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Effect of gamma radiations on *in vitro* regeneration in *Brassica carinata* A. Braun

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Abstract- Callus cultures were established from hypocotyl and cotyledon explants of 8-10 days old seedlings obtained from non-irradiated and irradiated seeds of *Brassica carinata* variety 'Jayanti' on MS medium supplemented with various combinations and concentrations of growth hormones. Shoot formation from the cotyledon explants was observed on MS medium supplemented with BAP (2.0 mg l^{-1}) and NAA (0.1 mg l^{-1}) obtained from non-irradiated seeds. Multiple shoots appeared at the base of cotyledon explants of non-irradiated seeds within 3 weeks. $AgNO_3$ in callus induction medium resulted in greening of callus. However, no significant effect of adding organic additive in shooting medium was observed. The hypocotyl explants showed good callusing but, no shooting response was observed. The callusing potential increased with increase in radiation dose up to 80 kR and afterwards started decreasing with increase in dose. The percentage of shoot formation decreased gradually with the increase in radiation dose up to 100 kR (except 60 kR) and the shoots developed were small, less vigorous with retarded growth and yellowish in color. Total suppression of shoot formation was observed in cultures derived from seeds treated with 110 kR dose.

Index Terms- Ethiopian mustard - *Brassica carinata* - Gamma radiations- Hypocotyl - Cotyledon - Explant - Growth regulators - Callus induction

I. Introduction

Ethiopian mustard (*Brassica carinata* A. Braun) or karanrai (BBCC, 2n=4x=34) is a natural alloploid from *Brassica oleracea* L. (CC, 2n=2x=18) and *Brassica nigra* L. (BB, 2n=2x=16) which has several agronomical important traits such as non dehiscent siliquae and a much more developed and aggressive root system than *Brassica napus*. The crop is a new introduction to north-west Himalayan regions. The variety 'Jayanti' of this species has higher seed yield and oil yield in comparison to other *brassica* species. This variety possesses tolerance to drought, shattering as well as bird attack due to its thick and hard siliquae covering. In general, all *Brassica* crops are attacked by a variety of pathogens culminating in huge losses in seed yield. About 10 to 70 percent yield loss due to *Alternaria* blight has been reported by Gupta et al. (*2003*) in rapeseed-mustard. The yield losses due to various biotic stresses such as pathogens and insect-pests are less in karanrai compared to other *brassica* crops.

Mutation Breeding has been considered to be a reliable approach to impart resistance/ tolerance against various diseases for which the resistance sources are not available. Tissue and cell culture techniques have been utilized to induce variability in many crop plants including *Brassica* crops (Kharb et al. *2002*, Larkin & Scowcroft *1981*, Jain et al. *1990*&Katiyar, *1997*). Tissue culture is one of the best methods to investigate direct effect of mutations on anyspecies and get mutants in short period of time. *In vitro* culture and shoot regeneration are key factors in developing an efficient transformation method in the genus *Brassica*. However, the studies on genotype variability for *in vitro* culture and shoot regeneration in *Brassica carinata* are confined to only a few genotypes (Javier et al. *2011*). This genotype dependence of *in vitro* culture is a limiting factor for the application of genetic engineering to a wide number of genotypes.

In view of this, the aim of present investigation was to initiate *in vitro* culture of karanrai variety 'Jayanti' using seeds with and without γ-radiations and optimize conditions for efficient callus induction and plant regeneration.

II. Materials and Methods

For radiobiological studies, the dry and uniform sized seeds of variety 'Jayanti'were exposed to 50, 60, 70, 80, 90, 100 and110 kR of gamma - radiations to induce resistance / tolerance to *Alternaria* blight, at Bhabha Atomic Research Centre, Mumbai. For the establishment of callus cultures and plant regeneration, the seeds (non-irradiated and irradiated with all doses) were surface sterilized for 5 minutes with aqueous solution of 0.1% $HgCl_2$ and few drops of Tween-20 for 10 minutes and rinsed repeatedly (3-4 times) with sterile distilled water. Seeds were dried on blotting paper and germinated on MS medium. Small segments of cotyledons and hypocotyls were excised from 10-12 days and 8-10 days old seedlings and cultured on MS basal medium (Murashige&Skoog, *1962*) containing sucrose (3 %), NAA (0.2 mg/l), BAP (0.2 mg/l) and agar (0.8 %) for callus initiation. The pH of the medium was adjusted to 5.8 before adding agar and autoclaving. All cultures were incubated under 16 hours light (1500 lx) and 8 hours dark cycles at a temperature of 25±1°C. For each treatment, 50 explants were utilized and all experiments were repeated at least twice. Both cotyledon and hypocotyl segments were utilized for establishment of callus and plantlet regeneration. Half of the calli were excised from undifferentiated explants, subcultured after every three weeks and maintained on the same medium for three months and half were simultaneously transferred onto MS medium supplemented with various concentrations of cytokinins and auxins for visualizing morphogenetic response.

III. Results and Discussion

Non irradiated seeds:

In order to evaluate callus induction ability, cotyledons and hypocotyl segments were cultured on MS medium supplemented with different plant growth regulator combinations (Table 1). The cultured cotyledons and hypocotyls derived from 8-10 days old seedlings formed callus at the cut ends within a week and simultaneously started differentiating into nodular structures from the base of cotyledons along with profuse root hair formation in callus zone after 2 weeks (Plate 1A). The cultured cotyledons and hypocotyls derived from 10-12 days old seedlings formed callus at the cut ends after 15 days and the callus turned brown with increase in dose (Table 2).Therefore, 8-10 days old seedlings derived callus were used for further investigation.

Shoot regeneration was observed on MS medium combination BAP (2.0 mg l^{-1}) + NAA (0.1 mg l^{-1}). Multiple shoots appeared at the base of cotyledon explants within 3 weeks. Yang et al. (*1991*) while establishing efficient tissue culture system for hypocotyl segments in *Brassica carinata* observed that the hypocotylar segments showed higher regeneration capacity at the distal end than proximal end and upper segment of hypocotyl was more regenerative than lower segment. Hypocotylar explants produced more shoots than cotyledon explants but, fewer roots in different cultivars of *Brassica oleracea* var. tronchuda (Msikita&Skirvin, *1989*). However, earlier studies had observed the failure of hypocotylar segments to differentiate shoots (George&Rao, *1980*, Jain et al. *1989*).

Table 1: Effect of various hormonal combinations on shoot regeneration

MS media	Hormonal combinations	Nature of response	
		Hypocotyl	Cotyledon
MS_0	Kinetin(2.0mg/l) + 2,4-D (0.2mg/l)	callus	callus
MS_1	Kinetin (4.0mg/l) + 2,4-D (0.01mg/l)	callus	callus
MS_2	BAP (2.0mg/l) + NAA (0.1mg/ l)	callus* + roots#	callus* + roots# + shoots
MS_3	BAP (2.0mg/l) + NAA (0.1mg/l+ $AgNO_3$ (4.0mg/l)	callus* + roots#	callus* + roots# + shoots

*Greening of callus, #Fibrous roots

Shoots appeared from the base of cotyledon explants

MS basal medium with varying concentrations of kinetin (2.0mg l^{-1} and 4.0mg l^{-1}) and 2, 4-D (0.01 mg l^{-1} and 0.2 mg l^{-1}) induced callus both from hypocotyl and cotyledon segments. However, MS basal medium with BAP (2.0 mg l^{-1}) + NAA (0.1 mg l^{-1}) induced callus and fibrous roots from hypocotyl while callus, fibrous roots as well as shoots were also induced from the base of cotyledon segments. BAP in combination with NAA had yielded no or reduced number of shoots in different *Brassica* species earlier (Jain et al.*1989*). Shoots were also regenerated on callus using NAA (0.1 mg l^{-1}) with BAP (2.0 mg l^{-1}) and $AgNO_3$ (4.0 mg l^{-1}) in MS medium. $AgNO_3$ in callus induction medium resulted in greening of callus.

Table 2: Effect of aging of young seedlings on callus induction from hypocotyl and cotyledon explants of *B. carinata* (control and irradiated)*

Doses	0 kR		50 kR		60 kR		70 kR		80 kR		90 kR		100 kR		110 kR	
10-12 days old seedlings																
Type of explant	**H**	**C**	**H**	**C**	**H**	**C**	**H**	**C**	**H**	**C**	**H**	**C**	**H**	**C**	**H**	**C**
Callus (%)	65.9	28.4	64.3	27.5	60.7	21.2	62.5	21.7	66	20.8	63.4	20.4	45.2	19.7	39.5	15.5
Weight (grams)	1.04	0.61	0.92	0.59	0.90	0.49	0.92	0.51	0.99	0.44	0.84	0.46	0.78	0.35	0.68	0.19
Color	W	W	W	W	L Br	L Br	L Br	L Br	L Br	L Br	Br	Br	Br	Br	Br	Br
8-10 days old seedlings																
Type of explant	**H**	**C**	**H**	**C**	**H**	**C**	**H**	**C**	**H**	**C**	**H**	**C**	**H**	**C**	**H**	**C**
Callus (%)	90.3	50	85	48.2	81.9	47.9	88.5	49	93	48.8	84	45	82.6	44.7	77.1	40.2
Weight (grams)	1.9	0.86	1.62	0.83	1.75	0.77	2.8	0.78	3.4	0.80	2.3	0.59	2.1	0.58	2.0	0.60
Color	L Gr	L Gr	W	W	L Br	W	Off W	W	Off W	W	L Br	L Br	L Br	Br	L Br	Br

Color: W-White, L Br-Light Brown, Br-brown, L Gr- Light Green, Off W- Off White H-Hypocotyl, C-Cotyledon

* Data scored after 25 days of culture

However, no superior effect of adding $AgNO_3$ as organic additive was observed in shooting medium. The results are in confirmation with earlier findings (Ali et al. *2007*). On the other hand, Lim et al. (*1998*) observed the optimum level of $AgNO_3$ for shoot regeneration from 0.5 to 1.0 mg l^{-1}. Addition of $AgNO_3$ at higher than 1.0 mg l^{-1} caused a high rate of vitrification of regenerated shoots. On MS_2 medium viz., MS + BAP (2.0 mg l^{-1}) + NAA (0.1 mg l^{-1}), 80 % of explants from the base of cotyledons developed shoots. Generally 8-9 shoots originated per explant within 4 weeks after culture (Plate 1B) but, growth was slow. Almost similar results were observed in both MS_2 and MS_3 media. Since a combination of BAP (2.0mg l^{-1}) and NAA (0.1mg l^{-1}) proved effective for shoot regeneration, further experiments were conducted using the same for inducing shoot regeneration from the cotyledon and hypocotyl explants of irradiated seeds.

Irradiated seeds:

To explore the morphogenetic potential of the cotyledon as well as hypocotyl explants excised from the seedlings, seeds from all doses were germinated in half-strength MS medium. Percent germination was not affected by γ-irradiations but, the germination was delayed by 2-3 days above 90 kR doses and seedlings exhibited slightly stunted growth. The seeds of all doses showed germination indicating a high radio resistance of Ethiopian mustard to gamma radiations. The morphogenetic response of the cotyledon and hypocotyl explants of mutants was similar to that of untreated seeds to some extent but, the degree of response and rate of growth varied with dose. 50 kR and 70 kR dose- derived explants showed less number of shoots per plant (Table 3) and fast growth rate as compared to untreated ones (Plate1C & D).

Table 3: Morphogenetic responses of callus in different cultures

Treatment	Nature of cultures response*	Shooting response (%)
0 kR	Good callus growth, fibrous roots and multiple shoot buds appear	80
50 kR	Moderate callus growth, shoot buds (2-3 buds per explant) appear	36
60 kR	Moderate callus growth, shoot initiation occurs but, very slow growth rate	6
70 kR	Better callus growth, fibrous roots and shoot buds (2 buds per explant) appear	20
80 kR	Excellent callus growth, shoot buds appear but, slower than lower doses (1bud per explant)	13.3
90 kR	Good callus growth, shoot initiation occurs with retarded growth	8
100 kR	Moderate callus growth, shoot initiation occurs with much retarded growth	4
110 kR	Limited callus growth, no shoot buds appear	0

*Explants were cultured on MS_2 (BAP (2.0mg/l) + NAA (0.1mg/l)

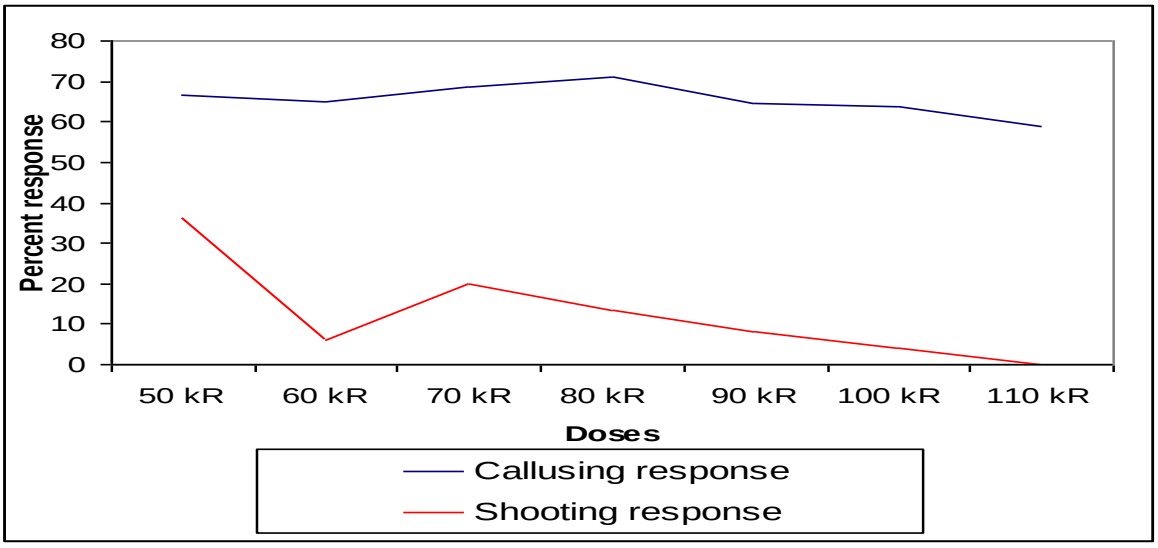


Fig.1. Comparison of callusing and shooting response with increase in dose of radiations

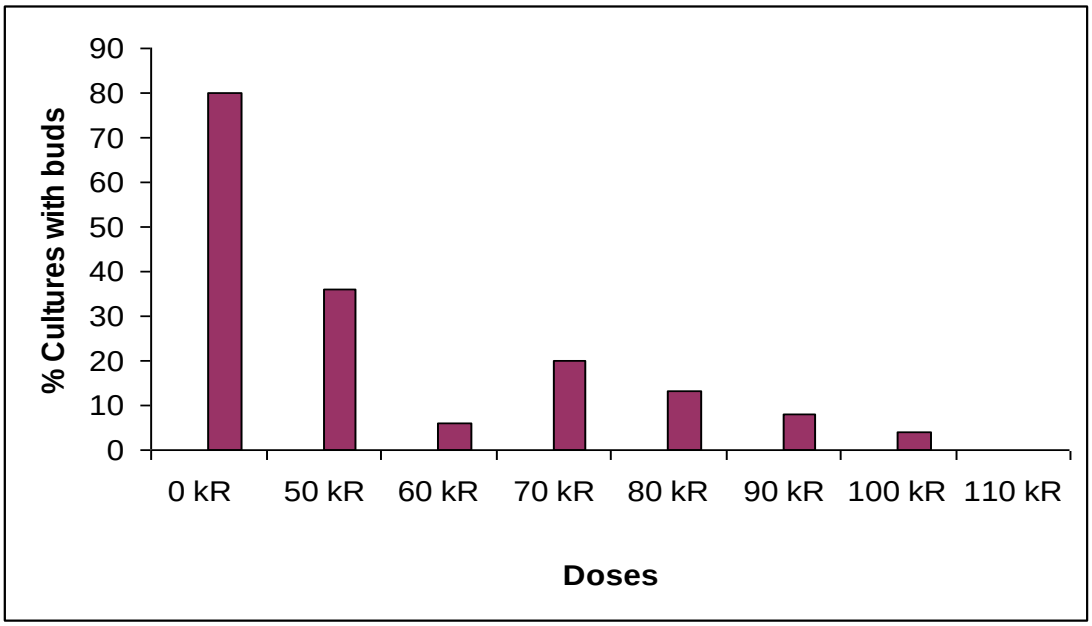
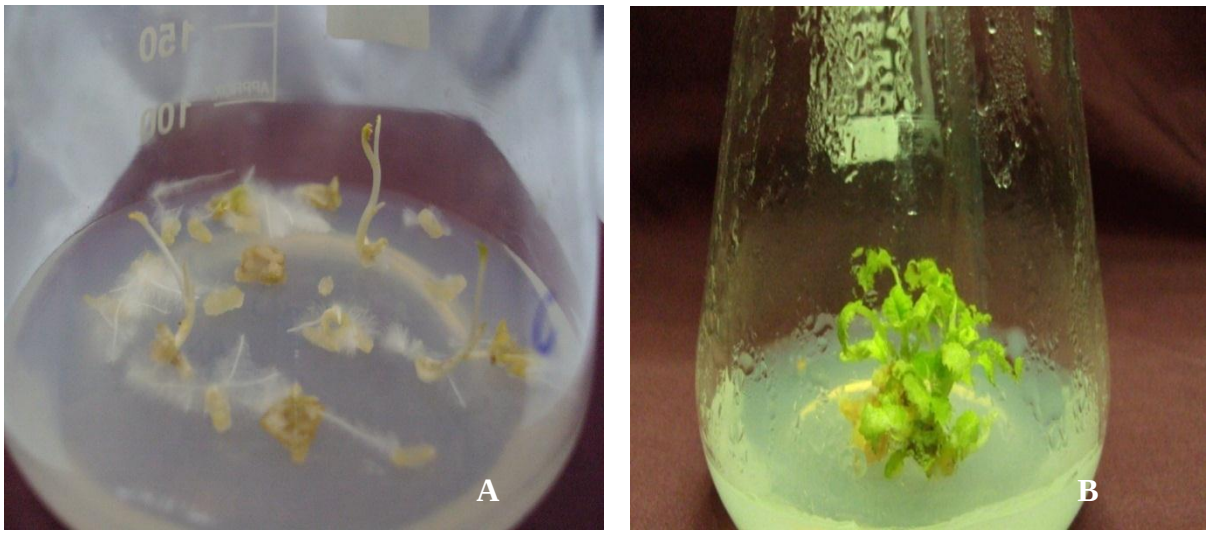


Fig.2. Effect of gamma radiations on shoot formation from cotyledon explants of Ethiopian mustard (*Brassica carinata* A. Braun). Each bar represents the mean of two separate experiments, scored after 4 weeks.

The callusing potential increased with increase in radiation dose up to 80 kR and afterwards started decreasing with increase in dose (except 60 kR). The percent shoot formation decreased gradually with the increase in radiation dose up to 100 kR (except 60 kR) and the shoots developed were small, less vigorous with retarded growth (Plate 1E & F, Figures 1& 2). Total suppression of shoot formation was observed in cultures derived from seeds treated with 110 kR dose. Earlier workers in mustard had demonstrated that the callus growth stimulated as the dose rate increased in cotyledon cultures obtained from seeds treated with higher doses and maximum growth was observed at 25 kR dose (George &Rao*1980*). Inhibition of shoot development following high doses of gamma radiation has been demonstrated earlier in mustard by George &Rao (*1980*). The highest inhibitory effect on shootregeneration was also observed by Singh et al. (*2007*) in all doses of gamma radiations (5 kR, 10 kR, 20 kR, 30 kR, 40 kR& 100 kR) except 54 kR along with total suppression of morphogenetic response by 100 kR.

Therefore, in addition to develop an efficient transformation method and generate material for somaclonal variation, these studies could also be used for screening somatic mutants tolerant to various stress conditions, where the stress can be created in culture vessels e.g. sodium sulfate stress (Chandler & Thorpe *1987*) as well as in *vitro* selections of disease resistant varieties / mutants (Willmot et al.1989).



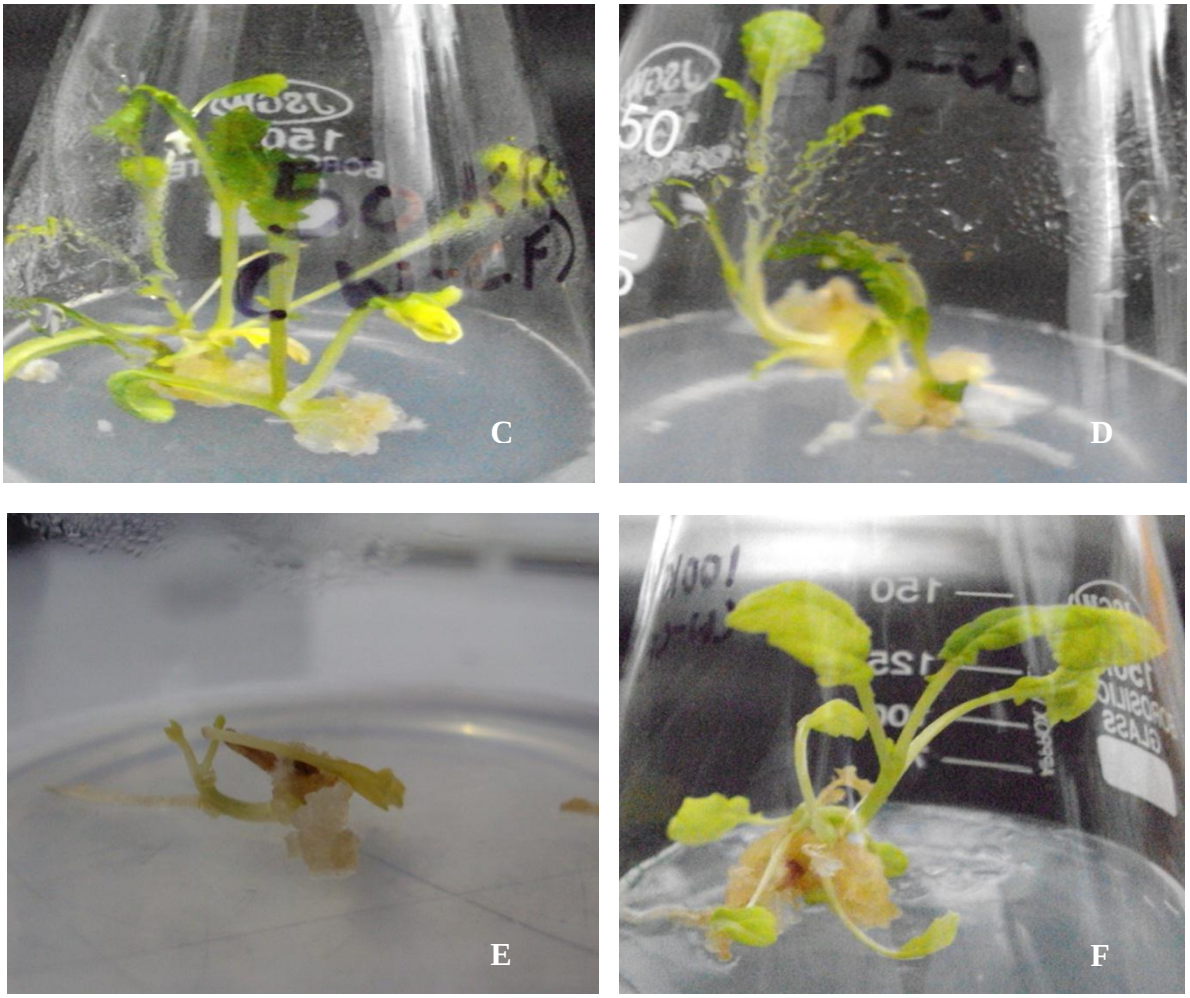


Plate 1: Morphogenetic response of non-irradiated and irradiated seeds explants
A Callusing from the hypocotyl and cotyledon explants of non-irradiated seeds with profuse rooting
B *Multiple shoots emerging from non-irradiated seed explants
C, D & E* Shooting response in 50 kR, 70 kR& 100 kR radiated seed explants, respectively
F Shooting response in100 kR radiated seed explants after 3 months
* Response was observed after 4 weeks

IV. Conclusion

The gamma radiations had showed almost nil effect on *in vit*ro germination up to 90 kR dose. For the doses above 90 kR, the germination was delayed by 2-3 days exhibiting slightly stunted growth with increase in dose indicating a high radio-resistance of Ethiopian mustard variety 'Jayanti' to gamma-radiations. The morphogenetic response varied with varying doses as the regenerative potential was highly influenced by the gamma-radiations. The callusing potential with increase in radiation dose increased up to 80 kR and afterwards started decreasing with increase in dose. The percentage of shoot formation decreased gradually with the increase in radiation dose up to 100 kR (except 60 kR) and the shoots developed were small, less vigorous with retarded growth and yellowish in color. Total suppression of shoot formation was observed in cultures derived from seeds treated with 110 kR dose.

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REFERENCES

[1] Ali H, Ali Z, Ali H, Mahmood S, Ali W,(2007) In vitro regeneration of *Brassica napus* L., cultivars (Star, Cyclone and Wester) from hypocotyls and cotyledonary leaves. PakJ Bot39 (4) 1251-1256.

[2] Chandler S F, Thorpe T A (1987) Proline accumulation and sodium sulfate tolerance in callus cultures of *Brassica napus* L. cv. Wester. Plan Cell Report 6:176-179.

[3] George L, Rao P S (1980)*In vitro* regeneration of mustard plants (*Brassica juncea* Var Rai 5) from cotyledon explants of non-irradiated, irradiated and mutagen treated seeds.Ann Bot46**:**102-112.

[4] Gupta R, Awasthi R P, Kolte SJ (2003) Influence of sowing dates and weather factors on development of *Alternaria* blight on rapeseed- mustard. Ind Phyto 56 (3):398-402.

[5] Jain R K, Sharma D R, Chowdhury J B (1989) High frequency regeneration and heritable somaclonal variation in *Brassica juncea*.Euphy 40: 75-81.

[6] Jain R K, Jain S, Nainawatee H S, Chowdhury J B (1990)Salt tolerance in *Brassica juncea* L.I. *in vitro* selection, agronomic evaluation and genetic stability.Euphy48 (2):141-152.

[7] Javier G H, Ahtonio M, Francisco B (2011) Characterization of a Collection of *Brassica carinat*a Genotypes for *in vitro* culture response and mode of shoot regeneration. American journal of Pl. Sci.(2): 27-34.

[8] Katiyar R K (1997) Exploitation of variability generated through somaclonal route in mustard (*Brassica juncea*).Ann Agril res18 (4):5152-517.

[9] Kharb P, Chaudhury J B, Jain R K (2002) Assessment of somaclonal variation in three tetraploid species of *Brassica.*Nat J Pl Improv4 (2): 30-34.

[10] Larkin P J, Scowcroft W R (1981) Somaclonal variation-a novel source of variability from cell culture for plant improvement. Theor Appl Genet 60:197-214.

[11] Lim H T, You Y S, Park E J, Song Y N, Park H K (1998) High plant regeneration, genetic stability of regenerates and genetic transformation of herbicides resistance gene (bar) in Chinese cabbage (*Brassica campestris* ssp. *Perkinensis*).ActaHort459: 199-208.

[12] Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue culture.PhysiolPlant15: 473-497.

[13] Msikita W, Skirvin R M (1989) In vitro regeneration from hypocotyl and seedling cotyledons of tronchuda (*Brassica oleracea* var. tronchuda Bailey).Plancell tissue organ cult19:159-165.

[14] Singh R R, Prasad B K, Singh H B (2007) Development of *Alternaria* blight tolerant lines through somaclonal variants in Indian mustard (*Brassica juncea* (L.) czern and coss). Brassi9(1/4): 21-27.

[15] Willmot D B, Nickell C D, Widhlom J M, Gray L E (1989) Evaluation of soybean resistance to *Phialophora gregata* culture filtrate in tissue culture. Theor Appl Genet 77:227-232.

[16] Yang M Z, Jia S R, Pua E C (1991) An efficient tissue culture system for high frequency of plant regeneration from hypocotyl explants of *Brassica carinata.*Plan cell tissue organ cult24:79-82.

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