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Improvement of Yield and Mineral Content in Two Cultivars of *Vicia faba* L. Through Physical and Chemical Mutagenesis and their Character Association Analysis

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Authors' contributions

This work was carried out in collaboration between three authors. Author Shahnawaz Khursheed performed the field experiment and drafted the manuscript under the supervision of author Samiullah Khan. Author Samiullah Khan finalized the manuscript with necessary corrections. Author AR helped in drafting the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

World is continuously facing the food insecurity and malnutrition problem. Scientists all over the world are busy in developing the latest and innovative approaches for reducing the hunger and malnutrition problem which are increasing at an alarming rate throughout the world. The current experiment was conducted to improve the yield and nutritional quality of two cultivars of faba bean viz., Vikrant and PRT-12. The seeds of two cultivars were treated with single and combined treatments of gamma rays and ethyl methanesulphonate (EMS) in M_1 generation. The seeds collected from M_1 plants were sown to raise the M_2 generation. High yielding plants from lower treatments of gamma rays, EMS and their combinations were analyzed for yield and different mineral elements like zinc, iron and manganese using atomic absorption spectrophotometer (AAS). Both cultivars showed significant increase in yield and mineral content. Variety Vikrant showed

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more increase in yield and mineral content towards mutagen treatments than PRT-12. This approach can be utilized on other plants to improve the yield and nutritional quality of plants. This will overcome the increasing rate of hunger and malnutrition problem throughout the world.

Keywords: Gamma rays; EMS; Atomic Absorption Spectrophotometer (AAS); Vikrant; PRT-12.

1. INTRODUCTION

Malnutrition and hunger problem are posing a severe threat to human civilization these days. India ranks 55th according to Global Hunger Index (2014). Millennium Development Goals (MDGs) is a commitment already set by different nations at Millennium Summit (2000) to reduce the hunger problem by half between 1990 and 2015. Pulses form the major part of food throughout world. Focus on growth study of pulses will be more effective especially for poor people because these form the major food of these below average people [1]. Pulses, with an ability to grow on wide range of climatic zones form an integral part of food throughout the world especially in Indian sub-continent. Pulses have a unique property of nitrogen fixing ability [2,3] along with higher percentage of proteins [4].

Faba bean, an important nutritional pulse crop, serves as an important source of proteins and minerals. Faba bean is a well known ancient crop that probably served as food for Mediterranean and Near East civilizations [5]. Due to its ability to grow under harsh climatic conditions, it is of urgent need to focus on this nutritious food crop. Induced mutagenesis through physical and chemical mutagens is an important and sophisticated approach to induce desirable variability [6,7]. Many workers have reported the desirable variability in cereals and pulses after induced mutagenesis [8,9].

The present experiment was conducted to induce the variability in yield and mineral content in two varieties of faba bean using single and combined treatments of gamma rays and ethyl methanesulphonate (EMS).

2. MATERIALS AND METHODS

Fresh and healthy seeds of two varieties of *Vicia faba* L. viz., Vikrant and PRT-12 were used for this experiment. 13 sets of each variety were used for this experiment. Eight sets of seeds were treated with four different doses of Gamma rays (100, 200, 300 and 400 Gy). The gamma ray treatment was performed at the NBRI, Lucknow using radioisotope ^{60}Co source. The rest of 5 sets of seeds were pre-soaked in distilled water for 9 hours. Out of these 5 sets, one set was taken as control and 4 sets of seeds were given chemical mutagen treatment (0.01, 0.02, 0.03 and 0.04%) for 6 hours. Four sets of seeds, which were already treated with different doses of gamma rays, were also given chemical treatment for 6 hours for combination treatments (100Gy+0.01% EMS, 200Gy+0.02% EMS, 300Gy+0.03% EMS and 400Gy+0.04% EMS). The solution of EMS was prepared in phosphate buffer of pH 7. For each treatment, 300 seeds were used. After chemical treatment, the seeds were thoroughly washed in running tap water to remove any residual effects of mutagens on seeds. The seeds were sown with three replications in a randomized complete block design, with each replication consisting of 100 seeds at the agricultural farm, at Aligarh Muslim University, Aligarh, India to raise the M_1 generation. The distance between seeds in a row and between the rows was kept 30×60 cms., respectively. Seeds collected from M_1 plants were sown to raise the M_2 generation. Mutants with higher yield from control isolated from lower doses/concentrations of single and combined treatments of gamma rays and EMS were analyzed for yield and different mineral elements like zinc, iron and manganese. Five replications for each treatment were used for analysis of yield and different mineral elements in M_2 generation. Higher doses/concentrations of single and combination treatments decreased the yield of plants compared to control, so the plants in these treatments were not analyzed for mineral content.

2.1 Atomic Absorption Spectrophotometer (AAS)

Mutant seeds were digested by wet diacid methodology and Zn, Fe and Mn contents were determined by atomic absorption spectrophotometer (AAS) [10]. Mineral element analysis dried seed samples were homogenized by grinding in a stainless steel blinder and then passed through sieves of 2 mm mesh size and kept at room temperature for further analysis.

2.2 Digestion of Samples

0.5 g grinded seed samples of mutants and controls were weighed and put into 50 ml digestion tube. 5 ml of acid mixture were added into each sample. The pyrex funnel was placed into the tubes and put into the block digestor. Samples were heated initially at 60ºC for 15 minutes till reaction completes which was confirmed on fumes eruption. Samples were then heated to 120ºC until samples became colourless. Once the samples get cleared the tubes were removed from the digestor and cooled. The blank was also prepared consisting of only acid mixture solution, without sample contents.

2.3 Estimation of Mineral Elements

Digested and blank samples was diluted with 50 ml deionized water and mineral elements (Fe, Mn, Zn) contents were detected using AAS (GBC Scientific Equipment Ptv. Ltd., Australia). The standard solution (MERCK manufactures, Mumbai, India) for each mineral element was used for calibration. The absorbance was read at the cathode lamp set up with slit width of 0.5 nm for the Zn and 0.2 nm for Fe and Mn estimation. The wavelengths of the lamp were kept 372.0, 403.1 and 307.6 for the estimation of Fe, Mn and Zn respectively. Same lamp wavelengths and width of the slit were kept for their respective standards and blank samples. The values for total Fe, Mn and Zn contents were directly assessed by the computer software AVANTA 2.0 version preinstalled in AAS computer equipment.

2.4 Statistical Analysis

The data obtained from AAS and from experimental field as yield was analyzed using SPSS 17.0 software. The data was analyzed using Dunkan's multiple range test and significance was tested at 0.05% level.

3. RESULTS AND DISCUSSION

Yield was calculated as the weight of 100 seeds in both control and treated plants. Results show a significant increase in yield of mutants in M_2 generation compared to control in both varieties (Tables 1 and 2; Figs. 1 and 2). But variety Vikrant showed more increase in yield compared to variety PRT-12. The highest yield was observed in 0.01% EMS (33.44 g) in variety Vikrant. Unlike other polygenic traits, this character has been reported by many workers to be controlled by small number of genes [11]. Some authors have reported that the shift of yield and its components depends on the favourable association between the components in response to mutagenic treatments [12]. Increase in yield and yield associated parameters has been observed more in M_3 generation than M_2 generation [13]. The result of this could be the direct selection for yield in M_2 generation [14].

Results also show a significant increase in the mineral content of mutant seeds compared to control (Tables 1 and 2; Fig. 1 and 2). In variety Vikrant, the more increase out of all treatments was observed in 0.01 and 0.02% EMS concentrations while as in variety PRT-12, it was observed more in 100 Gy dose, thus indicating the differential genotypic response of plants towards mutagenic treatments. The highest increase in zinc content was observed in 0.02% EMS (34.48 µg g-1) in variety Vikrant where as the highest increase in iron content was observed in 0.02% EMS (70.66 µg g-1) in variety Vikrant. The highest increase in manganese content was observed in 100 Gy (19.52 µg g-1) in variety PRT-12. Similar increase in the mineral content was observed by Kozgar et al. in *Chickpea* [15].

Table 1. Effect of single and combined treatments of gamma rays and EMS mineral content of *Vicia faba* L. var. Vikrant

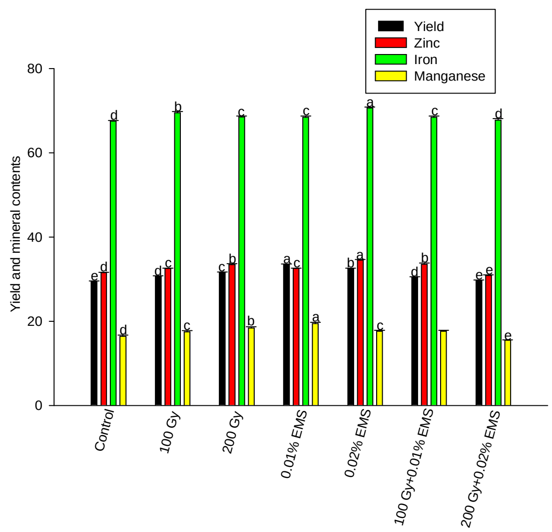
Treatments	Yield x̄ ± S.E	Zinc x̄ ± S.E	Iron x̄ ± S.E	Manganese x̄ ± S.E
Control	29.43^{e}±0.13	31.53^{d}±0.10	67.52^{d}±0.12	16.42^{d}±0.28
100 Gy	30.68^{d}±0.12	32.50^{c}±0.11	69.50^{b}±0.27	17.48^{c}±0.30
200 Gy	31.50^{c}±0.16	33.46^{b}±0.22	68.51^{c}±0.18	18.42^{b}±0.28
0.01% EMS	33.44^{a}±0.12	32.50^{c}±0.12	68.42^{c}±0.31	19.46^{a}±0.26
0.02% EMS	32.48^{b}±0.11	34.48^{a}±0.13	70.66^{a}±0.18	17.58^{c}±0.28
100Gy+0.01% EMS	30.46^{d}±0.10	33.54^{b}±0.27	68.44^{c}±0.32	17.58^{c}±0.28
200Gy+0.02% EMS	29.70^{e}±0.09	30.82^{e}±0.18	67.72^{d}±0.38	15.64^{e}±0.12

Different letters show significant difference at p≤0.05 (Dunkan's multiple range test)

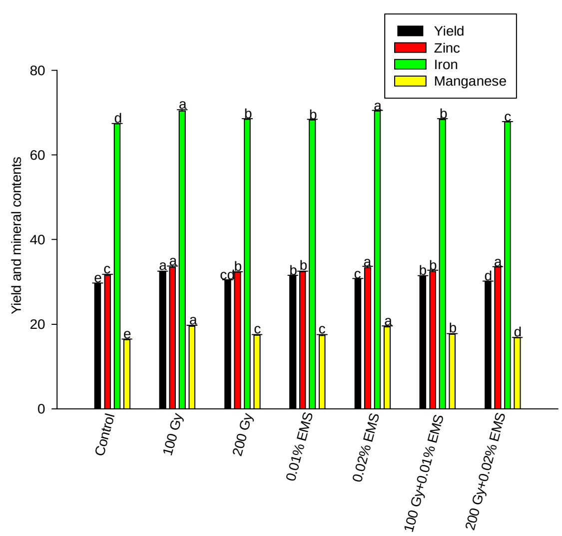
Table 2. Effect of single and combined treatments of gamma rays and EMS mineral content of *Vicia faba* L. var. PRT-12

Treatments	Yield x̄ ± S.E	Zinc x̄ ± S.E	Iron x̄ ± S.E	Manganese x̄ ± S.E
Control	29.64^{e}±0.09	31.53^{c}±0.26	67.32^{d}±0.15	16.32^{e}±0.14
100 Gy	32.48^{a}±0.08	33.48^{a}±0.25	70.41^{a}±0.27	19.52^{a}±0.19
200 Gy	30.36^{cd}±0.09	32.28^{b}±0.09	68.42^{b}±0.19	17.36^{c}±0.20
0.01% EMS	31.46^{b}±0.07	32.36^{b}±0.16	68.28^{b}±0.17	17.30^{c}±0.21
0.02% EMS	30.66^{c}±0.15	33.38^{a}±0.29	70.36^{a}±0.20	19.36^{a}±0.20
100Gy+0.01% EMS	31.34^{b}±0.10	32.48^{b}±0.25	68.36^{b}±0.20	17.62^{b}±0.17
200Gy+0.02% EMS	30.06^{d}±0.10	33.54^{a}±0.07	67.82^{c}±0.08	16.82^{d}±0.03

Different letters show significant difference at p≤0.05 (Dunkan's multiple range test)



(A)



(B)

Fig. 1. Effect of single and combined treatments of gmma rays and EMS on yield, zinc, rion and manganese contents of *Vicia faba* L. var. Vikrant (A) and PRT-12 (B) in M_2 generation

Table 3. Correlation coefficient matrix for yield and minral contents in high yielding mutants of *Vicia faba* L. in M_2 generation

	Yield	Zinc	Iron	Manganese
Yield	-	.526	.563*	.758**
	-	.054	.036	.002
	-	14	14	14
Zinc	-	-	.695**	.583*
	-	-	.006	.029
	-	-	14	14
Iron	-	-	-	.664**
	-	-	-	.010
	-	-	-	14
Manganese	-	-	-	-
	-	-	-	-
	-	-	-	-

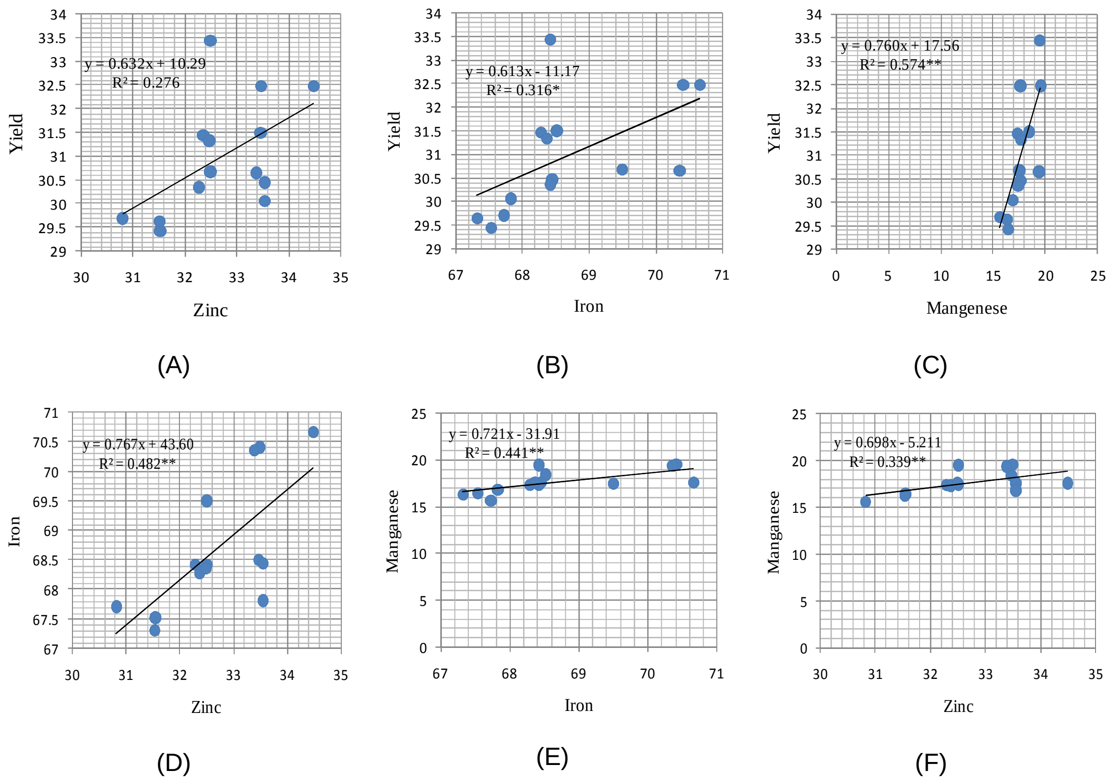


Fig. 2. Correlation and regression line graphs of different character pairs in high yielding mutants of *Vicia faba* L. in M_2 generation

Many efforts have been put on the induction of variability in plants using physical and chemical mutagenesis like growth habit, plant type and yield [16-20]. But not much work has been done on the increase in mineral content in seeds using physical and chemical mutagenesis. The present experiment was conducted as a new and an innovative approach using gamma rays and EMS treatments. It is possible to combine the high yielding trait of plants with high mineral trait [2].

3.1 Correlation Studies and Regression Analysis

Correlation and regression was observed between yield and mineral contents in the selected mutants in experiment (Table 3; Fig. 2). Significant positive correlation was observed between yield with iron and manganese. The regression line and square of correlation (R^2=0.316) indicates that there is a significant relationship between yield and iron whereas, regression line and square of correlation (R^2=0.574) indicates that there is a significant relationship between yield and manganese of mutants but no significant correlation was observed between yield and zinc. Significant positive correlation was also observed between mineral contents also. The highest significant positive correlation among mineral constituents was observed between iron and zinc and iron and manganese. The regression line and square of correlation (R^2=0.482) indicates that there is a significant relationship between iron and zinc whereas, regression line and square of correlation (R^2=0.441) indicates that there is a significant relationship between iron and manganese of mutants. Correlations studies using induced mutations has already been been reported by many workers [21,22]. The alteration of correlation whether in positive or negative direction using induced mutations has already been reported by Yadav et al. 2002; Shin et al. 2011 [23,24]. Significant correlation was observed in variety Vikrant than PRT-12 for yield and its component traits. The correlation for yield with other traits in pulses has already reported by many workers [25,26]. This might be due to the effect of pliotropic effects of mutated genes.

The current approach can be utilized in other plants like *Chickpea, Vigna* etc to increase the yield and mineral content. This can be much helpful in minimizing the hunger and malnutrition problem which the world is facing these days.

4. CONCLUSIONS

The current experiment was conducted to increase the yield and mineral contents in two genotypes of *Vicia faba* L. using gamma rays and EMS treatments. Significant increase in mineral contents was observed in mutant seeds compared to control. This innovative approach is a useful approach that can be used in future on other nutritious plants for reducing world hunger and malnutrition problem.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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