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Physiological response of two cultivars of faba bean using physical and chemical mutagenesis

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ABSTRACT

The present investigation was carried out to find the physiological response of two cultivars of faba bean viz., Vikrant and PRT-12 in M_1 generation using gamma rays and ethyl methanesulphonate as mutagens. Estimates of chlorophyll, carotenoid and nitrate reductase activity (NRA) were recorded in both the varieties using different doses/concentrations of both single and combination treatments of gamma rays and EMS. Both varieties showed decrease in chlorophyll and carotenoid contents and also a decrease in NRA with increasing doses/concentrations of both single and combined treatments of gamma rays and EMS. However, variety PRT-12 showed more decrease of pigment contents and NRA than the variety Vikrant. The maximum decrease in NRA, chlorophyll and carotenoid contents was 751.73 nmol. $h^{-1}.g^{-1}FW$, 1.96 $mg.g^{-1}FW$ and 0.14 $mg.g^{-1}FW$, respectively in combination treatment of 400 Gy gamma rays+0.04% EMS in the variety PRT-12. The effect of physical and chemical mutagens on the physiological parameters is an important parameter during mutation breeding and crop improvement programmes.

Key words: Vikrant, PRT-12, gamma rays, ethyl methanesulphonate, nitrate reductase activity (NRA).

I.INTRODUCTION

In Indian agriculture pulses play an important role in maintaining soil fertility and supplying protein to the large population of the country which consumes pulses. Pulse crops are endowed with unique property of fixing atmospheric nitrogen in their root nodules and improving soil physical property. However, with increase in population, per capita availability of pulses is getting reduced. As against recommended daily requirement of 50-60 grams, current per capita availability of pulses in India is less than 30 grams per day [1].

Faba bean (*Vicia faba* L.) is one of the most important grain legumes and hardy crop in the world. Faba bean seeds serve as a source of high content of proteins and starch along with good source of vitamins and minerals [2-4]. Faba bean is a partially cross-pollinated crop [5]. The lack of adequate pollination and reduced seed setting can be major constraint to yield [6]. Faba bean, world's one of the four important pulses, is treated as a minor pulse in India. The genotypes of faba bean in India have a low yield potential. In spite of its substantial production potential, no attention has been paid to its improvement and to increase the production of local

strains in different parts of the country. An optimum strategy for maintenance and utilization of locally available plant genetic resources is required for plant breeders to develop new varieties [7]. Induced mutations are found to be successful in yield characters, alteration in grain or seed quality is generally useful. Mutagenesis is a tool to increase variability in species in which natural variation is not large or, as often happens, where phenotypes desired by plant breeder are not available because they disappeared due to their poor competition ability in natural condition [8-9].

The present investigation was carried out to find the physiological response of two cultivars of faba bean viz., Vikrant and PRT-12 in M_1 generation using gamma rays and ethyl methanesulphonate as mutagens. Estimates of chlorophyll, carotenoid and nitrate reductase activity (NRA) were recorded in both the varieties using different doses/concentrations of both single and combination treatments of gamma rays and EMS. Mutations in genes controlling the biosynthesis of chlorophyll, carotenoid and nitrate reductase activity (NRA) are used as an important indices for measuring the potency of mutagens during the crop improvement programmes. They are useful in identification of threshold dose of mutagen that would increase the genetic variability.

II. MATERIALS AND METHODS

2.1. Varieties used

Two varieties of faba bean (*Vicia faba* L.) namely Vikrant and PRT-12 were used for the experiment. Seeds of both the varieties were obtained from the Indian Agricultural Research Institute (IARI), New Delhi, India. Both the varieties are well adapted to agroclimatic conditions of Uttar Pradesh (including the site of study).

Two varieties of faba bean chosen for the present study is mainly due to their low yield. Both the varieties are cultivated in and around Aligarh district. A brief description of both the varieties is given below:

2.1.1. Variety Vikrant

Released at Haryana Agricultural University, Hisar as a pure line selection from local material of Meerut (Uttar Pradesh), matures in 140-144 days, plant erect having an average height of 65-70 cm, seeds smooth and small size.

2.1.2. Variety PRT-12

Local collection from a village of Faizabad district (Uttar Pradesh), matures in 145-148 days, plant erect having an average height of 60-65 cm, seeds medium bold.

2.2. Mutagens used

Seeds of both the varieties of faba bean were treated with gamma rays, EMS and their combination treatments as detailed below:

2.2.1. Gamma rays (γ rays)

Dry seeds of each variety, with moisture content 12%, were irradiated with 100, 200, 300 and 400 Gy of gamma rays with a radioisotope ^{60}CO source (Gamma chamber Model-900 supplied by Bhabha Atomic Research Centre, Mumbai, India) at the National Botanical Research Institute, Lucknow, Uttar Pradesh, India.

2.2.2. Chemical mutagen: Ethyl methanesulphonate (EMS)

EMS ($CH_3SO_3C_2H_5$), a monofunctional alkylating agent is manufactured by Sissco Research Laboratories Pvt. Ltd., Mumbai, India. For EMS treatments, healthy seeds of uniform size of each variety were presoaked for 9 hours in distilled water and treated with 0.01, 0.02, 0.03 and 0.04 % of EMS for 6 hours with intermittent shaking at room temperature of 22±1^{o}C. The solution of EMS was prepared in the phosphate buffer of pH 7. Only freshly prepared solutions were used for all the treatments. The pH of the solution was maintained by using buffer tablets manufactured by MERCK manufactures, Mumbai, India. After treatment, the seeds were thoroughly washed in running tap water to remove the excess of mutagen.

2.2.3. Combination treatment: Gamma rays + EMS

For combination treatments, dry seeds of each variety were first irradiated with gamma rays at 100, 200, 300 and 400 Gy doses and then treated with 0.01, 0.02, 0.03 and 0.04 % EMS. (i.e. 100 Gy gamma rays+0.01% EMS, 200 Gy gamma rays+0.02% EMS, 300 Gy gamma rays+0.03% EMS and 400 Gy gamma rays+0.04% EMS). The procedure adopted was similar to that for the individual treatment.

2.3. Experimental procedures

2.3.1. Sample size

350 seeds were used for each treatment and control.

2.3.2. Controls

For each variety, 350 pre-soaked seeds were again soaked in phosphate buffer for 6 hours to serve as controls.

2.4. Estimation of nitrate reductase activity (NRA), chlorophyll and carotenoid contents

The estimation of NRA, chlorophyll and carotenoid contents was recorded from the fresh leaves taken from the plants grown in the field. The procedure of their estimation is elaborated below:

2.4.1. Assay for nitrate reductase activity (NRA)

The activity of nitrate reductase (EC 1.7.7.2) was measured as described by Jaworski (1971) in fresh leaf samples.

The leaves were cut into small pieces avoiding the mid veins. 200 mg of these chopped leaves were weighed and transferred to plastic vials. To each vial 2.5 ml of 0.1M phosphate buffer pH 7.4 and 0.5 ml of 0.2M potassium nitrate solution was added followed by the addition of 2.5 ml of 5% isopropanol. These vials were incubated in B.O.D. incubator for 2 hours at 30±2 ^{o}C in dark. 0.4 ml of incubated mixture was taken in a test tube (borosil) to which 0.3 ml each of 0.1% sulphanilamide solution and 0.02% NED-HCL were added. The test tube was left for 30 minutes, for maximum colour development. The mixture was diluted to 5 ml with double distilled water (DDW). The absorbance in terms of optical density was read at 540 nm on spectrophotometer (Spectronic 20D, Milton Roy, USA). A blank consisting of 4.4 ml DDW and 0.3 ml each of sulphanilamide and NED-HCL were used simultaneously for comparison with each sample. A standard graded concentration of sodium nitrite ($NaNO_2$) from standard aqueous solution of the salt were used. The absorbance

of each sample was compared with that of the calibration curve and NRA (nmol NO_2^- $g^{-1}h^{-1}FW$) was computed on fresh mass basis.

2.4.2. Estimation of chlorophyll and carotenoid contents

The chlorophyll and carotenoid contents of leaves on which measurement were made was estimated by the method of MacKinney [10].

1g of finely cut fresh leaves was ground to a fine pulp using a mortor and pestle after pouring 20 ml of 80% acetone. The mixture was centrifuged at 5,000 rpm for 5 minutes. The supernatant was collected in 100 ml volumetric flask. The residue was washed three times, using 80% acetone. Each washing was collected in the same volumetric flask and volume was made upto the mark (100ml) using 80% acetone. The absorbance in terms of optical density was read at 645 and 663 nm for chlorophyll and 480 and 510 nm for caretenoid against the acetone (80%) as blank on spectrophotometer (Spectronic 20D, Milton Roy, USA).

The chlorophyll and carotenoid contents present in the extracts of leaves was calculated according to the equation given by Arnon (1949).

$$\text{Total chlorophyll (mg g}^{-1}\text{leaf fresh mass)} = [20.2(OD_{645}) + 8.02(OD_{663})] \times \frac{V}{1000 \times W}$$

$$\text{Carotenoid (mg g}^{-1}\text{leaf fresh mass)} = \frac{7.6\ (OD_{480}) - 1.49\ (OD_{510})}{d \times 1000 \times W} \times 100$$

Where,

OD_{645}, OD_{663}, OD_{480}, OD_{510} = Optical densities at 645, 663, 480 and 510 nm, respectively

V = Volume of an extract

W = Mass of leaf tissues

d = Length of light path (d= 1.4 cm)

III. RESULTS AND DISCUSSION

Nitrate reductase activity, chlorophyll and carotenoid contents

Fresh leaves of the control and treated plants of Vikrant and PRT-12 were used for estimating NRA, chlorophyll and carotenoid contents. Both the varieties showed decrease in chlorophyll and carotenoid contents and also a decrease in NRA with increasing doses/concentrations of both single and combined treatments of gamma rays and EMS. However, variety PRT-12 showed more decrease of pigment contents and NRA than the variety Vikrant (Tables 1&2, Figs. 1&2). The maximum decrease in NRA, chlorophyll and carotenoid contents was

751.73 nmol. $h^{-1}.g^{-1}$FW, 1.96 mg.g^{-1}FW and 0.14 mg.g^{-1}FW, respectively in combination treatment of 400 Gy gamma rays+0.04% EMS in the variety PRT-12.

Variety Vikrant

The control plants showed the chlorophyll content of 2.78 mg.g^{-1}FW, while in the treated plants, it ranged from 2.14-2.62 mg.g^{-1}FW. The control plants showed the carotenoid content of 0.28 mg.g^{-1}FW, while in the treated plants, it ranged from 0.15-0.27 mg.g^{-1}FW. The NRA of control leaves were observed as 775.13 nmol. $h^{-1}.g^{-1}$FW, while in the treated plants, it ranged from 759-774 nmol. $h^{-1}.g^{-1}$FW (Table 1, Fig. 1).

Variety PRT-12

The control plants showed the chlorophyll content of 2.64 mg.g^{-1}FW, while the range of chlorophyll content in the treated plants was observed from 1.96-2.55 mg.g^{-1}FW. Carotenoid content in control leaves was 0.27 mg.g^{-1}FW, while in the treated plants, it ranged from 0.14-0.26 mg.g^{-1}FW. Nitrate reductase activity of control leaves were observed 771.93 nmol. $h^{-1}.g^{-1}$FW, while the range in treated plants was from 751.73-769.06 nmol. $h^{-1}.g^{-1}$FW (Table 2, Fig. 2).

Reduction in nitrate reductase activity (NRA), chlorophyll and carotenoid contents was observed in both single and combination treatments of M_1 seedlings than the control plants of both the varieties. Present findings are in agreement with the earlier reports on *Pisum sativum* [11], *Eruca sativa* [12]; *Satureja hortensis* [13]; *Oryza sativa* [14], *Triticum aestivum* [15], *Lens culinaris* [16-17], *Nigella sativa* [18] and *Vigna radiata* [19] in mungbean reported increase in chlorophyll contents compared to controls after gamma rays treatments. Decrease in chlorophyll contents after mutagenic treatments in the present study might be due to the increased activity of chlorophylase, an enzyme which is regarded as chlorophyll-degrading enzyme [20]. Hopkins [21] considered the decline in the activity of NR than the control might be due to the inhibition and/or metabolic dysfunctions of the enzyme protein.

REFERENCES

[1.] Assocham, The Associated Chambers of Commerce & Industry of India. Assocham Corporate, New Delhi, 2015.

[2.] Champ M, Foreword. British Journal of Nutrition, 2002, 88: S237.

[3.] Ofuya Z M, Akhidue V,. The role of pulses in human nutrition: a review. Journal of Applied Sciences and Environmental Management, 2005, 9: 99–104.

[4.] Khursheed S, Raina A, Khan S. Improvement of yield and mineral content in two cultivars of Vicia faba L. through physical and chemical mutagenesis and their character association analysis. Arch. Curr. Res. Int. 2016; 4(1):1-7.

[5.] Khursheed S, Laskar RA, Raina A, Amin R, Khan S. Comparative analysis of cytological abnormalities induced in Vicia faba L. genotypes using physical and chemical mutagenesis. Chromosome Science, 2015, 18(3-4):47-51.

[6.] Raina A, Laskar RA, Khursheed S, Khan S, Parveen K, Amin R, Khan S. Induce physical and chemical mutagenesis for improvement of yield attributing traits and their correlation analysis in chickpea. International Letters of Natural Sciences, 2017, 61;14-22 doi:10.18052/www.scipress.com/ILNS.61.14

[7.] Amin R, Laskar RA, Khursheed S, Raina A, Khan S. Genetic Sensitivity towards Mms Mutagenesis Assessed Through in Vitro Growth and Cytological Test in Nigella Sativa L. Life Sciences International Research Journal: ISSN.:2347-8691.

[8.] Raina A, Laskar RA, Khursheed S, Amin R, Tantray YR, Parveen K, Khan S. Role of mutation breeding in crop improvement-past, present and future. Asian Research Journal of Agriculture. 2016;2(2):1-3.

[9.] Khursheed S, Raina A, Parveen K, Khan S, Induced phenotypic diversity in the mutagenized populations of faba bean using physical and chemical mutagenesis. Journal of the Saudi Society of Agricultural Sciences, 2016, DOI: http://dx.doi.org/10.1016/j.jssas.2017.03.001.

[10.] Mackinney G. Absorption of light by chlorophyll solutions. J. biol. Chem. 1941 Mar;140(2):315-22.

[11.] Feenstra W J, Jacobsen E, Isolation of nitrate reductase deficient mutants of *Pisum sativum* by means of selection of chlorate resistance. Theoretical and Applied Genetics, 1980 58: 39-42.

[12.] Al-Qurainy F, Effects of sodium azide on growth and yield traits of Eruca sativa (L.). World Applied Sciences Journal, 7(2), 2009, 220-6.

[13.] Rahimzadeh P, Hosseini S, Dilmaghani K, Effects of UV-A and UV-C radiation on some morphological and physiological parameters in savory (*Setureja hortensis* L.). Annals of Biological Research, 2011, 2: 164-171.

[14.] Shereen A, Ansari R, Mumtaz S, Bughio HR, Mujtaba SM, Shirazi MU, Khan MA. Impact of gamma irradiation induced changes on growth and physiological responses of rice under saline conditions. Pakistan Journal of Botany, 2009, 41(5):2487-95.

[15.] Borzouei A, Kafi M, Khazaei H, Naseriyan B, Majdabadi A. Effects of gamma radiation on germination and physiological aspects of wheat (Triticum aestivum L.) seedlings. Pakistan Journal of Botany, 42(4), 2010, 2281-90.

[16.] Laskar RA, Khan S, Khursheed S, Raina A, Amin R. Quantitative analysis of induced phenotypic diversity in chickpea using physical and chemical mutagenesis. Journal of Agronomy, 2015, 14(3):102.

[17.] Laskar RA, Laskar AA, Raina A, Khan S, Younus H. Induced mutation analysis with biochemical and molecular characterization of high yielding lentil mutant lines. International journal of biological macromolecules, 2018, 109:167-79.

[18] Tantray AY, Raina A, Khursheed S, Amin R, Khan S. Chemical Mutagen affects Pollination and Locule Formation in Capsules of Black Cumin (Nigella sativa L.). International Journal on Agricultural Sciences, 2017. 8(1): 108-117.

[19.] Wani MR, Dar AR, Tak A, Amin I, Shah NH, Rehman R, Baba MY, Raina A, Laskar R, Kozgar MI, Khan S. Chemo-induced pod and seed mutants in mungbean (*Vigna radiata* L. Wilczek). SAARC Journal of Agriculture, 2017, 15(2):57-67.

[20.] Reddy MP. Changes in pigment composition. Hill reaction activity and saccharides metabolism in bajra (Penisetum typhoides) leaves under NaCl salinity. Photosynthetica. 1986;20:50-5.

[21.] HOPKINS W J, (Introduction to Plant Physiology, 438: John Wily and Sons Inc New York, 1995). SK.

Treatment	**NRA (nmol. $h^{-1}.g^{-1}FW$)** $\bar{x} \pm S.E$	**Cholorophyll (mg.g-1FW)** $\bar{x} \pm S.E$	**Carotenoid (mg.$g^{-1}FW$)** $\bar{x} \pm S.E$
Control	$775.13^{a} \pm 0.20$	$2.78^{a} \pm 0.008$	$0.28^{a} \pm 0.008$
100 Gy γ rays	$774.96^{a} \pm 0.17$	$2.62^{ab} \pm 0.07$	$0.27^{ab} \pm 0.007$
200 Gy γ rays	$773.93^{ab} \pm 0.49$	$2.55^{bc} \pm 0.11$	$0.25^{abc} \pm 0.01$
300 Gy γ rays	$773.36^{bc} \pm 0.69$	$2.47^{bcd} \pm 0.12$	$0.23^{cde} \pm 0.01$
400 Gy γ rays	$772.80^{bc} \pm 0.36$	$2.33^{cde} \pm 0.18$	$0.20^{ef} \pm 0.01$
0.01% EMS	$774.00^{ab} \pm 0.23$	$2.33^{cde} \pm 0.03$	$0.26^{ab} \pm 0.003$
0.02% EMS	$772.93^{bc} \pm 0.52$	$2.30^{de} \pm 0.05$	$0.24^{bcd} \pm 0.003$
0.03% EMS	$771.93^{c} \pm 0.32$	$2.19^{e} \pm 0.005$	$0.22^{de} \pm 0.01$
0.04% EMS	$770.43^{d} \pm 0.12$	$2.18^{e} \pm 0.003$	$0.17^{fg} \pm 0.003$
100 Gy γ rays+0.01% EMS	$773.33^{bc} \pm 0.52$	$2.19^{e} \pm 0.003$	$0.25^{bcd} \pm 0.005$
200 Gy γ rays+0.02% EMS	$769.03^{d} \pm 0.57$	$2.17^{e} \pm 0.005$	$0.22^{de} \pm 0.005$
300 Gy γ rays+0.03% EMS	$765.33^{e} \pm 0.88$	$2.15^{e} \pm 0.003$	$0.18^{f} \pm 0.008$
400 Gy γ rays+0 .04% EMS	$759.00^{f} \pm 0.57$	$2.14^{e} \pm 0.005$	$0.15^{g} \pm 0.005$

Table 1: Effect of single and combination treatments of gamma rays and EMS on nitrate reductase activity (NRA), chlorophyll and carotenoid contents in the leaves of *Vicia faba* L. var. Vikrant.

Different letters show significant difference at p≤0.05. Means with the same letter are not statistically different.

Treatment	NRA (nmol. $h^{-1}.g^{-1}FW$) $\bar{x} \pm S.E$	Cholorophyll (mg.g-1FW) $\bar{x} \pm S.E$	Carotenoid (mg.$g^{-1}FW$) $\bar{x} \pm S.E$
Control	$771.93^{a} \pm 1.02$	$2.64^{a} \pm 0.02$	$0.27^{a} \pm 0.005$
100 Gy γ rays	$769.06^{b} \pm 0.52$	$2.55^{b} \pm 0.02$	$0.26^{ab} \pm 0.005$
200 Gy γ rays	$767.53^{c} \pm 0.35$	$2.52^{bc} \pm 0.04$	$0.25^{bc} \pm 0.003$
300 Gy γ rays	$767.13^{cd} \pm 0.08$	$2.47^{c} \pm 0.005$	$0.24^{bcd} \pm 0.003$
400 Gy γ rays	$766.16^{de} \pm 0.08$	$2.38^{de} \pm 0.005$	$0.23^{cd} \pm 0.003$
0.01% EMS	$768.00^{bc} \pm 0.11$	$2.45^{cd} \pm 0.01$	$0.24^{bcd} \pm 0.003$
0.02% EMS	$766.20^{de} \pm 0.11$	$2.37^{de} \pm 0.05$	$0.23^{cd} \pm 0.008$
0.03% EMS	$765.20^{ef} \pm 0.11$	$2.31^{e} \pm 0.01$	$0.22^{d} \pm 0.003$
0.04% EMS	$764.43^{f} \pm 0.29$	$2.22^{f} \pm 0.01$	$0.20^{e} \pm 0.006$
100 Gy γ rays+0.01% EMS	$765.56^{ef} \pm 0.38$	$2.21^{f} \pm 0.008$	$0.22^{d} \pm 0.003$
200 Gy γ rays+0.02% EMS	$761.56^{g} \pm 0.38$	$2.06^{g} \pm 0.03$	$0.20^{e} \pm 0.008$
300 Gy γ rays+0.03% EMS	$756.80^{h} \pm 0.40$	$1.97^{h} \pm 0.005$	$0.18^{f} \pm 0.005$
400 Gy γ rays+0 .04% EMS	$751.73^{i} \pm 0.17$	$1.96^{h} \pm 0.006$	$0.14^{g} \pm 0.01$

Table 2: Effect of single and combination treatments of gamma rays and EMS on nitrate reductase activity (NRA), chlorophyll and carotenoid contents in the leaves of *Vicia faba* L. var. PRT-12.

Different letters show significant difference at p≤0.05. Means with the same letter are not statistically different.

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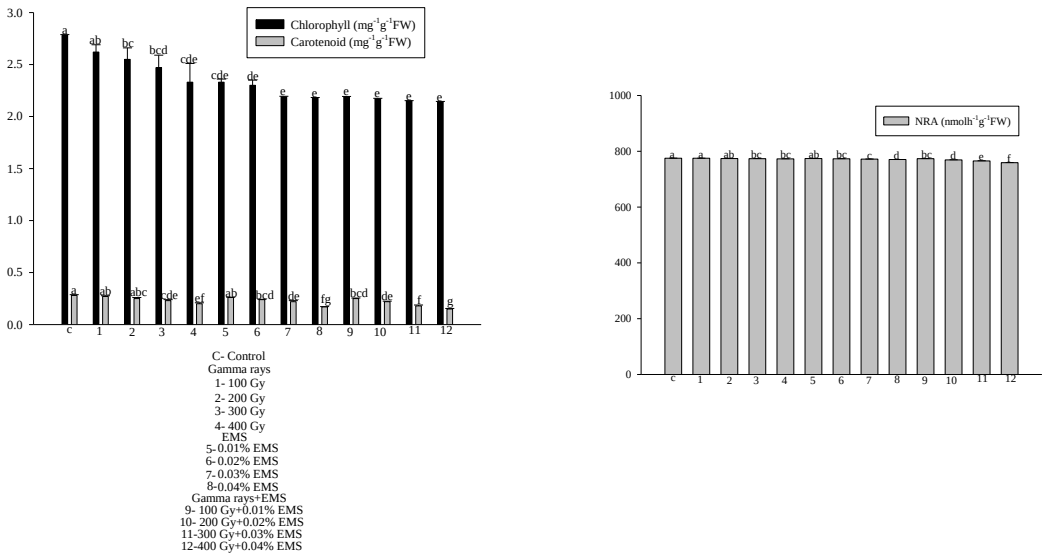

Fig. 1: Effect of single and combination treatments of gamma rays and EMS on NRA, chlorophyll and carotenoid contents of

***Vicia faba* L. var. Vikrant in M_1 generation.**

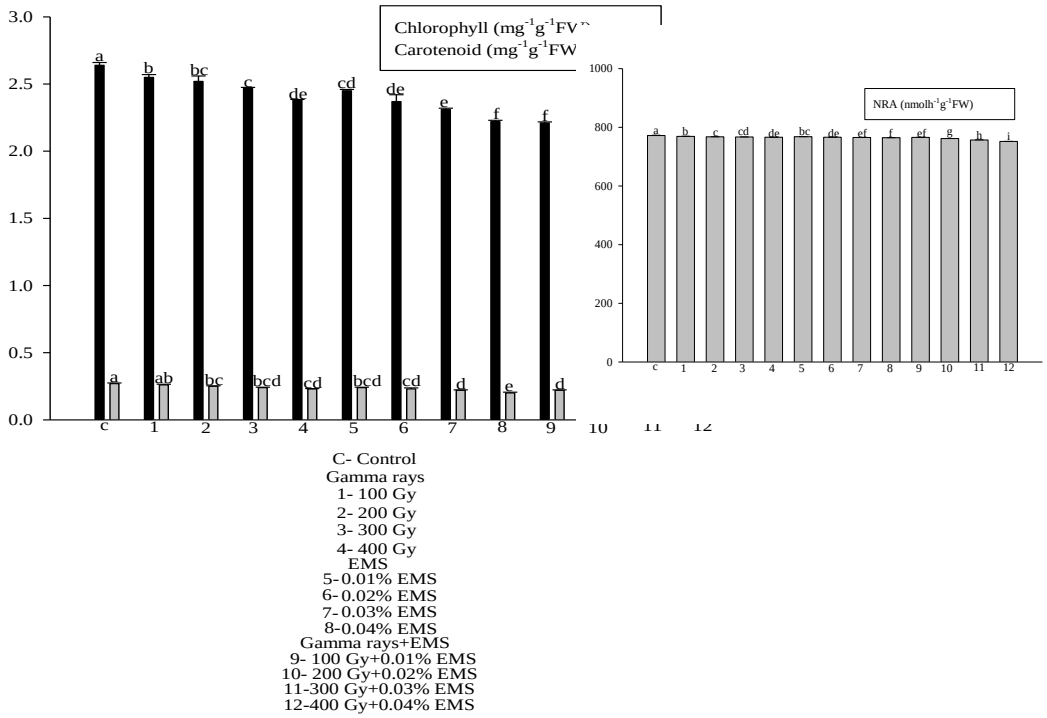

Fig. 2: Effect of single and combination treatments of gamma rays and EMS on NRA, chlorophyll and carotenoid contents of

***Vicia faba* L. var. PRT-12 in M_1 generation**