Garden, Nagpur (India). The LD_{50} dose, on the basis of vigour index, for EMS was 0.20% and for GR it was 250Gy. Therefore, only 50 seeds each with 0.15, 0.20 and 0.25% EMS (for 18h) and 200, 250 and 300Gy GR were treated. The untreated seeds served as control. These were surface sterilized and germinated to harvest shoot-tip explant and induce multiple shoots as described by Warhade and Badere (2015).

The explants harvested from 12 day(d)-old EMS-treated seedlings and inoculated over MS medium supplemented with 0.5µM NAA+6.6µM BAP, which was the most efficient medium according to Warhade and Badere (2015), failed to induce microshoot(s). Therefore, other PGR combinations to induce microshoots were attempted. First, we inoculated the 12d-old EMS-treated explants over basal MS medium, over which the explants induced a single microshoot with the frequency between 26.67 and 46.67% (data not shown). Four weeks later the explant was sub-cultured over the same medium after harvesting the induced microshoot. This was continued for 5 generations. However, each time 1 shoot/explant was induced (data not shown). Since this would not have served the purpose of amplification of mutated sector, we attempted other lesser efficient media reported by Warhade and Badere (2015). We tested the induction

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Published by the Indian Society of Genetics & Plant Breeding, A-Block, F2, First Floor, NASC Complex, IARI P.O., Pusa Campus, New Delhi 110 012; Online management by www.isgpb.org; indianjournals.com

of microshoot(s) in 12d-old explants over MS medium containing i) 0.4µM 2,4-D+6.6µM BAP and ii) 0.4µM 2,4-D+8.8µM BAP, and in 8d-old shoot-tip explants over MS medium containing 0.5µM NAA+6.6µM BAP.However, the explants failed to induce microshoots over these media (data not shown). Later, we inoculated 12d-old explants over MS medium containing 8.8µM BAP, where a single microshoot was induced per explant (Table 1). We sub-cultured these explants over the medium containing 0.5µM NAA+6.6µM BAP for next four generations upon harvesting the induced microshoots each time. Unlike the report of Warhade and Badere (2015) where NAA with BAP enhanced multiple shoot induction; in present case BAP alone induced the microshoots from EMS-treated explant. The auxin-dependence for regeneration was apparently abolished. Probably, the EMS treatment altered the endogenous levels of PGRs in the seedlings and inhibited regenerative processes over the media which otherwise supported the microshoot induction (Hussein et al. 2015). However, the explant displayed the expected regenerative capacity over the media containing NAA and BAP during the sub-culturing. This suggests the transient inhibitory effect of EMS on regenerative process in presence of NAA in cockscomb (Dai et al. 2011). Recurrent regeneration up to five sub-cultures was continued, wherein the number of shoots/explant in control of EMS treatment increased from 1 to 4.6 upto G_4 generation and later decreased. With 0.15% EMS the shoots/explant ranged between 1.0 and 1.7. In contrast, with 0.20 and 0.25% EMSmaximum i.e. 8.3 and 9.8 shoots/explant were induced in G_3 and G_4 generation, respectively (Fig. 1).

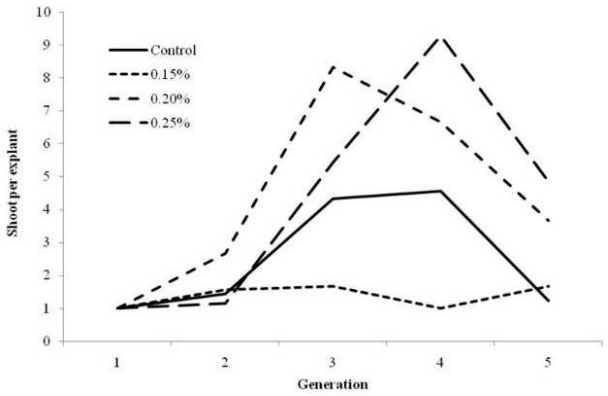

Fig. 1. Recurrent regeneration in 12d-old shoot tip explant harvested from seedlings raised from EMS treated seeds. *The explant was cultured over MS basal + 8.8 µM BAP in G_5 and subsequently cultured over MS basal + 0.5 µM NAA 6.6 µM BAP from G_6 to G_8.

The explants harvested from GR-treated 12d-old seedling and cultured over MS medium containing 0.5µM NAA+6.6µM BAP induced 1 shoot/explant with the frequency varying between 65 to 100% (Table 1). GR treatment adversely affected the process of recurrent regeneration. The drastic effect of GR was at the dose of 250Gy followed by 200 and 300Gy in that order. The maximum number of shoots/explant were induced in G_3 generation and then it decreased in the treated explant. In control, however, the maximum number of shoots/explant i.e. 1.2 were induced in G_4 generation (Fig. 2).

The total number of shoots induced during recurrent regeneration varied between 62 and 152 depending upon the mutagen. In the control of EMS cumulative shoots induced by an explant were 12.56, which reduced to 6.89 by the 0.15% EMS. However, at higher concentrations, the EMS treatment almost doubled the cumulative shoots per explant. In contrast

Table 1. Effect of mutagen treatment on the regeneration efficiency of shoot-tip explant

Treatment	Explants inoculated	Explants responded	Total no. of shoots regenerated	Shoots per explant	Regeneration frequency (%)	Regeneration efficiency (%)
			EMS (%) treatment			
Control	15	9	9	1.00	60.00	60.00
0.15	15	9	9	1.00	60.00	60.00
0.20	15	3	3	1.00	20.00	20.00
0.25	15	7	7	1.00	46.67	46.67
			GR (Gy) treatment			
Control	35	35	35	1.00	100.00	100.00
200	40	26	26	1.00	65.00	65.00
250	40	40	40	1.00	100.00	100.00
300	35	27	27	1.00	77.14	77.14

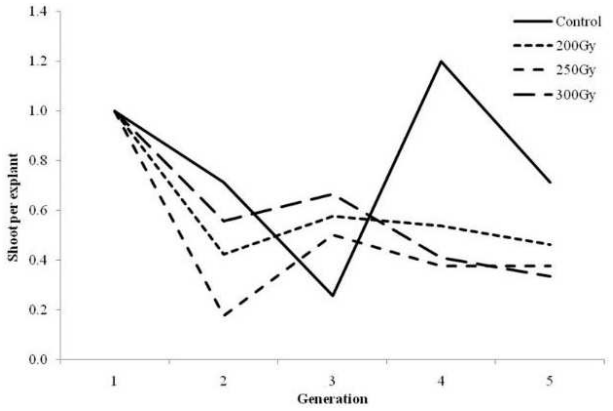

Fig. 2. Recurrent regeneration in 12d-old shoot tip explant harvested from seedlings raised from gamma ray treated seeds inoculated over MS media containing 0.5 μM NAA + 6.6 μM BAP

to this, the control of GR induced 3.89 cumulative shoots per explant, which decreased slightly in the GR-treated explants. The cumulative shoots/explant induced by the explants treated with 200, 250 and 300Gy dose of GR were 3.00, 2.43 and 2.96, respectively (Table 2).

Table 2. Data on the number of shoots induced by the explants in five generations

Mutagen	Treatment	Cumulative no. of shoots induced in five generations	Cumulative shoots per explant
EMS (%)	Control	113	12.56
	0.15	62	6.89
	0.20	67	22.33
	0.25	152	21.71
Gamma	Control	136	3.89
ray (Gy)	200	78	3.00
	250	97	2.43
	300	80	2.96

The present study suggests that the EMS and GR differentially affect the regeneration potential of the explant in terms of PGR requirement and regeneration efficiency. Therefore, we recommend culturing GR-treated 12d-old shoot-tip explant over MS medium containing 0.5μM NAA+6.6μM BAP and that from EMS-treated seedling over MS medium containing 8.8μM BAP. Therecurrent regeneration of these explants should be carried for four generations over MS medium containing 0.5μM NAA+6.6μM BAP. Such approach is likely to improve the mutation frequency thereby increasing the chances of the isolation of desired mutant.

Authors' contribution

Conceptualization of research (RSB); Designing of the experiments (PKR, RSB); Contribution of experimental materials (PKR, RSB); Execution of field/lab experiments and data collection (PKR); Analysis of data and interpretation (PKR, RSB); Preparation of the manuscript (PKR, RSB).

Declaration

The authors declare no conflict of interest.

Acknowledgements

RSB thank RTM Nagpur University, Nagpur for providing partial financial support to the present study (Grant No. Dev./RTMNURP/AH/2116 dated 18-1-2018).

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