**Lhb Mutant – A Novel Mutant of Mungbean (Vigna radiata (L.) Wilczek) Induced by Gamma Radiation**

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**ABSTRACT**

In the present investigation an attempt was made to induce mutations in mungbean (*Vigna radiata* (L.), Wilczek) var. ‘Vaibhav’ by employing gamma radiation with the objective of obtaining novel and desirable mutants. Mungbean seeds were irradiated with 30, 40 and 50 kR doses of gamma radiation and sown in the experimental fields to raise *M*<sub>1</sub>, *M*<sub>2</sub> and *M*<sub>3</sub> populations. Mutations were screened at *M*<sub>1</sub>, *M*<sub>2</sub> and *M*<sub>3</sub> generations. All three doses of gamma radiation effectively produced various morphological mutations. However, the 50 kR dose effectively produced a novel mutant that showed multiple morphological mutations such as large flowers with dark-yellow petals, dense and thick hairy pods and black seeds. In this study, SDS-PAGE analysis of seed proteins of large flower, hairy pods and black seeds revealed a difference in the banding pattern between the *Lhb* mutant, other mutants (tall mutant, dwarf mutant, high-yielding mutant and early maturing mutant) and control.

**Keywords:** gamma rays, morphological mutations, SDS-PAGE analysis

**Abbreviations:** kR, kilorad; *Lhb*, large flower - hairy pods - black seed coat; RBD, randomized block design; SDS-PAGE, Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis

**INTRODUCTION**

Pulses occupy an important position in world agriculture because of their high protein content, several essential amino acids and their capacity for fixing atmospheric nitrogen. Mungbean (*Vigna radiata* (L.), Wilczek) is an important pulse crop of semi-arid zones and sub-tropics. The genetic variability in most pulses, including mungbean, has been greatly reduced over the years, because of the role of natural selection under a low level of management (Singh and Singh 2003).

In mungbean, a self-pollinated crop, natural existing genetic variability might not be sufficient to achieve the desired improvement. As a consequence, lack of required amount of variability, limits the scope for the selection of better genotypes. Frequency of natural mutations is very low and information on mutagenesis induced population in mungbean is scanty (Singh 2008). Hence, artificial mutations are induced and genetic variability is best enhanced with the application of mutagens. Artificially induced mutations are the best way to enlarge genetic variability, considerably within a short time (Patil et al. 2003; Singh and Singh 2003).

Mutation breeding has been proved to be one of the important techniques to develop and release new genotypes and high yielding cultivars in mungbean. Mutation breeding is a supplement to conventional breeding and is useful for creation of desirable variability in crop and could be a driving force for evolution besides selection in mungbean (Tah 2009). A closer examination of the type of mutants used and the number of mutant cultivars released in India indicates that the largest number of mutant varieties (65%) have been induced by physical mutagens, gamma (γ) rays (168 cultivators) being the most commonly used and successful (Kharkwal et al. 2004). According to the Food and Agricultural Organization/International Atomic Energy Agency (FAO/IAEA) Mutant Varieties Database (http://www.mvd.iaea.org) (December 2011), 3191 plant varieties were released worldwide which were derived from RT (radiation and isotope techniques) and chemical mutagens during the past 75 years, increasing by approximately 500 since 2007 (Kang et al. 2007). To increase production and productivity, several approaches have been used to develop 43 high-yielding mungbean varieties during 1985-2006 (Reddy and Dhanasekar 2007). Mutation breeding resulted in the development of 15 high-yielding and disease-resistant varieties during 1979-2006. BARC contributed 7 varieties which are under popularization programme and the average yield is expected to increase (Reddy and Dhanasekar 2007).

Although traditional mutation breeding has lost its prominent position, induced mutations continue to be in great demand for various biotechnological applications (Chopra 2005). New molecular approaches have greatly simplified forward genetic approach with conventionally derived mutants. Saturated molecular maps are now being used to delimit mutant locus with molecular markers and the gene is cloned through positional cloning or chromosome walking (Brown et al. 2003). In addition stable mutants can be used as source material for tagging of specific loci with DNA markers and for molecular analysis for gene function. Induced mutagenesis, which later was to become the most important tool in locating genes on chromosomes, studying gene structure, expression and regulation, and for exploring genomes. The sequencing of some plant genomes are completed but assigning functions to many of the DNA sequences would not be possible, but for the mutants induced with gamma rays, fast neutrons or chemical mutagens, the identification and analysis of mutants using molecular techniques of DNA fingerprinting and mapping with PCR based markers such as RAPD, AFLP and STMS and mutant tagging could bring a new dimension in gene technology (Ahlloowalia and Maluszynski 2001).

Induced mutations contributed significantly to plant improvement programs, even though most of the induced mutations are recessive and deleterious from a breeding point of view (Maluszynski et al. 1995). Significant in-
duced variability among various mungbean genotypes for various traits have been reported by Patra (1997), Khan et al. (2004) and Lal and Mishra (2006). Mutation breeding has become increasingly popular in recent times as an effective tool for crop improvement (Acharya et al. 2007). The present investigation was undertaken with the objective of inducing mutations in locally adapted mungbean variety ‘Vaibhav’ employing γ radiation to obtain novel mutants with desirable and economically important characters.

MATERIALS AND METHODS

Healthy seeds of mungbean (Vigna radiata (L.), Wilczek) var. ‘Vaibhav’ (released by Mahatma Phule Agricultural University, Rahuri, India) selected for the present study was procured from Pulses Improvement Division of Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri (Ahmednagar District, Maharashtra State, India). Dry and uniform seeds were irradiated with γ radiation at 30, 40 and 50 kR. γ-Rays, an ionizing radiation from a ⁶⁰Co (Cobalt-60) source, fixed in the Gamma Cell 200 installed at the Department of Biophysics, Government Institute of Science, Aurangabad, India, was used in the present work.

For each irradiation dose, 700 seeds were used. 100 seeds from each treatment were used for germination studies. The remaining lot of treated seeds from each dose was sown in an experimental field in a randomized block design (RBD) with three replications together with non-treated seeds (control) for raising the M₁ generation. The spacing between rows and plants was 30 × 10 cm, respectively. All the surviving M₁ plants were individually tagged. Seeds of single plants from each treatment were harvested and kept separately. The M₁ plants were harvested individually and the seeds obtained were used to raise the M₂ generation as plant-to-row progenies. The M₂ population was carefully screened and the seeds obtained were used to raise the M₃ generation as plant-to-row progenies. The M₃ generation was also observed that these effects were dose dependent.

For each irradiation dose, 100 seeds from each treatment were used for germination studies. The remaining lot of treated seeds from each dose was sown in an experimental field in a randomized block design (RBD) with three replications together with non-treated seeds (control) for raising the M₁ generation. The spacing between rows and plants was 30 × 10 cm, respectively. All the surviving M₁ plants were individually tagged. Seeds of single plants from each treatment were harvested and kept separately. The M₁ plants were harvested individually and the seeds obtained were used to raise the M₂ generation as plant-to-row progenies. The M₂ population was carefully screened for different morphological mutations. Different types of mutants like tall mutant, dwarf mutant, high yielding mutant, early maturing mutant and Lhb mutant has been recorded. Lhb mutant showed poor performance in all agronomic characters over all obtained mutants and control plant. Present paper gives comparison of Lhb mutant with only control plant (Table 2).

The mutant plants which showed multiple mutagenic effects in the form of a large flower, hairy pod, black seed coat, leaf modification and semi-dwarf habit were selected. The name of the mutant was assigned on the basis of flower size, presence of thick hairs on the pod and black colour seed coat. Lhb (large flower - hairy pods - black seed coat) mutant plants (25) were selected and from each plant 25 seeds were sown in the field together with the control to raise the M₂ generation in order to multiply the mutant and confirm its stability.

Seeds of the Lhb mutant were analysed for protein, albumin and globulin content (Lowry et al. 1951). Albumin and globulin were isolated by using dialysis bags. The protein profile of the Lhb mutant was analysed by SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis) following the method of Dadlani and Varier (1993).

Statistical analysis

Morphological and agronomical data obtained from 20 mutant plants was analysed for range, mean, standard deviation, coefficient of variation and one-way analysis of variance to assess quantitative differences in the various characters of the mutant and the control using SPSS 9.0 software (SPSS Inc., Chicago, Illinois, USA). Significant differences between mutant and control means were determined using least significant difference (LSD) values at P = 0.05, 0.01 and 0.001.

RESULTS AND DISCUSSION

In the present investigation, mungbean variety ‘Vaibhav’ proved to be very sensitive to all employed doses of the γ radiations (30, 40 and 50 kR). The mutant also exerted inhibitory effects on the percent germination, seedling survival and increased the rate of sterility in M₁ generation. It was also observed that these effects were dose dependent. The dose levels of 40 to 50 kR were critical for inducing mutations (Sharma and Haque 1997). The M₁ population showed various morphological variations included different leaf, flower, pod and seed mutations. In M₂ generation three types of chlorophyll mutations namely striata, xantha and chlorina was noted. Maximum number of chlorina and xantha types was observed. Average chlorophyll mutation frequency for γ radiation is 2.02%. In this variety as dose of the γ radiation increased, the effectiveness was decreased. 30 kR dose of γ rays was found to be more effective in inducing chlorophyll mutations and 50 kR γ dose induces prominent mutations.

Along with simple viable mutations, multiple mutagenic effects on two or more characters were also found in all the mutagenic treatments. However their frequencies differed with dose of mutagen and variety. In the present work 50 kR dose of γ radiation induced a novel mutant that showed multiple morphological mutations like large flowers with dark yellow petals, dense thick hairy pods and black coloured seeds (Fig. 1). It is named as large flower, hairy pod and black seed mutant (Lhb mutant). Such a mutant has not been reported earlier in mungbean. These Lhb mutants were also recovered in M₃ populations of mungbean. Lhb mutant is homozygous in nature and whether it is recessive or dominant is yet to be established.

These mutants also showed multiple mutagenic effects on various other traits. Important among them are semi-dwarf habit, late flowering, large-sized flowers, dark-yellow petals, late maturing, broad and short pods, thick dense hairs on pods. The large flower, hairy pod and black seed mutant (Lhb mutant) raised through γ radiation induced mutagenesis, seems to be promising at least for two characters i.e. semi-dwarf habit and presence of thick dense hairs on the pods. The semi-dwarf habit of the plant enables it as lodging resistant. The thick dense hairs on the pod act as a mechanical barrier and helps in protecting them from insects and caterpillar predators and seems to be promising economical important character (Pfeiffer et al. 2003). It can be used or incorporated in breeding programmes that are aimed at genetic improvement of mungbean. Large flowers minimize the consequent difficulties of emasculation during the crossing practices. Large flower mutant can be used for crossing to exploit heterosis (Jones 1945). The mutant heterosis has been described in many plant species (Maluszynski et al. 1989).

A comparative account of morphological characters of control and large flower, hairy pod, black seed mutant (Lhb mutant) is presented in Table 2.
Hypogaea semi-dwarf mutants were also reported earlier in (Arachis 2005), sometimes because of alterations in number of nodes was found to be due to changes in the internodal length and (2009). The mutants affecting the plant height in many crops such as tomato (Patil 2009; Borkar and More 2003; Badigannavar and Murty 2007; Barshile et al. 2005, 2011; Wani 2011), Cicer arietinum (Kharkwal 2000; Barshile et al. 2005; Priya Tah 2006; Reddy and Danasekar 2007; Anand Kumar 2009; Reddy 2009). Glycine max (Ahire 2008; Tambe and Apparao 2009; Manjaya and Nandanwar 2007; Nadaf et al. 2009), Phaseolus vulgaris (Patil 2009; Borkar and More 2010; Mahamune and Kothekar 2010) and in Vigna unguiculata (Kumar et al. 2010, 2011), Cicer arietinum L. by the treatment with three different concentration of ethyl methane-sulphonate (EMS) (8, 12 and 16 mM), SA (2, 3 and 4 mM) and gamma rays (400, 500 and 600 Gy). Pod mutations (small, roundish, gigas and narrow elongated. Flower colour mutations were recorded by Manjaya (2009) in soybean variety ‘VLS-2’ by the use of 250 Gy gamma rays, Nadaf et al. (2009). Macoumba et al. (2010) recorded flower mutation in sesame cultivar ‘32-15’ and ‘38-1-7’ and Turkish cultivar ‘Birkan’ by the irradiation with 300-400 Gy dose of gamma radiation. Flower mutant showed split corolla. This character is useful for open pollination and constitutes another mechanism for hybrid seed production. High sterility was noted in the mutant of var. ‘Birkan’, due to rudimentary floral parts. Wani (2011) reported flower mutants in two varieties of Cicer arietinum L. from EMS, gamma rays and combination (EMS + gamma rays) treatment. Mutants isolated in this category included double flower, white-flower and non-flowering vegetative mutants. POLLEN sterility ranged from 40 to 50% and very few seeds were produced. The frequency of these mutants was 0.06% in ‘Pusa-212’ and 0.02% in ‘Pusa-372’. One true breeding mutant was isolated from 0.3% EMS in ‘Pusa-372’. This mutant was characterized by violet flowers which gradually turned white instead of pink flowers in the parent variety. Seed set in this mutant was normal. In addition to the above, some non-flowering mutants with no

Table 1 Comparative account of control and Lhb mutant on the morphological characters.

<table>
<thead>
<tr>
<th>Characters</th>
<th>Control Plant – ‘Vilahav’</th>
<th>Large flower, hairy pod and black seed color (Lhb) mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habit</td>
<td>Herbaceous, erect</td>
<td>Herbaceous, erect</td>
</tr>
<tr>
<td>Root</td>
<td>Tap system provided with nodules</td>
<td>Tap system provided with nodules</td>
</tr>
<tr>
<td>Stem</td>
<td>Green with pigments</td>
<td>Green with pigments</td>
</tr>
<tr>
<td>Leaf</td>
<td>Trifoliate, thick leaves; no. of leaves: 6.8, leaf area index: 5.82; leaf area: 165.5 cm²</td>
<td>Trifoliate, thick leaves; no. of leaves: 6.9, leaf area index: 5.10; leaf area: 155 cm²</td>
</tr>
<tr>
<td>Flower</td>
<td>Bisexual, zygomorphic, Faint yellow, medium sized flower</td>
<td>Bisexual, zygomorphic, dark yellow color, large sized flower</td>
</tr>
<tr>
<td>Pod</td>
<td>Green pod with prominent constriction, pod length medium, produced green seeds</td>
<td>Small pod with dense thick hairs on the pod, produced black seeds</td>
</tr>
</tbody>
</table>

Table 2 Comparative account of control and Lhb mutant on their agronomic and biochemical characters.

<table>
<thead>
<tr>
<th>Characters</th>
<th>Control</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
<th>Lhb</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agronomic traits</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant height (cm)</td>
<td>45.5</td>
<td>51.9</td>
<td>48.2</td>
<td>1.9</td>
<td>4.0</td>
<td>36.1</td>
<td>42.1</td>
<td>38.8</td>
<td>#</td>
<td>1.7</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>Days to flowering</td>
<td>32.0</td>
<td>35.0</td>
<td>33.4</td>
<td>1.2</td>
<td>3.5</td>
<td>40.0</td>
<td>45.0</td>
<td>42.3</td>
<td>***</td>
<td>1.3</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Days to first pod bearing</td>
<td>42.0</td>
<td>46.0</td>
<td>44.4</td>
<td>1.3</td>
<td>2.9</td>
<td>48.0</td>
<td>54.0</td>
<td>51.1</td>
<td>***</td>
<td>1.8</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>Days to maturity</td>
<td>74.0</td>
<td>78.0</td>
<td>74.9</td>
<td>1.2</td>
<td>1.6</td>
<td>80.0</td>
<td>84.0</td>
<td>81.9</td>
<td>***</td>
<td>1.4</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>Pods/plant</td>
<td>18.0</td>
<td>28.0</td>
<td>21.5</td>
<td>3.1</td>
<td>14.2</td>
<td>13.0</td>
<td>18.0</td>
<td>16.2</td>
<td>**</td>
<td>1.5</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>Pod length (cm)</td>
<td>5.7</td>
<td>7.2</td>
<td>6.4</td>
<td>0.4</td>
<td>7.0</td>
<td>4.2</td>
<td>5.2</td>
<td>4.6</td>
<td>#</td>
<td>0.3</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>Grains/pod</td>
<td>4.0</td>
<td>6.0</td>
<td>5.0</td>
<td>0.7</td>
<td>14.8</td>
<td>4.0</td>
<td>5.0</td>
<td>4.3</td>
<td>#</td>
<td>0.5</td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td>100 seed weight (g)</td>
<td>3.2</td>
<td>3.9</td>
<td>3.5</td>
<td>0.2</td>
<td>5.2</td>
<td>2.4</td>
<td>2.9</td>
<td>2.6</td>
<td>#</td>
<td>0.2</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>Yield/plant (g)</td>
<td>4.5</td>
<td>4.7</td>
<td>4.6</td>
<td>0.1</td>
<td>1.7</td>
<td>2.5</td>
<td>3.5</td>
<td>3.0</td>
<td>#</td>
<td>0.3</td>
<td>9.4</td>
<td></td>
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<tr>
<td>Harvest index</td>
<td>18.4</td>
<td>20.5</td>
<td>19.7</td>
<td>0.6</td>
<td>3.2</td>
<td>14.2</td>
<td>23.0</td>
<td>17.9</td>
<td>#</td>
<td>2.2</td>
<td>12.1</td>
<td></td>
</tr>
<tr>
<td>Number of nodules</td>
<td>21.0</td>
<td>24.0</td>
<td>22.0</td>
<td>1.1</td>
<td>5.2</td>
<td>14.0</td>
<td>18.0</td>
<td>16.1</td>
<td>###</td>
<td>1.2</td>
<td>7.2</td>
<td></td>
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<tr>
<td>Biochemical traits</td>
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<td></td>
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<tr>
<td>Total protein</td>
<td>21.8</td>
<td>22.2</td>
<td>20.0</td>
<td>0.2</td>
<td>1.0</td>
<td>17.6</td>
<td>18.9</td>
<td>18.6</td>
<td>***</td>
<td>0.7</td>
<td>3.8</td>
<td></td>
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<tr>
<td>Globulin</td>
<td>12.2</td>
<td>12.9</td>
<td>12.6</td>
<td>0.4</td>
<td>2.9</td>
<td>9.8</td>
<td>10.5</td>
<td>10.1</td>
<td>***</td>
<td>0.4</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>9.9</td>
<td>10.2</td>
<td>10.1</td>
<td>0.2</td>
<td>1.5</td>
<td>6.4</td>
<td>6.5</td>
<td>6.4</td>
<td>***</td>
<td>0.1</td>
<td>0.9</td>
<td></td>
</tr>
</tbody>
</table>

Lhb: Large flower, hairy pod and black seeds; Min: minimum; Max: maximum; SD: standard deviation; CV: coefficient of variation
Values given are mean of 10 independent samples; *** Significantly higher than control at P < 0.001; #, ##, ### Significantly lower than control at P < 0.05, 0.01 and 0.001, respectively
seed set (0.11% in var. ‘Pusa-212’ and 0.18% in var. ‘Pusa-372’) were isolated from higher dose treatments of EMS, gamma rays.

Sangsiri et al. (2005) reported flower and pod mutations in mungbean varieties ‘KPS 2’ and ‘VC 6468-11-B’ with γ rays (Cs-137 source) at a dose of 50 kR. The flower mutant was cock’s comb raceme while the pod mutant was a lobed one, with fewer seeds per pod. This trait may associate with partial sterility, causing constriction at the point where there was undeveloped seed.

Barshile (2006) has recorded white coloured flower mutant, seed coat colour and seed size mutations in chickpea variety ‘Vijay’ induced by 60 kR dose of γ radiation. Many of these were associated with various leaf and plant type mutations. Atta et al. (2003) reported that white flower mutants in chickpea showed an increase in yield as compared to control. Barshile (2006) did not observe any increase in yield with white flower mutations. White flower mutants were reported by Diana et al. (2010) and Manjaya and Nandawar (2007) in Glycine max by the use of 150 to 200 Gy dose of gamma radiation. Flower colour mutation can be exploited as genetic markers in breeding experiments (Datta and Sengupta 2002; Atta et al. 2003). Patil (2009) and Borkar and More (2010) has recorded different flower colour mutations in Phaseolus vulgaris by the treatment of EMS and irradiated with gamma radiation.

In soybean the mutants with off-colour flower (Ahire et al. 2005; Manjaya 2009) and pink flower (Smutkupt 1996; Ahire et al. 2005; Manjaya and Nandawar 2007; Barshile and Apparao 2009; Manjaya 2009; Tambe and Apparao 2009) were obtained in the M2 population. Different flower colours in soybean were used in germplasm characterization, evolution studies, and cultivar identification (Buzze1 et al. 1977). Pigmentation in the flowers is controlled by a number of Mendelian loci in the anthocyanin pathway. Many of these loci act pleiotropically (Fasoula et al. 1995). According to Datta and Banerji (1995), chromosomal aberrations, changes in chromosome number, gene mutations, rearrangement of different histogenic layers and changes in biochemical pathways leading to pigment formation, may be prime cause for flower colour mutations. Flower color mutation appeared or produced due to qualitative and quantitative changes in pigments during pigment biosynthetic pathways due to γ radiation (Datta 1994).

In the present investigation pod colour, pod size, pod with dense pubescence and pod shape mutants were obtained in the M2 generation. Pod mutants were obtained from γ rays treatments in Cicer arietinum (Barshile 2006; Barshile and Apparao 2009), Vigna unguiculata (Kumar et al. 2007; Aheire et al. 2008), Glycine max (Ahire 2008) and Vigna radiata (Anand Kumar 2009) and Cynomposis tetragonoloba (More and Shinde 2010).

Pods of the Lhb mutant showed dense hairs on its surface. Pubescence density can be an important factor in controlling Soybean mosaic virus (SMV) infection because increased pubescence density can bring a delay in time of infection by acting as a mechanical barrier to aphid probing (Parker et al. 2004). Bhasale and Hallale (2011) have recorded lobed and hairy pod mutation in Vigna mungo (L.) Hepper. Lobed pod mutations contained fewer seeds per pod, this trait may associated with partial sterility, causing constriction at the point where there was undeveloped seeds. Diouf et al. (2010) reported 8 mutants in Sesame. Two mutants from a “Birkani” background had dense hairs both on capsules and stems whereas 6 from -32-15’ and 38-1-7’ had the highest frequency (5.9 × 10^-3) while the lowest frequency was observed in 32-15’ (1.6 × 10^-3). Hairiness may be related to drought tolerance. Goyal and Khan (2010) has recorded pod mutations in Vigna unguiculata. Pod mutations e.g. small, roundish, gigas and narrow elongated has reported by Barshile and Apparao (2009) in Cicer arietinum by irradiating the seeds with gamma radiation and EMS. Manjaya (2009) and Tambe and Apparao (2009) in MACS-450 cultivar of the use of the 250 Gy gamma rays, and 10, 20, 30 and 40 kR dose of γ radiation. Kumar et al. (2009) have reported non-hairy mutant and short pod mutant in Vigna mungo cv. ‘Pant URD-19’. A non-hairy mutant line was isolated from the combination treatment of EMS (2%) and γ rays (30 kR). Short pod mutant was isolated from 10 kR.

Pundir and Reddy (1989) obtained a glabrous mutant from ethyl methanesulphonate (EMS)-treated chickpea seeds. The characteristic trait of this mutant is glabrousness (Pundir et al. 1968). This mutant can be useful in certain pathological studies. Glabrous plants are more damaged by insects; on the other hand there is a cost for the plant to develop these structures (Karkkainen and Agren 2002). Production of trichomes is a variable character and the inheritance acts in a simple Mendelian fashion, where glabrousness is recessive to trichome production (Karkkainen et al. 2004). From Anna Engstrom (2006) studied molecular and morphological analysis of genetic polymorphisms causing glabrousness in wild populations of Arabidopsis thaliana. Trichome mutants were first used as convenient genetic markers (Marks 1997). The glabrous1 (gll) mutant, which lacks trichomes on most surfaces, was used in early gene mapping studies (McKelvie 1965). Trichome mutants were used to calculate mutation frequencies generated using several different treatments (Koonsmore et al. 1986). Uhrig and Hulskamp (1994) used the Arabidopsis mutants to define steps in a pathway to trichome development. Trichomes in Arabidopsis are single-celled hairs that develop from epidermal cells regularly distributed on leaves, sepals and stems. Trichome formation in Arabidopsis is emerging as a genetic and molecular model system for the analysis of the spatial regulation of cell type specification in plants (Uhrig and Hulskamp 2010). Interaction between mutant alleles of two genes gl3 and sst which causes trichome development. The mutation in the gl3-sst protein modifies its ability to form a complex with the GL1 protein (a MYB transcription factor required for trichome formation), leading to changes in gene expression compared with wild type during gl3-sst mutant trichome development (Mark et al. 2007; Uhrig and Hulskamp 2010).

In the present investigation a large number of seed coat colour (brown, dark green, yellowish green and black) seed size (small, bold) seed shape (round, wrinkled and elongated) mutants were obtained from different mutagenic treatments in the M2 generation. The various seed-coat colour and seed size mutants by using γ rays have been reported by The various seed-coat colour mutants by using gamma rays have been reported by Auto and Apparao (2009) in Vigna radiata, Kharkwal (2000) and Bashile and Apparao (2009) in Vigna arietinum (2000) and Mahamune and Kothekar (2010) in Phaseolus vulgaris and Kumar et al. (2007) in Vigna unguiculata, Manjaya (2009) and Tambe and Apparao (2009) in Glycine max and Satpute and Suradkar (2011) in Arachis hypogaea.

Bold and small seeded mutants in soybean were induced by γ ray and EMS treatments individually (Karthika and Lakshmi 2006). Increased seed size is generally attributed to an increase in cell number (Koonsmore et al. 1986) and to an increase in cell size (Koonsmore et al. 1986). Production of trichomes is a variable character and the inheritance acts in a simple Mendelian fashion, where glabrousness is recessive to trichome production (Karkkainen et al. 2004). "VLS-2’’ by irradiation with 250 Gy γ rays. Maha-mune and Kothekar (2010) has recorded seed coat colour mutant in Phaseolus vulgaris L. by the treatment of 0.05, 0.10 and 0.15% concentrations of EMS. Highest mutation frequency was induced by 0.10% EMS. Diouf et al. (2010) recorded seed coat colour mutations in sesame (Sesamum indicum L.) - the seed coat color of all these mutants was also different from that of the source cultivars of sesame cv. ‘Birkani’. Eight of them had brown seed coat while the source parents
were whitish. The other one had whitish seed coat while the parent source (‘Birkan’) seed coat was yellowish light brown (data not shown). This trait is related with day length sensitivity and ‘Birkan’ is an insensitive genetic background in comparison to the African cultivars of this study, which are very sensitive to photoperiod and causes no capsule set in the first half of the plant. Consequently, it should be considered a useful drought escape mechanism. Satpute and Sundarar (2011) have reported different colour in TAG-24 variety of Arachis hypogaea by irradiating seeds with different doses of γ radiation. The frequency of plants carrying different seed coat colour was highest in 15 kR dose of γ-rays treated seeds. Seeds are sometimes big or small, long, round or oval, colour can also vary, some are yellow, others are green but can also be brown and some are even black, buff or with spots. Although some of these differences are determined by growing environments, others are genetic and stable and therefore can be used for cultivar identification (Liu 1997). According to Moh (1972) seed coat colour in beans is controlled by genetic factors especially modifying factors which do not produce colours, but influence the colour produced by other factors.

Chandlee and Vodkin (1998) reported the r-m (ring-molted) allele of soybean that produces a mottled or variegated seed coat colour such as spots and/or concentric rings of black pigmentation superimposed on an otherwise brown seed coat. The r-m allele is the mutable allele reported in soybean contains an autonomous transposable element. There are 2 other mutable alleles reported in soybean are the Y18-m allele and the wp-m allele (Xu and Palmer 2005). The mutable line with the Y18-m allele displays variegated green/yellow leaves and the mutable line with the wp-m allele produces variegated flower color.

The seed coat is a maternally derived tissue and should reflect the genotype of the parent plant. Todd and Vodkin (1996) reported that the dominant l gene inhibits accumulation of anthocyanin pigments in the epidermal layer of soybean seed coats. It was suspected that a transposon was responsible for the k2 (tan-saddle seed coat) mutant phenotypes (Palmer et al. 1989; Xu and Palmer 2005).

In the present investigation the 50 kR dose of gammy rays treated seeds. Seeds are sometimes big or small, long, round or oval, colour can also vary, some are yellow, others are green but can also be brown and some are even black, buff or with spots. Although some of these differences are determined by growing environments, others are genetic and stable and therefore can be used for cultivar identification (Liu 1997). According to Moh (1972) seed coat colour in beans is controlled by genetic factors especially modifying factors which do not produce colours, but influence the colour produced by other factors.

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Hilum color as well as other physical appearances has become important factors in determining the type of food application for a particular soybean cultivar (Liu 1997). However, yellow hilum cultivars are more susceptible to low temperatures, resulting in reduced seed yield and poor seed quality compared with brown hilum cultivars (Takahashi 1997).

In the present investigation the 50 kR dose of gammy rays might be affecting the genes, which are causing changes in metabolism of mutant characters or causes gene mutations or various chromosomal aberrations may be the reason for semi-dwarf habit, flower mutation and seed mutations in mungbean. Rearrangement of different histogenetic layers and alternations occurring in biochemical pathways leading to pigmentation formation, may be the reason for flower mutations (Datta and Banerji 1995).

**Total protein profile of Lhb mutant**

In the present study the SDS-PAGE analysis of seed proteins of tall mutant, dwarf mutant, high yielding mutant, early maturing mutant and Lhb mutant and their controls showed interesting results. Lhb mutants did differ with other mutants and controls in their protein-banding pattern (Fig. 2). No variation could be observed in the number, position and molecular weight of polypeptides of control and tall mutant, dwarf mutant and high yielding mutant except Lhb and Early maturing mutants. Lhb mutants differ from other mutants and controls in their protein-banding pattern (Fig. 2).

The Lhb mutant had 7 polypeptide bands. These bands differed from those of control in position and molecular weight. Molecular weight of these polypeptides ranged from 94,406 to 11,885 Da. The molecular weight of the nine bands was 39,811, 28,184, 25,119, 22,387, 17,783, 14,125 and 11,885 Da. Lhb mutant was characterized by the presence of dense, narrow and light bands. Band number 7 and 3 in Lhb mutant was narrow and light as compared to 7th band of other mutants. Band number 3 was dark and broad in all the mutants and controls.

Mutation breeding is an important method used for the improvement of crops through the induction of mutations at loci that control economically important traits and/or by eliminating undesirable genes from elite breeding lines (Patil 2009). In common with other plant breeders, the legume breeder’s prime objective has been to develop genotype capable of producing optimal yield of satisfactory quality. Improvement in either single or few polygenically controlled economic traits and quality attributes is not normally achieved by hybridization within shortest possible time. Furthermore, although selection for a economically useful spontaneous mutations still takes place with considerable success, purposeful induction of a specifically desired mutations at specific time and place and in selected genotype can be a much more attractive option (Harten 1998). One of the chief advantages of traditional mutagenesis is that it can give rise to many different mutant alleles with different degree of trait modification. This variation in expression is very useful in many basic studies, such as identification of amino acid residues critical for enzyme activity (Chopra 2005).

Mutation techniques have been successfully used in generating vast genetic variations and breeding thousands of new crop varieties (Harten 1998; Ahloowalia et al. 2004; Shu and Lagoda 2007). Recent advances in genomics have further stimulated great interest in induced mutations for functional genomic studies in plants (Waugh et al. 2006). In recent years, a couple of mutant germplasm panels have been developed for forward and reverse genetic studies (Wu et al. 2005; Jia et al. 2006).

**CONCLUSION**

The Lhb mutant raised through γ radiation induced mutagenesis, in the present investigation, seems to be promising at least for two characters i.e., semi-dwarf habit and presence of thick dense hairs on the pods. The semi-dwarf habit of the plant enables it as lodging resistant. The thick dense hairs on the pod helps in protecting them from insects and caterpillar predators and seems to be promising economical important character. It can be used or incorporated in breeding programmes that are aimed at genetic improvement of mungbean.

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