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Genetic Improvement in Different Crop Plants using Induced Mutagenesis with Special Reference to Pulses and Cereals: A Review

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ABSTRACT

Current world scenario of hunger and malnutrition problem is posing a great threat to the human civilization. Millions of people are undernourished globally according to the reports of Food and Agriculture Organization (FAO, 2015). Pulses and cereals have been used for their high percentage of proteins and carbohydrates in seeds. Pulse crops also have the ability to enrich the soil fertility due to their nitrogen fixing ability of their root nodules. Further, it is not only the high protein content of pulses for which they are important, but the amino acid profile of these proteins is such that the mixed diet of cereals and pulses has superior biological value than either of the components alone. However, with increase in population, per capita availability of pulses is getting reduced. As against recommended daily requirement of 50-60 grams, current per capita availability of pulses in India is less than 30 grams per day. India is the largest producer and consumer of pulses and depends mostly on pulses and cereals for their food. Urbanization and drastic climatic changes like drought, global warming, different environmental stresses *etc*. have led to considerable loss of crop productivity throughout the world leading to food insecurity. Scientists all over the world are using sophisticated approaches in developing improved varieties of crop plants that are high yielding and show considerable resistance to drastic environmental changes. Physical and chemical mutagenesis has been proved to be a convenient tool compared to other conventional processes in inducing desirable variability in crop plants related to yield and other components. In the light of above all, it is necessary for a scientist to study in detail the all aspects of different approaches that are used to increase the yield and productivity in different crop plants. The current review covers all aspects of study during the different approaches of physical and chemical mutagenesis for increasing food security and developing high yielding and stress resistant plants.

Key words: Cereals, Environmental stress, Food insecurity, Pulses.

The continuous increase in population, urbanization and climate changes are the major causes of food insecurity in India which ranks 2nd after china in population. The FAO (2013) has reported that mankind is facing worse problems of food insecurity and consistent malnutrition and this might be reduced through interventions in agricultural research policies. Different strategies and work plans are needed to increase the food production. Conventional and modern plant breeding strategies are being adapted to enhance the production levels of crops (WHO, 2005; Kozgar *et al*., 2014), Whereas modern methods of breeding have significantly increased the crop yields over the past 50 years, the future potential of these methods is constrained by the limitations in the natural diversity of trait genotype within the crop species and sexual-compatibility boundaries between crop types (WHO, 2005). In these conditions, the role of induced mutagenesis is a novel as compared to conventional breeding methods such as selection, polyploidy and hybridization, which increases the genetic diversity of various interesting traits without altering the actual genetic makeup (Parry *et al*., 2009; Khan and Kozgar, 2012). Due to their ability to grow on harsh climatic conditions and in different agro-climatic zones, the pulses can prove better crops in overcoming world food insecurity (Wani *et al*., 2011).

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Dose effect and genotypic sensitivity

Different genotypes show different degrees of sensitivities towards mutagens. Study of lethal dose of mutagen is important in mutation breeding experiments. Lethal dose is the dose of mutagen at which 50% treated individuals die. Usually in plant breeding experiments, the over dose kills number of cells and under dose produce few mutants. So, an optimum dose is necessary for production of desirable mutants. Careful experimentation is necessary for finding out the optimum dose of any mutagen (Acquaah, 2007). The optimum dose is the one which kills the minimum

individuals and produce maximum frequency of desirable mutations (Solanki and Waldia, 1997). The genetic differences of different plant materials are responsible for the differential response of genotypes towards mutagens and thus LD_{50} of particular genotype varies towards different mutagens (Goyal and Khan, 2010). With regard to LD_{50}, differences in genotypic sensitivity at inter-varieties level has been reported by Kharakwal (1981 a,b) in *Cicer arietinum*. Different workers have reported different explanations for the differential sensitivity of genotypes towards mutagens. The difference in the recovery process involving enzyme activity has been reported by Akbar *et al.* (1976) in various rice varieties to be the cause of differential sensitivity towards mutagens.

Bio-physiological damages

Bio-physiological damages (seed germination, seedling height, plant survival, pollen fertility *etc*.) caused by mutagens have been used in mutation breeding programmes to assess the potency of mutagen. Many authors have reported the mutagenic effects of different mutagens on different biological parameters like seed germination, seedling height, plant survival at maturity, pollen and seed fertility, cytological abnormalities, estimation of chlorophyll and biochemical contents, leaf aberrations and the activities of certain enzyme assays *etc*. in M_1 generation in *Oryza sativa* (Cheema and Atta, 2003), *Triticale* (Edwin and Reddy, 1993a), *Triticum* spp. (Xiuzher, 1994), *Brassica juncea* (Singh *et al*., 1993), *Capsicum annum* (Dhamayanthi and Reddy, 2000), *Nicotiana tabacum* (Amarnath and Prasad, 1998), *Plantago ovata* (Sareen and Kaul, 1999), *Vigna mungo* (Goyal and Khan, 2010), *Lathyrus sativus* (Kumar and Dubey, 1998), *Lens culinaris* (Khursheed and Khan, 2014; Laskar and Khan, 2014), *Vigna radiata* (Rehman *et al.,* 2000; Khan and Wani, 2005a), *Chenopodium quinoa* (Gomez-Pando and Barra, 2013), *Cicer arietinum* (Laskar *et al*., 2015), *Hordeum vulgare* (Khursheed *et al*., 2014, 2015) and *Vicia faba* (Laskar and Khan, 2014; Khursheed and Khan, 2015). Many workers have reported that the reduction in seedling height, seed germination, pollen and ovule sterility and meiotic abnormalities after mutagen treatments in M_1 generation is an indicator of biological damage (Kodym and Afza, 2003; Al-zahrani *et al*., 2012; Kamble and Patil, 2014), whereas others have described chlorophyll contents (Verma *et al*., 2010), photosynthetic activities (Kim *et al*., 2005; Seung *et al*., 2007; Moussa and Jaleel, 2011), carbohydrate metabolism (Joshi *et al*., 1990; El-Fiki *et al*., 2003) and nitrate reductase activity (Barshile *et al*., 2009) are effective indices to determine the mutagenic action.

Cytological aberrations

Cytological analysis is considered to be one of the most dependable indices to estimate the potency of mutagens and to elucidate the response of various genotypes to a particular mutagen. Although meiotic chromosomal aberrations have been studied in different plants, comparatively less attention has been given to them in faba bean. Cytological abnormalities, induced by single and combined mutagen treatments, have been reported in some cereals (Suganthi and Reddy, 1992), *Triticale* (Edwin and Reddy, 1993b), *Hordeum vulgare* (Kumar and Singh, 2003; Khursheed *et al*., 2014, 2015), *Capsicum annuum* (Dhamayanthi and Reddy, 2000), *Solanum melongena* (Zeerak, 1991), *Nigella sativa* (Kumar and Gupta, 2007), *Cicer arietinum* (Kamble and Patil, 2014), *Vicia faba* (Fatima and Khan, 2009; Laskar and Khan, 2014; Khursheed *et al*., 2015; Khan *et al*., 2015) and *Vigna* spp. (Goyal and Khan, 2009). Many workers have reported an increase in different chromosomal aberrations with increasing doses/ concentrations of both single and combination treatments of physical and chemical mutagens in *Vicia faba* (Bhat *et al*., 2007), *Vigna mungo* (Goyal and Khan, 2010),*Vicia faba* (Al-zahrani *et al*., 2012),*Cicer aietinum* (Kamle and Patil, 2014), *Hordeum vulgare* (Khursheed *et al*., 2015; 2016).

Chlorophyll mutations

Chlorophyll mutations frequency and spectrum is one of the reliable methods of evaluating the potency of mutagens used in mutation breeding programmes. Chlorophyll mutations have been reported by Khan and Tyagi (2009) in *Glycine max*, Kumar *et al*. (2003) in limabean, Toker and Cagirgan (2004), Barshile *et al*. (2006) in *Cicer arietinum*, Wani *et al*. (2011) in *Vigna radiata*, Bawankar and Patil (2001) in *Lathyrus sativus*, Paul and Singh (2002) in *Lens culinaris*, Girija and Dhanavel (2009) in *Vigna unguiculata*, Devi *et al*. (2002) in *Vigna umbellata* and Gomez-Pando and Barra (2013) in *Chenopodium quinoa.* Many workers have reported that chemical mutagens induce more chlorophyll mutations than physical mutagens (Waghmare and Mehra, 2001; Karthika and Subbalakshmi, 2006; Lal *et al.,* 2009). Shah *et al.* (2006) and Wani *et al.* (2011) have reported that EMS produced a greater number of chlorophyll mutants in comparison to other chemical mutagens. Cheema and Atta (2003) in *Oryza sativa* and Karthika and Subbalakshmi (2006) in *Glycine max* reported that gamma rays produce more number of albina mutants than chemical mutagens. However, the occurrence of higher number of chlorina and xantha than albina in gamma rays treated population have been reported by Hemavathy and Ravindran (2005) in *Vigna mungo*. Different chlorophyll mutants like chlorina, albina, xantha, viridis, aurea, tigrina and maculata were observed in M_2 generation have been reported by Khursheed and Khan (2016) in *Vicia faba*. Various workers have reported that the frequency and spectrum of chlorophyll mutations depend on the dose of mutagen (Das and Kundagrami, 2000), whereas others reported that spectrum of chlorophyll mutations is not necessarily dependent on the dose of mutagen (Yamaguchi *et al*., 2009). Ganapathy *et al.* (2008) reported that the highest dose of mutagen may not be the most effective dose. The combined physical and chemical mutagen treatments have been used to induce and alter the frequency and spectrum of chlorophyll mutations and were found to be more effective (Gautam *et al*., 1992) in

Vigna mungo, compared to the individual mutagenic treatments (Khan and Tyagi, 2009) in *Glycine max*.

Chlorophyll mutations were scored when the seedlings were 15-25 days old. They were identified and classified according to Gustafsson (1940). The frequency of chlorophyll mutations was calculated by the following formula:

$$\text{Mutation frequency (\%)} = \frac{\text{Number mutant of seedlings}}{\text{Total number of } M_2 \text{ seedlings}} \times 100$$

To evaluate the effect of combined treatments on chlorophyll mutation frequency the data were analyzed following the method of Sharma (1970).

$$\text{Coefficient of interaction (k)} = \frac{(a + b)}{(a) + (b)}$$

Where

(a+b) = The mutation frequency induced by the two mutagens in combination treatments.

(a) + (b) = The mutation frequencies induced by the two mutagens when applied alone.

k = Hypothetical interaction coefficient.

The value of 'k' should be one, if the interaction is additive. Any deviation from this value would show synergistic or less than additive effects.

Mutagenic effectiveness and efficiency

Mutagenic effectiveness indicates the rate of mutations per unit dose of mutagen, while the mutagenic efficiency indicates the genetic damage in relation to the total biological damage caused in M_1 generation (Khan and Wani, 2006; Singh, 2011). In mutation breeding programmes, the mutagenic effectiveness and efficiency are necessary for obtaining the desirable mutations in plants (Smith, 1972). Mutagenic effectiveness and efficiency depend upon the type of genotype used and the mutagen applied on it. Different workers have reported different effectiveness and efficiency of mutagens on different plants as in grasspea (Waghmare and Mehra, 2001), fenugreek (Koli and Ramkrishna, 2002), lentil (Gaikward and Kothekar, 2004), limabean (Kumar *et al.*, 2003), clusterbean (Velu *et al.*, 2007), cowpea (Dhanavel *et al.*, 2008; Girija and Dhanavel, 2009), soybean (Kavithamani *et al.*, 2008; Pavadai *et al.*, 2009; Khan and Tyagi, 2010), garden pea (Sharma *et al.*, 2010), mungbean (Wani *et al.*, 2011b). Different workers studied mutagenic effectiveness and efficiency in various varieties of *Helianthus annuus* (Banakar *et al.*, 2013), *Catharanthus roseus* (Mangaiyarkarasi *et al.*, 2014), millet (Ambavane *et al.*, 2015), *Zea mays* (Gnanamurthy *et al.*, 2011), winged bean (Kolthe and Mogle, 2014) and *Pisum sativum* (Govardhan and Lal, 2013) in both single and combined treatments of physical and chemical mutagens. The mutagenic effectiveness was calculated on the basis of chlorophyll mutations in M_2 generation, while mutagenic efficiency was calculated on the basis of biological damage in M_1 generation. Both mutagenic effectiveness and efficiency were observed to be highest at the lower concentrations of mutagens. Different varieties showed varied sensitivity to mutagenic treatments. Mutagenic effectiveness and efficiency are affected by various factors like biological, chemical and environmental ones (Kodym and Afza, 2003) and many workers are also of the view that an effective mutagen may not necessarily be an efficient tool (Koli and Ramkrishna, 2002; Gaikwad and Kothekar, 2004; Khan *et al.*, 2005). Methylating agents have been reported to be more toxic in causing damage at higher concentrations. So, these chemicals have been reported by many workers to be more effective and efficient at lower concentrations (Khan and Siddiqui, 1992). The least damager and highly useful yielder are the most desirable mutagens (Kaul, 1989).

Formulae suggested by Konzak *et al*. (1965) were used to evaluate the mutagenic effectiveness and efficiency of the mutagens used.

$$\text{Mutagenic effectiveness (Gamma rays)} = \frac{\text{Rate of mutation (Mp)}}{\text{Dose in Gray (Gy)}}$$

$$\text{Mutagenic effectiveness (EMS)} = \frac{\text{Rate of mutation (Mp)}}{\text{Concentration} \times \text{Duration of treatment}}$$

$$\text{Mutagenic effectiveness (Combination)} = \frac{\text{Rate of mutation (Mp)}}{\text{Dose of physical mutagen (Gy)} \times \text{Concentration of chemical mutagen} \times \text{Duration of treatment}}$$

$$\text{Mutagenic effectiveness} = \frac{\text{Rate of mutation (Mp)}}{\text{*Biological damage in } M_1 \text{ generation}}$$

*Biological damage = For measuring the biological damage, three different criteria were used;

(i) Injury - *i.e.* percentage reduction in seedling height (Mp/I).
(ii) Sterility - *i.e.* percentage reduction in pollen fertility (Mp/S).
(iii) Meiotic abnormalities - *i.e.* percentage of meiotic abnormalities (Mp/Me).

Morphological mutations

Morphological mutations, having desirable traits, play a key role in plant breeding. The development of new varieties and making of ideotype are the result of modifications of plant parts during morphological mutations. Many workers have reported that some morphological mutations which have desirable traits can be used to develop improved varieties when these mutants are used in cross breeding programmes (Pawar *et al.,* 2010). Different morphological mutations like leaf, pod, seed forms and other plant forms were reported by many workers to be induced by single or combination treatments of physical and chemical mutagens as in cereals (Khursheed *et al*., 2014 and 2015), pulses (Toker and Ceylan, 2013; Khursheed and Khan, 2015) and other economically important plants (Datta and Sengupta, 2002; Cagirgan, 2006). Multiple mutations are mutations

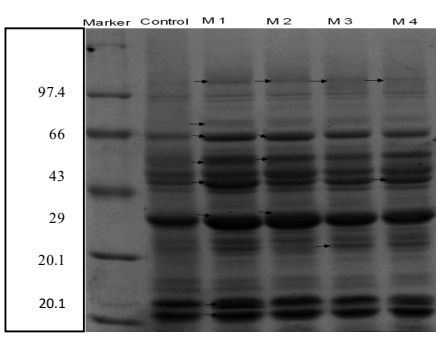
which are present more than one type in a single plant (Sharma, 1969). Multiple mutations can prove handy tool in inducing desirable mutations in a plant. Many workers have reported 'multiple mutations' in *Vigna radiata* (Auti and Apparoa, 2009). There are many reports which suggest that the frequency and spectrum of morphological mutations increase with increasing doses of mutagens (Jain and Khandelwal, 2009; Mishra *et al*., 2013). Datta and Sengupta (2002) observed higher frequency of morphological mutations at lower doses of mutagens in *Coriandrum sativum*. Vanniranjan *et al.* (1993) reported higher mutation frequency at intermediate doses/concentrations of gamma rays and EMS in *Vigna mungo*. Thus, the differential genetic makeup of different organisms plays an important role in recoverable frequency and spectrum of morphological mutations (Sharma, 2001) and the frequency and spectrum also vary with the dose/concentration of mutagen applied. Many morphological mutants like tall, dwarf, prostate, bushy, semi-dwarf and bold seeded mutants which breed true in subsequent generations are under the influence of many genes (Konzak *et al*., 1969).

Quantitative traits

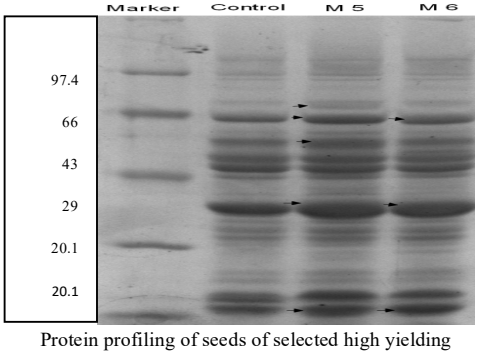
Micro mutations as genetic changes resulting in a small effect that, in general, can be detected only by help of statistical methods (Van Harten, 2007). Micromutations involve changes in quantitative traits. Quantitative traits in most cases show continuous variation and always a scattering around a phenotypic mean. Their variations are 'continuous' for two reasons, 1) because multiple genes are affecting the trait, 2) because of the superimposition of multiple variation arising from non-genetic causes. Micromutations produce desirable characters in plants are of the most important point of focus for plant breeders. Gaul (1965) on signifying the micromutations has stated that "there appears to be no doubt that micromutations may affect virtually all morphological and physiological characters as do large mutations and they might have higher mutation rate than the macromutations". Micromutations have been proved successful in creating the desired variation in plants as reported by many workers *viz*., cereals like barley (Khursheed *et al*., 2014, 2015), triticale (Viswanathan *et al*., 1994), wheat (Jamil and Khan, 2002; Sakin and Yildirim, 2004), rice (Ishiy *et al*., 2006), medicinal and ornamental plants (Datta and Sengupta, 2002; Cagirgan, 2006) and pulses like chickpea (Kozgar and Khan, 2009; Laskar *et al*., 2015), lentil (Khursheed and Khan, 2014), cowpea (Pandey, 2002), urdbean (Singh *et al.,* 2001), mungbean (Mathew *et al.,* 2005; Khan and Goyal, 2009) and faba bean (Joshi and Verma, 2004). However, opinions differ regarding the shifting of mutations in positive or negative direction during polygenic mutations in M_2 and later generations (Siddiqui and Singh, 2010). Due to the heritable nature of mutations in quantitative traits, many workers are of the view that by inducing mutations in genes controlling quantitative characters, the appropriate selection can be made thereafter for proper improvement of plants (MacKay, 2010). Induced mutations occur more or less randomly in the genome and cannot be directed. Only one of the two alleles of a locus are affected, inheritance is almost ever recessive, therefore, homozygosity is required for expression (Micke, 1999). The variability induced in different quantitative traits like plant height, flowering and maturity period, pods per plant, number of seeds per pod, pod length, number of fertile branches, seed weight and yield using single and combination treatments of physical and chemical mutagens have been reported by Yaqoob and Rashid (2001)and Wani *et al*. (2014). Many workers have reported that traits like pods per plant, seeds per pod, pods bearing branches, seed weight have close association with plant yield in mutant lines (Raut *et al*., 2004; Makeen *et al*., 2009; Giri *et al*., 2010). Significant genetic variability has been observed by many workers in agronomic traits (Kozgar and Khan, 2009; Barshile *et al*., 2009).

Protein profile of mutants

With an aim to improve the seed protein content coupled with high seed yield of legumes and cereals the genetic fortification through induced mutagenesis has been done in the past and an international programme was started by the joint FAO/IAEA, Vienna to improve the seed protein quantity and quality of legumes and cereals through induced mutations (Gottschalk, 1986). Gottschalk and Wolf (1983) and Gottschalk (1986) are of the view that high protein



Protein profiling of seeds of high yielding mutants of *Vicia faba* L. var. Vikrant



Protein profiling of seeds of selected high yielding mutants of *Vicia faba* L. var. PRT-12

Protein profiling of high yielding mutants (Khursheed and Khan, 2017).

content is difficult to combine with yield as these two traits reveal almost negative correlation. However, high yielding mutants coupled with high protein contents were reported by Ignacimuthu and Babu (1989) in urdbean, Naik *et al*. (2002) in mungbean, Hassan *et al*. (2001) in chickpea and Hiremath *et al*. (2010) in groundnut. Gottschalk (1990) explained that there is no doubt that these traits are controlled by genes and mutations in these genes can alter the protein makeup of the genotypes. However, it is very difficult to predict their action reliably because protein production in plants is highly influenced by the interaction of gene(s) and environmental factors (Gottschalk and Wolf, 1983; Gottschalk, 1990).

The profiling of proteins in the isolated mutants via the electrophoresis involves the separation of different protein polypeptides on the basis of their molecular weights and the net charge they carry (Anitha *et al*., 2008). The proteins can be reliably fractioned by SDS-PAGE (Laemmli, 1970). The protein profile could be used as molecular markers to identify them from other mutants and also from the controls (Auti and Apparao, 2009) and the mechanism therein involved. Protein profiling of the mutants has been reported in beans (Belele *et al*., 2001), tomato (Mahmoud and Al-Twaty, 2006), wheat (Das and Bhagwat, 2009), mungbean (Barshile *et al*., 2009), soybean (Nakagawa *et al*., 2011), mothbean (Khadke and Kothekar, 2011) and chickpea (Kozgar *et al*., 2014).

Mutations and salinity stress

Plants face different types of stresses like salinity, cold, temperature *etc*. that considerably reduce the productivity of the crop and hence the yield (Newton *et al.,* 2011; Lobell *et al.,* 2011). Salinity is a major and increasing problem in irrigated areas world-wide. The deleterious effects of salinity on plant growth are associated with low osmotic potential of soil solution (water stress), nutritional imbalance, specific ion effect (salt stress), or a combination of these factors (Mantri *et al.,* 2012). Induced mutations have played a key role in by their utilization for the development of new mutant varieties and hence meeting challenges related to world food and nutritional security by way of mutant germplasm enhancement. Mutation breeding has played a significant role in developing the improved varieties facing challenges against different types of stresses (Takeda and Matsuoka, 2008).

Mineral elements

It would be of great significance if the plants under experiment could be induced to increase mineral constitution in their seeds. This would be more helpful in limiting the increasing malnutrition problem to a great extent. So far not much work has been done to increase the mineral content in plants after treatments with physical and chemical mutagens. Alteration in the profile of seed mineral elements has been identified in pea (Wang *et al*., 2003a). It is possible to combine the high yielding trait of plants with high mineral nutrient trait (Gregoria, 2002) as per the reports of International Agriculture Research Micronutrient Project (CGIARMP). Kozgar *et al.* (2012) have reported an increase in the mineral content of high yielding mutants of chickpea var. Pusa-256 after treatments with gamma rays and EMS alone or in combination.

Conflict of interest

All authors declare that they have no conflicts of interest.

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