

BIOCHEMICAL AND MOLECULAR EVALUATION OF MUNGBEAN (*Vigna radiata* L.) GENOTYPES UNDER DIFFERENT GAMMA RAYS TREATMENTS

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The gamma rays treated seeds of four mungbean varieties (V1=NM-98, V2= KM1, V3= Inqilab mung and V4= Sona mung) were studied for both biochemical and molecular analysis. Randomized Complete Block Design (RCBD) was used for the experiment with four treatments using three replications (T0= Control, T1= 5 krad, T2= 10 krad, T3= 15 krad and T4= 20 Krad). Maximum biochemical and molecular based variability was recorded among genotypes. Higher amount of total sugar and proline contents were recorded in the plants grown from the seeds treated with T1, T2, T3 and T4 as compared to the control (T0) plants. On the other hand, effect on the protein content was inversely proportional to the increasing range of the radiations *i.e.* in T4 treated plants the protein content was low in comparison to the T3, T2, T1 and T0, respectively. However lower protein content was recorded in all treated samples with increasing of gamma radiation doses. The Random Amplified Polymorphic DNA (RAPD) primers were used to check molecular based variability in the allelic pattern at different gamma irradiation treatments. The three primers (OPS-03, OPB-1 and B-11) amplified four extra alleles in

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the treated plants. These extra bands were observed in NM-98 (after T1, T2 and T3 treatments), KM-1 (after T2, T3 and T4 treatments), Inqilabmung (after T1 treatment) and Sonamung (after T2, T3 and T4 treatments). Variations in physiological, biochemical and more interestingly the molecular (amplification of extra alleles/bands) attributes of the treated plants grown could possibly be due to the changes in the genome of these varieties. However, this study serves as a model to check the effect of gamma rays on other economically important crop species.

Keywords: Biochemical traits; genetic diversity; gamma rays; mungbean; molecular marker.

INTRODUCTION

Mungbean (*Vigna radiata* L.), generally called green gram belongs to family leguminaceae and is a vital crop among the pulses in Pakistan (KHATTAK *et al.*, 2004). Apart from the seeds being used as food, it is also used as a vegetable at the green stage and as fodder for animals. In comparison to other pulses, mungbean are easily digestible and therefore becomes first choice for every age people. In addition to dietary significance Mungbean have as well medicinal importance. The seeds are good for patients having heart diseases, nervous system disorders, paralysis, rheumatism and are also used as appetizer, aphrodisiac, tonic and diuretic (KHATTAK *et al.*, 2004).

The abiotic stresses affect plant growth and other morpho-biochemical process (JAN *et al.*, 2016). However, Mungbean plants have the ability to tolerate drought and therefore can grow under variable agro-climatic conditions (WRIGHT, 1935). It ranks second to drought confrontation after soybean. ALI *et al.* (2001) and GHAFOR *et al.* (2003) and therefore is grown at low to average elevations in the tropics where rain water is enough for the growth of plants. In Pakistan Mungbean is sown in two seasons i.e. summer and spring and require 2-5 months for maturity. Moreover, the optimum temperature for its growth ranges from 65-75°F (FIELD, 1985). Mungbean is a rich source of carbohydrates (38-50 %), proteins (24 %) and fats (0.67 %) (LEE *et al.*, 1997) and therefore its vegetal proteins are used as a cheaper replacement for proteins of animals meat, eggs and milk (MCTCALFE and DONALD, 1972; LEE *et al.*, 1997).

Mutation breeding is the effective breeding method for the required traits to produce genetic distinction in the germplasm. Gamma radiations, fast neutrons, X-rays, thermal neutron as well as beta radiations and ultraviolet rays are physical mutagens causing mutations. Usually, seeds are mostly used for studying the effects of radiations, while in some of the cases part of plant or entire plant is also used that can be irradiated by doses of radiations. The seeds are picking up, kept, stored and well-preserved with no trouble for a long period of time MALIK (2009). Induced mutation is the mutation, playing an important role in crop development with the help of inducing desired genetic changes for enhancing both the quality and yield (SINGH and SHARMA, 1982). The present study might be the first attempt to investigate the effect of gamma radiations on molecular and biochemical processes of mungbean genotypes. The effect of gamma radiations on biochemical and molecular parameters of mungbean genotypes are discussed.

MATERIALS AND METHODS

The experiments were conducted at Agricultural Research Station Ahmad Wala Karak in Spring 2017. Each experiment was repeated three times through split plot preparation. Seeds were exposed to gamma radiations (5, 10, 15 and 20 krad) using CO60 gamma radiations source at NIFA, Peshawar, Pakistan. The land was uniformly prepared and divided into subplots following agronomic practices and the gamma irradiated seeds were sown in comparison to the control seeds. Each subplot contained plants grown from differently treated seeds. In each subplot (containing T0, T1, T2, T3 and T4 treated plants) five plants were randomly selected and labeled for examining their biochemical and molecular traits. Different parameters like total sugar contents, total proline contents and total proteins contents were analyzed. In addition molecular characterization of the treated and untreated plants was also performed. For the extraction of proline SINGH *et al.* (1973) and for determination BATES *et al.* (1973) methods were followed. For sugar quantification DUBOIS *et al.* (1956) method while for total protein estimation JAN *et al.* (2013) procedures were used. Means were calculated using ANOVA and LSD (statistix8.1). WEINING and LANGRIDGE (1991) method was used for DNA extraction while for studying the genetic diversity DEVOS and GALE (1992) procedure was followed for amplification of the desired regions using six Randomly Amplified Polymorphic DNA (RAPD) primers (Table 1).

Table 1. Name and sequence of RAPD primers

S. NO	Primer Name	Sequences
01	OPS_3	CTATGCCGA
02	OPAJ_20	ACACGTGGTC
03	OPS_07	TCCGATGCTG
04	E_02	GGTGCGGGAA
05	OPB_1	GTTTCGCTCC
06	B_11	GTAGACCCGT

Statistical Analysis

Four mungbean genotypes along with their controls were investigated using six RAPD primers for the detection of genomic polymorphism due to the exposure of gamma rays. Analyses involved the prominent bands. Single band was measured as a single locus/allele for all the genetic studies. RAPD loci pattern was recorded as presence (01) or absence (0) as bivariate data matrix. Based on data matrix, the homology identity was calculated and homology tree was constructed. Moreover, cluster analysis was performed based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and by using DNAMAN (5.2) software following the procedure of NEI and LI (1978).

RESULTS AND DISCUSSION

A randomly induced mutation is one of the strategies used for the improvement of crop quality and yield which is mostly attained through exposure of seeds to radiations (SINGH and SHARMA, 1982). Gamma radiations are largely used for bringing genetic changes in the genome of different crops. Generally, the effect of these radiations depends on the magnitude and

exposure time. Higher exposure of gamma radiations on seeds germination of angiosperms and gymnosperms produces inhibitory effects (KUMARI and SINGH, 1996) while occasional stimulatory effect is caused due to lower exposures (RAGHAVA and RAGHAVA, 1989).

The present study showed that the magnitude of gamma rays was directly proportional to the amount of detected sugar contents in the radiations treated plants i.e. at T3 0.0376 $\mu\text{g/g}$ while at T0 0.232 $\mu\text{g/g}$ total sugar contents were accorded (Fig. 1). A study by BŁASZCZAK *et al.* (2002) also showed a direct relationship of radiations magnitude with total sugar contents. In contrast an inverse relationship has been shown between the gamma rays magnitude and sugar contents but in a different crop like *Eruca vesicaria* sub sp. *Sativa* (MOUSSA, 2006).

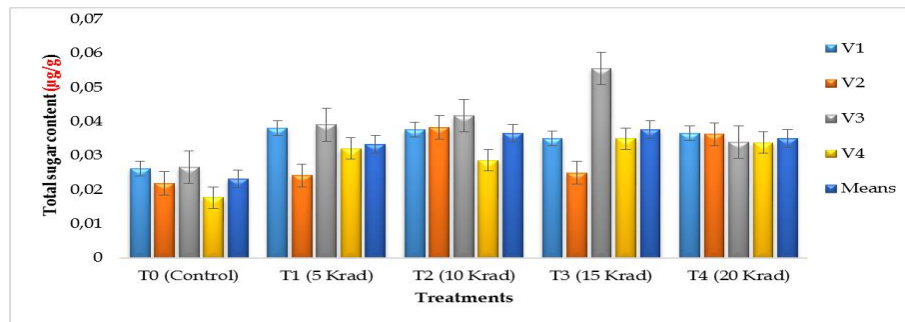


Fig.1. Effect of gamma radiation on total sugar content

The amount of proline increased with the increase of gamma radiation. The proline amount was found maximum at T2 (0.1103 $\mu\text{g/g}$) while found minimum at T0 (0.0534 $\mu\text{g/g}$). All the genotypes showed varied response to different gamma treatment (Fig. 2). The steady increase might be a response to the stress developed by different range of gamma radiations. In a similar study by BORZOUEI *et al.* (2010), a gradual increase of proline contents were reported in relation to the magnitude of gamma radiations while studying the germination and functional features of wheat seedlings.

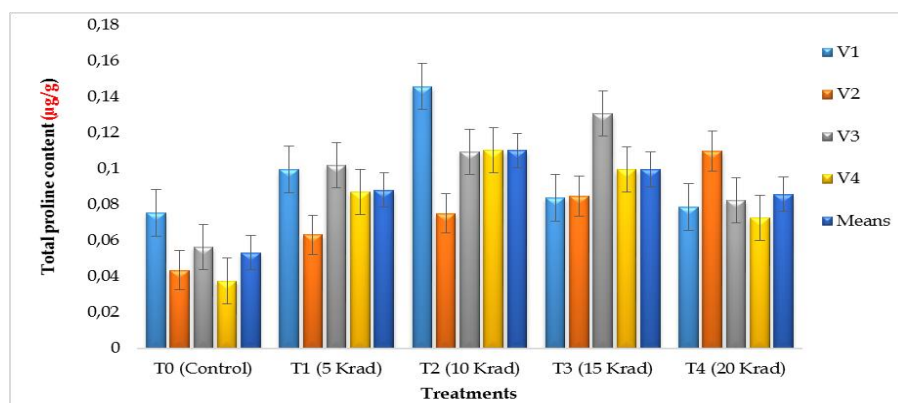


Fig. 2. Effect of gamma radiation on total proline content

Unlike, the total sugar and proline contents, the protein contents showed an inverse relationship to the magnitude of gamma rays. As per varietal effect in V1 highest value (23.838 mg/g) was recorded at T0 followed by T2 (23.637 mg/g) whereas minimum value (21.766 mg/g) was obtained at T3 (Fig. 3). The T4 significantly decreased the protein content as compared to control plants. The results of the present study are not in agreement with the study by PRIYA (2006) who showed maximum amount of protein contents gamma rays treated mungbean in comparison to the healthy controls.

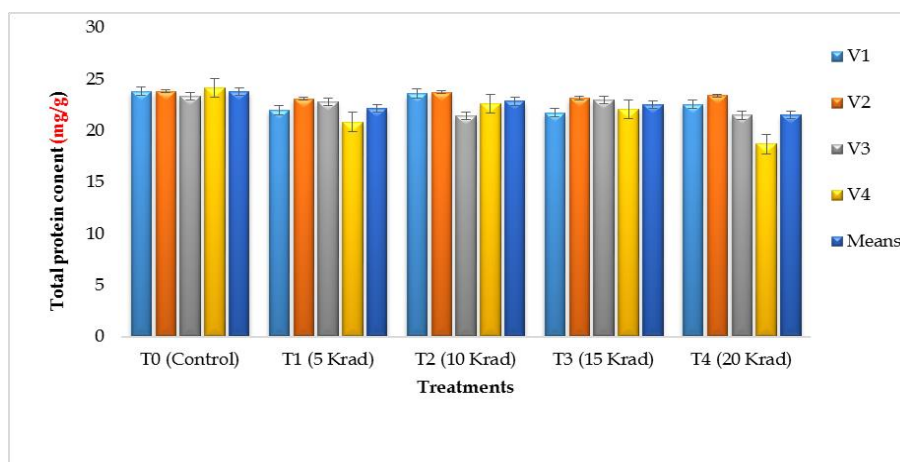


Fig. 3. Effect of gamma radiation on total protein content

The molecular based diversity was envisaged with help of six RAPD primers at different gamma rays treatments. From results we noted the presence of extra alleles in experimental samples. The presence of extra alleles might be due to mutation occurring due to gamma rays treatments or may be the regulation/expression of some silent gene. The level of polymorphism was found maximum in treated plants with primers OPS-03, OPAJ 20 and E 02 (Fig. 4a-f). The genetic similarity values among treated and control samples varied from 89% to 100%. Phylogenetic tree classified all tested genotypes with different events classified into four major groups with help of RAPD markers. Among these the genotypes V3 at treatment 3 and genotype V1 at treatment T0 are highly diverged from the rest ones (Fig. 5). These findings showed similarity with the results of KAWAR (2009) who recorded maximum polymorphism in sugarcane genotypes using 38 molecular markers. HAMIDELDIN and ELIWA (2015) also recorded genomic variability in *Brassica alba* L. seeds treated with 10, 20, 30, 40 and 50 Gy). Our findings are also supported by FADIA *et al.* (2011), who reported that using RAPD PCR on *Hibiscus subdariffa*, DNA variations in treated seeds with comparison to control were successfully assessed. The morpho-biochemical and molecular response varies with type of genotype (IBRAHIM *et al.*, 2019; SHAH *et al.*, 2018; IBRAR *et al.*, 2018; JAN *et al.*, 2017; QADIR *et al.*, 2017; HALDANIN *et al.*, 2016).

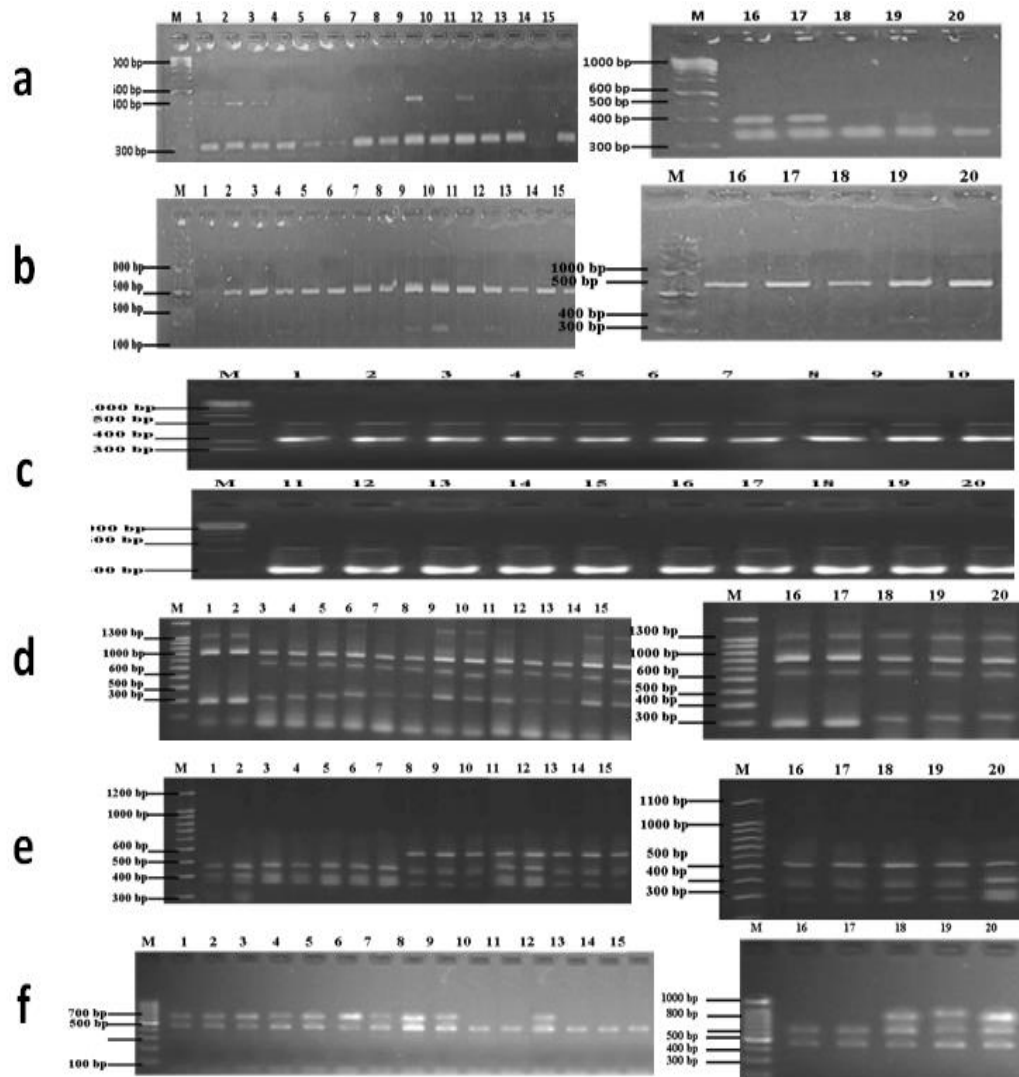


Fig. 4a-f. PCR amplification profile of four mungbean genotypes using primers (a) OPS-3M, (b) OPAJ-20M (c) OPS-07M (d) E-02M (e) OPB-1M (f) B-11M. M = molecular size marker (1kbp), V1xT0-T4 (1_5), V2XT0-T4 (6_10), V3x T0-T4 (11_15) and V4x T0-T4 (16_20).

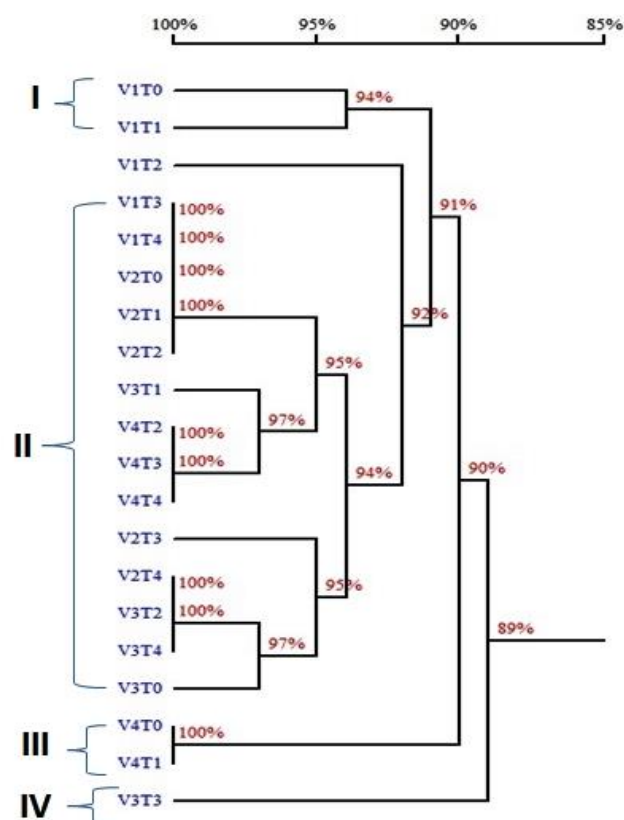


Fig. 5. (Dendrogram) Homology genetic tree constructed by using the genetic matrix. The four varieties of mungbean with five treatments were grouped based on RAPD primers.

CONCLUSION

Gamma rays induced novel variations in mungbean crop. The variable sugar, protein and prolin amount was recorded in different genotypes at different treatments. The presence of extra alleles in treated samples showed that novel mutation may occur due to gamma rays. However, maximum variability was observed in all genotypes at T3 (15 krads), and this dose is recommended to irradiate mungbean seeds. It is also recommended that long term research at biochemical as well as molecular level using more specific primers (SSRs and SNPs, *etc.*) is required to confirm the sustainability of the mutant plants.

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**BIOHEMIJSKA I MOLEKULARNA PROCENA MUNGO PASULJA (*Vigna radiata* L.)
GENOTIPOVA POD RAZLIČITIM TRETMANIMA GAMA ZRACIMA**

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Izvod

Seme četiri vrste mung pasulja (V1 = NM-98, V2 = KM1, V3 = Inkilab mung i V4 = Sona mung) tretirano gama zracima analizirano je na molekularnom i biohemijskom nivou. Randomiziran kompletan blok dizajn (RCBD) korišćen je za eksperiment sa četiri tretmana I tri ponavljanja (T0 = kontrola, T1 = 5 krad, T2 = 10 krad, T3 = 15 krad i T4 = 20 Krad). Zabeležena je maksimalna biohemijska i molekularna varijabilnost među genotipima. Utvrđena je i veća količina ukupnog sadržaja šećera i prolina u biljkama izraslim iz semena tretiranog T1, T2, T3 i T4 u odnosu na kontrolne (T0) biljke. S druge strane, uticaj na sadržaj proteina bio je obrnuto proporcionalan rastućem opsegu zračenja, tj. u biljkama tretiranim T4 sadržaj belančevina bio je nizak u poređenju sa T3, T2, T1 i T0. Međutim, zabeležen je niži sadržaj proteina u svim tretiranim uzorcima sa povećanjem doza gama zračenja. Random amplifikirani polimorfni DNK (RAPD) prajmeri su korišćeni za proveru varijabilnosti na molekularnom nivou pri različitim tretmanima gama zračenja. Tri prajmera (OPS-03, OPB-1 i B-11) amplifikovala su četiri dodatna alela u tretiranim biljkama. Ove dodatne tarke evidentirane su kod NM-98 (posle T1, T2 i T3 tretmana), KM-1 (posle T2, T3 i T4 tretmana), Inkilabmung (posle T1 tretmana) i Sonamung (posle T2, T3 i T4 tretmana). Varijacije na fiziološkom, biohemijskom i, što je još interesantnije, molekularnom (amplifikacija dodatnih alela / traka) nivou kod tretiranih biljaka mogu biti posledica promena u genomu ovih sorti. Ova studija može poslužiti kao model za proveru uticaja gama zraka na druge ekonomski važne vrste useva.

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