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Induced Macromutation in Mungbean [*Vigna radiata* (L.) Wilczek]

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Abstract: Mutation breeding has been recognized since the beginning of this century as one of the driving force of evolution, besides selection and evolution. Though mutations are generally recessive in nature and are lethal to the organism, in some cases sudden changes in DNA due to mutagenesis can be harmless. Reversion of a fixed mutation can be eventually possible depending on the presence or the absence of the original DNA. Environmental stress due application of mutagens or tissue culture conditions may activate the transposons which should be the reason of acquired genetic diversity. Development of morphological mutants due to methylation on the restriction sites (epigenetic mutation) may play a more significant role in the evolution. However, these epigenetic mutations have proved to be less stable than normal DNA sequence alterations. Hence conventional methods of mutation breeding remain much acceptable means of crop improvement. Visible macromutants in mungbean are generally the variation in the morphological parameters. The higher doses of physical and chemical mutagenic irradiation in mungbean will provide enough scope to develop a wide range of morphological variation in desirable plant attributes such as multifoliation, variation in leaf lamina, sterility etc. Observation of visible macro mutants such as synchronously maturing and large seeded multifoliate may be progressed in the M2 and M3 generation through directed selection and these stable mutants can be used as donar for restructuring mungbean genotypes. Further analysis of the visible macro mutants can be followed through the use of suitable molecular marker systems such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeat (SSR) and Single Nucleotide Polymorphisms (SNPs). High-density mapping and its application in map-based cloning can be useful technique for isolation of useful genes liked with morphological attributes.

Key words: Induced mutations, γ-irradiation, mungbean,

INTRODUCTION

Mutation breeding, a much heralded short cut breeding technique, mainly based on conventional breeding approach, brings novel and high yielding genotypes through heritable changes in genotype and phenotype of a particular trait. Induced mutagenesis has been employed to create desired genetic variability, the base of crop improvement. Genetic variation among genotypes and relationships between major yield contributing traits/characters is of vital importance to breeding programmes that aim to produce important cultivars in crops like mungbean. Mutation induction with radiation was most frequently used method to develop direct mutant varieties, as improvement by acclimatization, selection and hybridization have proven to be time consuming, laborious with limited genetic variation (Elliot, 1958; Yaqoob and Rashid, 2001). Thus mutation breeding has been recognized since the beginning of this century as one of the driving force of evolution, besides selection and evolution.

The heritable change occurs through modifications in the DNA of an organism. They are random, can occur in any gene and are recurrent in nature, which mean they can occur again and again. Most induced mutation does show pleiotropy, which may be due to heritable changes in closely linked genes. Hence it is important to use large populations preferably more than 10,000 for the selection of best possible mutants. Random modification in DNA will certainly change the function of gene or the number of genes resulting significant heritable changes. Though mutations are generally recessive in nature and are harmful/lethal to the organism, mutations can be dominant as well. Hence the effects are normally perceived until the second generation of the treatment.

However, some of the effects of mutations can be beneficial as well. However, out of all possible mutations, the useless mutations far outnumber than those having significant and beneficial effects. In some cases, useless changes in the DNA may have a significant outcome as well. However, genome of most living beings especially plants has the self-repairing phenomenon, which actually

corrects these harmful mutations during the process of DNA replication. Detection of damage in the DNA template or incorrect base paring or copying are few reasons of unwanted mutations and can be detected through this mechanism.

Reversion of a fixed mutation can be eventually true depending on the presence or the absence of the original DNA. Usually mutations revert back to original sequences are rare however, depending on large population size, it can be possible through the isolation of reverse mutation possible. Abiotic factors such as stress may be a factor leading to reversion of mutations (Weigel and Jurgens, 2005). Environmental stress due application of mutagens or tissue culture conditions may activate the transposons which should be the reason of acquired genetic diversity (Zhang *et al*., 2002). These transposable elements have the capability of modifying major gene functions, variation in gene size and major changes in gene and/or genome structure. Retrotransposons are well established as highly prevalent components of plant genomes in general and are also called as 'jumping genes' as they increase their number by transposition within the genome. Retrotransposons are thought to be analogous with retroviruses and believed to have common origin and are very often also referred to as parasitic or selfish DNA (Bennetzen, 2000; Vicient *et al*., 2001). Discovery of these transposable elements (jumping genes) in maize by Barbara McClintock, have named these as controlling elements having a major role in the evolution and bringing genetic diversity.

The phenomenon of reverse mutation has been previously reported in hothead plants carrying the mutant gene *Hothead*, which undergoes a process of reversion of the genome sequences in the DNA. The reversion has been found in the F3 generation, where the genome sequence of the DNA has been changed to exactly what present in the original grandparent, but not same with the immediate parent. This may be due to genome wide mutations resulting from extrachromosomal inheritance or non-mendelian inheritance of the DNA sequence (Lolle *et al*., 2005). However the exact mechanism of reversion stills unknown and mystery (Weigel and Jurgens, 2005).

Contradictions have also arised from differences in generation of morphological mutants in the laboratory and those related to mutants in natural population, mainly because of the environmental variation and different breeding strategies leading to problems in limitation in natural selection (Cubas *et al*., 1999). Studies also indicated that development of morphological mutants due to methylation on the restriction sites (epigenetic mutation) may also play a more significant role in the evolution. Such kind of epigenetic mutations have been first observed in naturally occurring mutants of *Linaria vulgaris*, where the mutant actually carries a defect in *Lcyc*, a homologue of the cycloidea gene, which actually controls the dorsoventral asymmetry in *Antirrhinum* and has been extensively methylated. Interestingly the authors have pointed out demethylation as one of reason for reversion of mutations and hence restoration of gene expression. However, these epigenetic mutations have proved to be less stable than normal DNA sequence alterations. Hence conventional methods of mutation breeding remain much acceptable means of crop improvement. As such the basic advantage of traditional method of mutagenesis is that it can give rise to various modification in the traits and also can give rise to different mutant alleles, which should help in an unbiased natural selection (Chopra, 2005).

CHOICE OF THE MUTAGEN DOSE

Today the plant breeder has at hand a number of effective physical mutagen. The choice of the mutagen is not nearly related to its effectiveness in terms of frequency of desired mutations, but to the kind of material to be treated and to the availability of a mutagen. The frequency and the spectrum of mutations differ somewhat depending on the mutagen used and the dose applied. The physical mutagens, X-rays and gamma rays (sparsely ionizing radiations) are widely used. They have the advantage of good penetration and precise dosimetry. Whilst, Ultraviolet Light (UV) has low penetration power and effectively used with materials such as pollen or *in vitro* cultured cells in a thin layer. Chemical mutagens are known to produce a higher rate of gene mutations generally preferred. However, chemical mutagens present particular problems such as uncertain penetration to the relevant target cells, poor reproducibility and persistence of the mutagen or its metabolites in the treated material and finally the risk of safe handling (Singh *et al*., 1997).

Mutagenic efficiency with gamma rays can be increased through irradiating seeds at extremely low temperature. Moreover many findings proved that the mutation frequency obtained by the various ways can be positively influenced by specific kinds of pre or post treatment. With regard to the mutagenic efficiency, these chemicals are comparable to the physical mutagens used, or better. The efficiency of all the mutagens mentioned can be improved with the help of combined treatments. Whilst, treated plants can be followed by the treatment with other mutagen. It has been established that the radiations as well as chemical mutagens may provide opportunities in increasing genetic variability of the quantitatively inherited characters (Sharma *et al*., 1991).

Physical mutagens namely X-rays, gamma rays, fast neutrons, thermal neutrons, ultraviolet and beta radiations have been frequently used for induced mutagenesis (Elliot, 1958). Except ultraviolet rays, all radiation types were found to ionise atoms in a tissue by detaching electrons from the atoms (Anonymous, 1977). Apart from physical mutagens, several chemical mutagens were also frequently used for induced mutagenesis in crops, respectively, Ethyl Methane Sulphonate (EMS), Ethylene Amine (EA), Methyl Nitroso Urea (MNU), N-nitroso-N-methyl urea (NMU), Ethyl Nitroso Urea (ENU) and Methyl Nitroso Urea (MNU). Approaches related with combined effects of mutagen were also point of interest. Interms of the preference of the mutagens, EMS has been the potent options for studies related with chlorophyll mutations. However, some mutagens Hydrazin Hydrate (HZ) may have a mutational repairing process leading to a significant drop in the mutation through combined treatment.

Frequency of chromosome aberrations and chlorophyll mutations registered a significant drop when there is considerable evidence that mutations are induced in polygenic traits and that there is a genetic gain under selection. In the M2 generation, macro mutations may be observed particularly following radiation treatment.

INDUCED MUTAGENESIS

There is considerable evidence that mutations are induced in polygenic traits and that there is a genetic gain under selection. Irrespective of mutagens used, seed treated plants usually come out the recessive mutants in the second (M2) or third (M3) generation after the treatment (Ahloowalia and Maluszynski, 2001). In the M_2 generation, macromutations may be observed particularly following radiation treatment. The macromutants are usually undesirable due to accompanying genetic instability. Micromutations that alter quantitatively inherited characters are more useful to the breeders since they are least deleterious although they are more difficult to detect. The micromutations increase variability in yield, protein content, plant height, flowering, pod production, seed weight, or other yield related traits that are quantitatively inherited. In case of vegetative propagation, mutagen treatment produces *Chimera*, which is basically the mixture of one or more genotypes and hence needs to be dissolved. These chimeras are unstable in clonal crops hence several are needed to extract true morphological mutants (Ahloowalia and Maluszynski, 2001). Through mutation breeding attempts may be made to broaden the variation spectrum to facilitate selection of lines with improved nutritional qualities, especially with respect to protein associated with high yield.

PAST ACHIEVEMENTS

Previous workers have reported significant changes in the desirable characters in crop plant by using gamma rays as a physical mutagen, which has been used to develop 64% of the radiation-induced mutant varieties followed by X-rays (22%) (Ahloowalia *et al*., 2004). Isolation of mutants of agronomic and economic significance was a major goal of mutation breeding. Consequently, the high yielding varieties rice (RD6, RD15, PNR-102, PNR-381, Kashmir Bashmati, NIAB-IRRI-9) were derived from mutation, while several dwarf mutants were also induced following chemical and physical mutagenesis. Similarly in wheat, a light coloured grain mutant 'Sarbati Sonora' was obtained from 'Sonora 64' whilst a similar light grain colour mutant 'Pusa Lerma' was derived from another popular line. 'Lerma Rojo 64A'. 'Pusa Lerma' with its high resistance to stem rust and semi hard white grains were also released to stem rust and semi-hard white grains was released for cultivation in peninsular India. Among the other mutants, adult plant resistance was also found in these mutants for yellow rust, black rust and brown rust was highlighting, proving the ability to ward off rust infection. Other wheat mutants are 'Jauwar 78', 'Soghat 90', 'Kiran 95', respectively. Barley (diamant, golden promise), chickpea (CM-88, CM-98), mungbean (NIAB Mung 92, NIAB Mung 98, NIAB-M51 and NIAB-M54), Cotton (NIAB-78, NIAB-999, NIAB-111, NIAB-86, NIAB-Karishma, AC-134), have been developed through γ-irradiation, which act as an effective alternate method to conventional breeding (Ahloowalia *et al*., 2004; Malik, 1991; Siddique *et al*., 1999). Exhaustive research at National Institute of Agriculture and Biology (http://www.niab.org.pk/) has resulted high yielding cultivars developed through mutation breeding. A Jassid-resistant line was also isolated through mutation breeding of the highly susceptible variety 'Mescilla Acala'.

MUTATION BREEDING IN MUNGBEAN

Among the pulses, mungbean has been favoured by children and elders due to its easy digestibility, rich source of protein and low flatulence problem. However, it is expected to be used as an additional source of protein to replenish the deficiency of cereals, not to compete with it (Khattak *et al*., 2002). It is widely grown in Asian countries and is also being widely cultivated in Australia with major export goes to European and North American markets (Miyagi *et al*., 2004). Average protein content in the seed is around 24%. The protein is comparatively rich in lysine, the amino acid predominantly deficient in cereal grains. Mungbean protein is deficient in methionine, cystine and cysteine, sulphur bearing amino acids and

cereal grains compensates for the deficiencies in protein quality in either grain alone and also provides a balances amino acid diet.

Attempts have been made to develop improved varieties through selection and hybridization, but the yield potentiality of mungbean remains static due to shortage of germplasms. Having characteristically yield potentiality within existing germplasms, plant breeders for a long time had tried to increase the variation spectrum through induction of mutation for isolation of high yielding lines with reduced shattering habit and comparatively resistant to various diseases, which come into the way in developing of high yielding variety. Mungbean varieties MUM1, MUM2 and MUM3, highly tolerant to Mungbean yellow mosaic bigeminivirus (MYMV) and were synchronous in maturity were also developed through γ-irradiation and chemical mutagens like Ethyl Methane Sulphonate (Gupta *et al*., 1996). Fluctuation in the germination%, plant height, number of grains per plant, grain yield in the wheat were observed through γ-irradiation (Jamil and Khan, 2002). Linear reduction in plant height with increase in gamma ray dosage has also been obtained in the Mungbean variety Pusa Baisakhi treated with 10, 20, 30, 40 Gy gamma rays (Pande and Raghuvanshi, 1988; Sarkar *et al*., 1996). Mutants of dwarf habit in advanced generation of gamma ray mutagen treated populations in Mungbean was noticed by Tickoo (1987). But Khan *et al.* (1995), Singh *et al*. (1979) observed an increase in the mean values of plant height in M2 generation. Similarly, an induced dwarf mutant of grasspea was observed by inducing polyploidy with Colchicine treatment of grasspea (Talukdar *et al*., 2001).

Both macro and micromutants in mungbean have been isolated previously, however this review will only be restricted within the visible macromutants/morphological mutants in mungbean and finer details about future prospects of them.

MORPHOLOGICAL MUTANTS

Visible macromutants are generally the variation in the morphological parameters. Morphological parameters are mainly the variation in lamina shapes of leaves and leaflets with respect to lobes and serration on lamina surface. Leaf multifoliation is also a type of morphological character and a visible macromutation, usually observed in the M2 generation. Among the other visible leaf mutation, orientation of the leaf can be another important morphological character selected for mutation studies because leaf orientation is the genotypic feature showing variations among genotype with respect to photosynthetic efficiency. This can be done in the M2 generation by taking the measurement of leaf angle of a standard leaf with respect to the main stem. Similarly, petiole length can be another morphological character selected for the mutation studies. Visible macromutant like synchronous maturity (all the pods mature at a time and has a significant difference from the normal control lines) may provide agronomically desirable lines.

Among the other morphological mutants common in the M2 generation are tall, erectoid, unifoliate, dwarf, bushy, trailing, clustered pod sterility etc. The inheritance of these morphological mutants in the M3 generations indicated that these are monogenic recessive in nature (Saini and Mahna, 1989). Tall mutant with high leaf areas and yields were selected in the M2 and M3 generations and supposed to have a substantial effect on the seed yield with greater leaf area (Chow *et al*., 1987). Some of the other qualitative traits included leaf mutants (dark green, waxy, multiple and lobed), pod mutants (large and top podding) and semi-dwarf plants (Srinives *et al*., 1999). Visible morphological dwarf mutants can be generated by inducing polyploidy with chemical mutagens like Colchicine. Trait dwarfism can be obtained in the M2 generation and generally exhibit a slower growth rate and reduced plant height from early seedling stage until maturity. Internodes of a dwarf mutant can be winged and the main axis can be perpendicular to the soil. The orientation of early-formed branches may also be found very close and inclined to the main axis compared to the control. Dwarf mutants can be late in initiation and days to 50% flowering, however it can mature earlier compared to the control. The total number of branches, flowering nodes, pods per plant and seed yield per plant decreased significantly, but the number of seeds per pod, pod size, 100-seed weight and harvest index decreased marginally (Talukdar *et al*., 2001).

VARIATION IN LEAF CHARACTER

Trifoliate leaf with three leaflets in each leaf is the usual condition in mungbean. Though mungbean is trifoliate in nature, increase in multi-foliation will certainly increase the biomass production (as well for spreading trailing mutant type), which could make a positive impact on seed yield, if the translocation activity in the genotypes were increased by genetic manipulation (Bhagat and Chakravarty, 1989). Multifoliation is one of the visible macomutants found in Mungbean. Though not all, but some leaf mutants appeared to be useful for breeding as it will increase the net photosynthetic area by increasing the concentration of sink to the developing seeds with an increase in transfer of assimilates to the grain responsible for a positive effect in seed yield (Khan, 1987; Quintero *et al*., 1990; Saini and Mahna, 1989).

Changes in the net photosynthetic rate, leaf area development has also been found to be correlated with chlorophyll a/b ratio of the leaves (Babu *et al*., 1993). Previous research by Chhabra and Singh (1988) have concluded that the number the leaflets in the pentafoliate mutant is controlled by a single recessive gene, designated as *'ll'*. Similarly, Satyanarayana *et al.* (1989) identified a mutant in the M_2 generation of *Vigna radiata* cv. Pant mung-2 through 40 Gy gamma rays having each leaflet of its trifoliate leaf substituted by a trifoliate leaf, giving 9 or more leaflets per leaf. The mutation established as a true breeding line was found to be controlled by a single recessive gene designated *mf1*. Treatment with physical mutagens supposed to increase the contents of flavonoids and anthocyanins along with high Phenylalanine Ammonia - Lyase (PAL) activity in mungbean leaves. Prior work in this respect by Pal *et al.* (1999) had concluded that though leaf soluble proteins get decreased, total free amino acids were found to be higher in physical mutagen exposed plants. Changes in the net photosynthetic rate, leaf area development has also been found to be correlated with chlorophyll a/b ratio of the leaves (Babu *et al*., 1993).

Singh and Singh (1995), isolated a small leaf mutant of mungbean cv. Pant Moong-3, having broad ovate trifoliate leaves. They also studied the inheritance of leaf type in the F1, F2 and F3 generations of a cross between the mutant plants, (female) and long Pant moong-3 plants. The F1 hybrids having normal leaves, suggesting large leaf size is dominant over the small leaf mutation.

Isolation of leaf mutants in mungbean is also possible through combined seen treatment with gamma rays and EMS. Significant increase in dry matter production and increase in chlorophyll content take place in contrast to parents in the segregating (M2 and M3) generations. These leaf mutants can be hybridised in the succeeding generation through single plant selection to judge the inheritance of the multifoliation (Vasanthi, 2003). Prior results of large seeded multifoliate *Vigna radiata* mutant PAEC-2 has resulted with 4 recommended varieties successfully (Grafia *et al*., 1987).

VARIATION IN LEAF LAMINA

Variation in leaflets/leaf has also resulted variation in lamina shape of leaves. Variation in lamina shape was also very much found prevalent in the leaves of mungbean. Most of the variation in the lamina shape was observed in the trifoliate leaves of higher doses of gamma ray irradiation, which turned into tetrafoliate or pentafoliate with much variation in shape and size and lamina like crumpled, lanceolate, ovate, lobed, were observed in both the varieties. Previous study carried by (Ramamoorthi *et al*., 1994) concluded that trifoliate, lobed leaf character controlled by a single dominant gene. Recessive morphological mutations like narrow leaf trait, controlled by two recessive genes, designated as nl_1 and nl_2. Whilst, Narrow lanceolate leaves, is also one of recessive mutation, which was observed by Dwivedi and Singh (1985) in the M_2 generation from 10 Gy treatment of gamma ray irradiation to the mungbean variety T-44.

Reduction in leaf size was also found common in most leaf mutants, due to small leaf mutation and is monogenic recessive in nature (Singh and Singh, 1995). This means large leaf size in mungbean, is dominant over small-leaf mutation. Recessive morphological mutations like narrow leaf trait, controlled by two recessive genes, designated as nl_1 and nl_2. Whilst, Narrow lanceolate leaves, is also one of recessive mutation, which was observed by Dwivedi and Singh (1985), in the M_2 generation from 10 Gy treatment of gamma ray irradiation to the mungbean variety T-44.

VARIATION DUE TO CHLOROPHYLL MUTATION

The spectrum and frequency of chlorophyll and morphological mutations are usually studied in the M2 and succeeding generations. Chlorophyll mutations in mungbean consisted of albina, chlorina, viriscence, viridis and xantha, alboviridis, viriscens, albescens and maculata are controlled by recessive gene (Santos and Bahl and Gupta). Usually the frequency and spectrum of chlorophyll mutations as well as mutagenic efficiency and effectiveness was the highest at lower doeses. Chemical mutagens are usually preffered for chlorophyll mutations in terms of efficiency and effectiveness with respect to the biological damage and high frequency of chlorophyll mutations (Mehrajuddin *et al*., 1999). Using sodium azide and hydrazine hydrate chlorophyll mutations were obtained in mungbean and found that xantha type was predominant in both the mutagenic treatments (Mehrajuddin *et al*., 1999). HZ was the most effective and effective mutagen with respect to the biological damage and high frequency of chlorophyll mutation. Whilst, Khan and Siddiqui (1993) reported chlorophyll mutants' viz., albina, chlorina and viridis in mungbean by using EMS, Methyl Methane Sulphonate (MMS) and SA, mutations induced in two mungbean (*Vigna radiato* (L.) Wilczek) varieties viz., PS-16 and 'Pusa Baisakhi'. Interms of effectiveness, EMS produced highest frequency of mutations followed by MMS and SA. All the three mutagens were found to be effective and efficient at the lower mutagen concentrations. Hence frequency of mutagenic doses does an influence on chrolophyll mutation.

Chemical mutagen like Colchicine, also behave as a stress factor and may lead to historical modifications in chlorophyll and carotenoid content of leaf segments. However, variation in chlorophyll and carotenoid may not affect the growth of the mutant as it may be due to the level of chlorophyll being at threshold-point or due to the increased efficiency of chlorophyll (Hossain and Hossain, 2003).

This can be also a visible macromutant in the segregating generation and may lead to significant changes in the protein content, changes in the isozyme pattern and higher peroxidase activity in the shoots. These biochemical changes in the plant results in induction of oxidative cell damage and inhibition of essential biosynthetic processes may lead to the visible macromutation in the segregating genetation. However, physical mutagens like gamma rays also can be effective for chlorophyll mutation studies (Sharma and Haque, 1997).

REDUCTION IN POLLEN FERTILITY

Reduction in pollen fertility can be one of the major effects due to the application of mutagenic treatments (chemical and physical). This can lead to visible sterile macromutants. The reduction in fertility due to different treatments might be due to the disturbances at the meiotic level as a result of irradiation in the M2 generation. However, this may not be true in M1 generation, which may provide comparatively high level of pollen fertility. Hence, selection of plants showing higher pollen fertility and simultaneous rejection of plants at lower ranges of pollen fertility could help to overcome the depression of the fertility. The frequency of pollen sterility may vary from 6-95% in the M5 generation from gamma ray treated seeds of mungbean. Observation of male sterility in the M2 generation through higher treatment doses (40 kR treatment) with narrow leaves and compact habit has been observed from gamma ray irradiated seeds of mungbean cv. T-44 (T1xT49) (Yadav and Singh, 1987). However, in some occasion, reduction in pollen fertility can also be obtained in the M_1 generation of mungbean var. 'Pusa Baisakhi' treated gamma rays (Sarkar *et al*., 1996).

Reduction in pollen fertility also possible in the variegated mutants of mungbean derived through X- ray and neutron-irradiated seeds. Observation of pollen fertility also can be possible in dormant seeds of mungbean cultivar treated with 5-100 kR gamma rays. Whilst, Singh and Chaturvedi (1993) treated seeds of mungbean cv. PS-16 and cv. Sona with 3 concentrations (0.2, 0.3 and 0.4%) of the mutagenic chemicals, EMS and HA. They isolated some plants having low plant fertility and found a linear relationship between doses of mutagen and the trait. Observation of reduction in the pollen fertility in the M1 generation as compared to control by treating seeds of *Vigna radiata* variety PS-16 with 0.02, 0.04 and 0.06% Diethyl Sulphate (DES) (Khan *et al*., 1995. Marked decrease in pollen fertility may also be possible with increasing correlation between chromosomal abnormality and pollen sterility (r = 0.82-0.98). Pollen sterility in the M1 and M2 generation has also been found different among the species of mungbean (highest in *Vigna radiata* and lowest in *Vigna sublobata)*.

VARIATION IN POD MATURITY

Synchronously maturity can also be considered as one of the visible macromutant and can be used for practical used in development of important variants. Synchronous maturity signifies the simultaneous ripening of pods in mungbean (Yadav and Singh, 1988). An induced synchronized mutant in mungbean is earlier in maturity (by at least a week), erect and gives a significant increase (at least 40%) in the seed yield (Saikh *et al*., 1988). Previous work on inheritance of early flowering, early pod maturity and synchrony in pod maturity has been studied by using a diallel crossing so as to pyramid the difference factors responsible for pod maturity.

Both additive and dominant gene effects are responsible controlling the synchrony in pod maturity though, the effect of additive component is predominant and decides the heritability (narrow and broad sense) of the trait than the dominance (Khattak *et al*., 2001). Combined treatment with chemical mutagens can also be effective in obtaining synchronous mutants in mungbean. Chaturvedi and Singh (1980) obtained some synchronously maturing mutants in the M2 generation from treated seeds of pusa baisakhi of mungbean with different concentration of aqueous solutions of EMS and NMU where treatment with 0.1% EMS failed to produce such mutant. Tickoo (1987) also observed mutation for synchronous maturity by exposing mungbean cv. Pusa baisakhi with gamma rays. Yadav and Singh (1988) obtained a synchronous mutant in M2 generation from 40 kR treatment gamma ray with seeds of mungbean variety PS-16.

VARIATION IN SEED SIZE, SEED COAT, POD COLOUR

Induced variation for the seed coat colour and seed size was also can be observed in some plants in the M_2 generation. The control plants of mungbean produce seeds of dull green colour but some plants of gamma ray treated can produce dark greenish with greenish black seeds. Dahiya (1973) has observed seed colour as a result of mutation in mungbean following gamma ray irradiation.

Variation in pod size and shape can be observed in mungbean. Variation in pods of different shapes and sizes showing constricted appearance or curvature with loops also among the frequently observed visible macromutant. This is one of the most visibly observed variations in the M2 population.

Similar variations in the mature pod colour have also raised the possibility that pod colour could be used as a genetic marker in breeding programmes (Biswas and Bhadra, 1997). Mature pod colour such as black, blackish straw or straw) could also be used as a genetic marker in breeding programmes (Biswas and Bhadra, 1997). Small seeded mutants needs to be selected because of its resistance to mungbean yellow mosaic germini virus (MYMV) and also found to be partly dominant over large seededness and is controlled by 7 gene pairs (Malik *et al*., 1987).

GRAFT INDUCED GENETIC VARIATION

Grafting of mungbean also can induce inheritable variations and the phenomenon of inducing genetic variation from the scion after grafting is termed as graft-induced genetic variation (Zhang *et al*., 2002). Grafting of plants is not new and has been reported since 1960s such as work on male sterile Petunia

Seedling of mungbean has been grafted into the stem of sweet potato (*Ipomoea batatas* (L.) Lam.), resulted in inheritable variation induced by the graft, as per cytoplasmic and genomic DNA analysis. Molecular markers such as Random Amplified Polymorphic DNA (RAPD) have been used for the analysis of variation, resulting polymorphisms and differences in the cytological DNA differences. However, no such results have been found by using Restricted Fragment Length Polymorphism (RFLP) in the cytoplasmic DNA between the original scion and the graft.

Hence induced mutation related with stress can be a factor leading to genetic variation other than transformation of DNA. Further grafting of mungbean can be used for further improvement of this crop. Previous works proved the inheritance of male sterility from stocks of male sterile Petunia to the progenies of the scion. Further, studies have been done on red pepper, egg-plant and Soybean respectively. Minor changes in the morphology of the seeds can be obtained due to graft induced genetic variation. The appearance of the seeds has resulted in irregular in shapes with deep (brown) in colour.

Induced variation due to grafting can be criteria for improvement in mungbean, which may not be possible through gene transformation and variation may not be possible because of inability of greater distance mobility of DNA.

FUTURE PROSPECTS

The higher doses of physical and chemical mutagenic irradiation in mungbean will provide enough scope to develop a wide range of variation in desirable plant attributes which may facilitate to select high yielding mutants with other desirable characteristics like early maturity, shore stature etc. Observation of visible macro mutants such as synchronously maturing and large seeded multifoliate may be progressed in the M2 and M3 generation through directed selection and these stable mutants can be used as donar for restructuring mungbean genotypes. Among the morphological traits in mungbean, growth habit and shape are important, however not much work has been done, though these can be acceptable as genetic markers.

Further analysis of the visible macro mutants can be followed through the use of suitable molecular marker systems, which can detect DNA differences between the mutants and normal plants. Some of the most common molecular techniques are southern blot technique, Restriction Fragment Length Polymorphism (RFLP), basic PCR methods such as Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeat (SSR), (Rafalski and Tingey, 1993). However, with increasing sequence information of different plant genomes, STSs and SNPs have become latest trend of marker research and can be useful in further analysis of these visible macromutants particularly any useful gene and more specifically, marker-assisted selection (Pal *et al*., 2002). Further the problem of analysis with RFLPs due to lack of reproducibility may be overcome by the development of Sequence Characterized Amplified Regions (SCARs) or Sequence Tagged Sites (STS) from RFLP markers, leading to higher throughput and less requirement of genomic DNA (Langridge *et al*., 2001)

Major revolution in marker-assisted selection (MAS) for visible macromutants will be by using the smallest unit of variation, the Single Nucleotide Polymorphisms (SNPs), abundant in the genome and form the basis of the markers. Though SNP markers hard to detect, application of these for further analysis of mutants can be effective (Wittenberg *et al*., 2005). Previous research resulted DNA components of Mungbean Yellow Mosaic Virus (MYMV) analyzed through the above suitable marker systems like RFLPs, based on southern blot and other PCR based methods (Karthikeyan *et al*., 2004). Once molecular markers closely linked to mutants are identified, marker-assisted selection can be performed in early segregating populations and at the early stages of plant development. Marker-assisted selection or identification can be used to pyramid the major genes including resistance genes, with an ultimate goal of producing varieties with more desirable

characters such a synchronous maturity or multifoliate. Thus with MAS, it is now possible for the breeder to conduct many rounds of selection in a year (Mohan *et al*., 1997).

Further, polymorphic markers identified after analysis of mutants should be validated to a different population of mungbean to test their efficiency in determining target phenotype in independent populations and different genetic backgrounds (Cakir *et al*., 2003; Collins *et al*., 2003; Sharp *et al*., 2001). Marker validation will further allow the reliability of markers for predicting a target phenotype to be determined and thus the usefulness of such markers in routine screening for marker assisted selection for these macromutants (Sharp *et al*., 2001).

Further, high-resolution linkage mapping may be created using a larger mungbean population for identification of tightly linked markers for mutants. Isolation of desirable gene of interest can be done through map based cloning approaches. This can be useful as it helps in accuracy in marker-assisted selection for these macromutants and also helps in finding the flanking markers of a specific trait of interest. The permissible distance between tightly linked markers is 1cM or less and helps in increasing the marker density (Mohan *et al*., 1997). Futher bulked segregant analysis using different DNA samples of mutants and normal plants also can be useful in increasing marker density as it helps in identification of more marker to a particular chromosomal segment (Michelmore *et al*., 1991; Xu *et al*., 2000).

One of the applications of high-density mapping is in map-based cloning useful technique for isolation of useful genes from complex traits. Hence, map based cloning of the mutants can be useful. Identification of molecular markers associated with trait of interest through a high-density mapping can be compared through compared with a genomic DNA library. This process is called gene tagging. Hence the linked markers can be screened with an appropriate genomic library like Yeast Artificial Library (YAC), Bacterial Artificial Chromosome (BAC) libraries to extract polymorphic clones (Mohan *et al*., 1997; Xu *et al*., 2000). This process of construction of genomic libraries of tightly linked markers is an essential step in map based cloning methods namely chromosome walking and 'chromosome landing' (Tanksley, 1993). High-resolution mapping is also helpful in finding syntenous regions (and to test the microcolinearity) across different species consisting of homologous regions associated with trait of interest, if not the exact gene of interest through map based cloning approaches. Recently, it has been found that BACs have been a preferred option for construction of mungbean BAC library compared to cosmid and YACs. So far the only mungbean BAC library created for bruchid (*Callosobruchus chinesis*) resistance (Miyagi *et al*., 2004). Further the polymorphic BAC clones can be used for creating PCR based markers specific to a particular locus conferring resistant to any of the visible macro mutants. Molecular map based cloning for Mungbean Yellow Mosaic Bigeminivirus (MYMV) has also been done.

Location and identification of unique genetic changes at the nucleotide level of the morphological mutants can be another future prospect. As such conventional method of mutation may be time consuming, availability of transformation technique such as induction of mutation through artificial insertion of transposons or T-DNA, which should help in the identification of mutant line based on the presence and absence of extra/foreign DNA sequences. Hence based on the additional time of field screening can be avoided and this may also result in further accuracy of tracking the macromutants. Considering the mobility of transposons, in some cases it may lead to the disruption of the mutant allele and hence leading to loss of function of the gene responsible for macromutation.

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