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Studies on induction of genetic variation through seed mutation in cowpea (*Vigna unguiculata* L. Walp.) by gamma irradiation

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Abstract
The present study was carried out to induce seed mutants in cowpea for getting desired mutants for increased yield, bold seed size. Dry seeds of cowpea varieties were exposed to gamma irradiation from cobalt 60 source at 250 Gy dosages. The non-irradiated parent was used as the control. Identification and selections were carried out at the second generation (M_2). The treated and untreated plants were self-fertilized for five generations to observe different morphological characters in M6 generation. Several unique and interesting mutants were isolated in thisinvestigation. Cowpea mutants were screened for useful agronomic traits. These independent mutants have different phenotypes from control. The most distinct mutants included were different pod size, pod shapeshape and coat color of seeds were also observed. The mutants selected from 250 Gy population included plants with increased vegetative growth of plants, increased number of pods, increase in size of pods, curved pods, early maturing plants with broad leaflets, Plants resistant to thrips, black seed colourand bold seed size. The selected mutants were found to possess useful agronomic traits capable of conferring on them selection advantage for increased yield, easy harvesting and insect tolerance.

Keywords: Cowpea, gamma rays, mutation frequency, mutants

Introduction

Cowpea is one of the major sources of plant protein. The advent and subsequent improvement of legumes such as cowpea through induced mutation could make it possible to develop new germplasms. The use of induced mutation had long been recognized as a rapid source of producing genetic variability in crops (Harris, 1979) [25]. This is because existing genes can change into different alleles, linkage groups can separate and gene(s) can change positions or be eliminated (IAEA, 1977) [26]. Variations have been generated in inherited characters in many crops such as yield, maturity time and nutritional quality (FAO/IAEA, 2004) [27]. By inducing mutations in cowpea, it may be possible to identify new genes and thus broaden the spectrum of inheritable changes and expand cowpea germplasm. Plant architecture is recognized as one of the major trait for higher yield in grain legumes. Alterations of plant type characterized by modification of leaves into tendrils have been extensively used in developing new cultivars in pea. However information on morphological mutations in cowpea is very limited. This study thus aims at identifying and selecting mutants with useful morphological attributes and agronomic traits. Mutation induction is an important complementary method of breeding crop species. The utilization of induced mutations for the improvement of crop plants has yields several mutants which have been used directly as new cultivars (12). Mutation breeding is accomplished by chemical or physical treatments followed by selection for heritable changes of specific genotypes, and this method has been used successfully in the genetic improvement of crop plants (19). Mutagenesis has been widely used as a potent method of enhancing variability for crop improvement (27). The induced mutations are of considerable value for comprehension evolution and accelerating the process of plant improvement. Induced mutants constitute a valuable resource for research aimed at understanding the process in governing plant development (5). One of the most important breakthroughs in the history of genetics was discovered that mutations can be artificially induced in organisms (29). Present paper reports data on flower color and seed mutants induced by different doses of gamma rays and EMS in cowpea.

Materials and Methods

This research was conducted at the Breeder Seed Production Centre of the G.B. Pant University of Agriculture & Technology, Pantnagar. Seeds of five improved varieties and one advanced line were irradiated with 250 GY gamma rays in the Kharif season of 2016 for generating mutants for resistance to thrips and other desirable traits.

Cowpea variety Pant Lobia-1, Pant Lobia-2, Pant Lobia-3, Pant Lobia-4 and Pant Lobia-5 was obtained from GBPUAT, Pantnagar. Healthy and uniform seeds of cowpea were dried in sunlight to decrease the moisture content below 13%. Seeds were irradiated with a mutation dose of 250 GY. The treatments were induced at Bhabha Atomic Research station, Bombay. The treated seeds were planted in the field according to randomized block design with three replications. The non-irradiated parents of the same varities was used as the control. Each plot consisted of 4 rows with a distance of 45 cm between the rows and 20 cm between the plants. Each M1 plant was harvested separately and the seeds were sown in the next season in plant progeny rows, to raise M2 generation in a randomized block design with three replications followed by M3, M4, M5 and M6 generation. Mutants affecting pod colour, size and shape were detected by observing the plants from budding to podding stages in all generation. The generation plants were examined for visible changes from the parent such as changes in plant height, plant architecture, leaf colour, leaf length, leaf texture. Also, changes such as earliness, flower colour, pod texture, pod shape, pod angle, pod colour and seed coat colour, presence of pigment and growth habit were examined. Mutants affecting color, size and shape of seeds were determined by opening six to eight pods from each M5 plants. A wide variation in sensitivity of varieties to irradiation dose was observed in the M1 generation during summer 2017. The subsequent M2 and M3 generations showed very interesting mutants which are being further evaluated. During summer and Kharif 2018, M4 and M5generations were evaluated. In addition to this, 250 gm of Pant Lobia-2 was also irradiated with 250 GY and planted as M1 genertation during Kharif 2018.

Results & Discussion

The following mutations were identified with different morphological attributes from the parent. Shape of leaflets of mutants ranged from broad to ovate. Some mutants were also identified with growth habit which differs from that of the parent. Some mutants were identified with pods, which differs from that of the parent. The parent has smooth textured, straight and pendant pods. However, rough textured pods were identified in mutant. Pod shape ranged from slightly curved of mutants while curved shaped pods were found in mutant. Pod position varied from pendant to slightly upright. Mutations are phenotypically classified into two groups (10); macro mutations: These are easily detectable in individual plants, phenotypically visible and morphologically distinct and they are qualitatively inherited genetic changes, and occur in major genes or oligogenes; and micro mutations: These result in a small effect that, in general, can be detected only by help of statistical methods and quantitatively inherited genetic changes, and occur in minor genes or polygenes. In the present investigation, some of the morphological (viable) mutants were observed in M6 generation. Flower mutants such as early flowering, white color flower, blue color and pink color flower were also observed in all mutagenic treatments. Similarly (Fig 1). Similar results were observed in chick pea (13); chrysanthemum (17); Artemisia (24); sesame (6); chickpea (3); sesame (4); French bean (18). The macro mutations of seed color were induced in the M6 generation. Such seed mutations for different seed color were reported in pulse crops, for example, buff and black in Arhar (1), Yellow in Soybean (28) and golden yellow (26) in mung bean. The brown color, white color and bold size seed mutant was observed (Fig 2). Similar reports of seed color were isolated by sesame (22), (23) and (9); Chick pea (13), Mustard (30), French bean (18). Although desired variation is often lacking, mutagens can be used to induce mutations and thereby generate genetic variation. Morphological mutations affecting different plant parts can be of enormous practical utility and many of them have been released directly as crop varieties (25). Gamma rays are belonging to ionizing radiation and interact with atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and affect different morphology, anatomy, biochemistry and physiological characters in plants, mainly depending on the level of irradiation. These effects could changes in plant, the cellular structure and metabolism, like dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative and accumulation of phenolic compounds (15 and 31). Phenotype of flower color and seed mutants in M6 progenies was observed. For flower colour different colours observed are, Purple, Pink, Light blue, White, dark purple, dark blue, light blue. For Seed colour: mutatnts observed were of White, brown, red, black, bold size seed were also observed.

Cowpea with broad leaflet shape may have yield advantage over the narrow one. This is because light penetration may be deeper into the leaf canopy and this may enhance photosynthetic activities of such plant (IITA, 1989) [28]. It may also imply high leaf area index, which may result in higher photosynthetic rate. The rough texture of leaflets may be an escape mechanism from leaf feeding insects. Mutants with dark green leaflets may be more efficient in photosynthetic activities due to increase in chlorophyll pigments. It may also serve as genetic marker for easy identification of cowpea.

As for the growth mutations identified, the mutants with short branches and erect growth habit are mostly determinate in nature. This may confer the advantage of planting at higher density. Such high population tends to result in significant yield improvement. Similar results have been observed by Awoleye (2000) [29] in cowpea. On the other hand, higher branching may favour higher production of pods which may result in higher seed yield. Fawole *et al*. (1985) [30] reported the identification of a cowpea mutant with branched peduncles which is potentially capable of carrying more than usual number of pods. Extra early maturing mutants may be selected for the many advantages it may confer on the plant. These include ability to escape or tolerate insect damage due to short duration of reproductive period. Short duration of flowering has been known to prevent insect population from building up on such plants. Hence, such plants may suffer less yield reduction (Jackai, 1982) [31]. Early maturing mutants are also suitable for late planting in areas with short rainfall duration as they show better tolerance to drought. The dwarf mutant that was identified in this study may possess the advantage of lodging resistance due to their short basal branches. Pod mutations such as rough texture suggest the presence of trichomes. Cowpea plant with trichomes has been found to suffer less damage by *Marucavitrata* (Veeranna and Hussain, 1997) [32]. Pod position may also confer selection advantage on the plant. Mutants with upright pods and pods above plant canopy may be selected for mechanical

harvesting. Such findings have been reported by Wien and Summerfield (1980) [33]. Furthermore, mutant with pods at wide angle has the potential of suffering less damage from insect. This is because *Maruca* damage has been reported to vary inversely with pod angle. Pods that were held closely at narrow angle were found to be more susceptible to *Maruca* damage than those held widely apart (Oghiakhe *et al*., 1992) [34].
Cowpea pods with dark green coloration have been reported to show less damage to *Maruca* (Ojomo and Cheda, 1975) [35]. Also, flower mutation as observed in mutant has been found to make plants more attractive to foraging by insects particularly when they are large in size. Such plants are more susceptible to insect damage when left unprotected (Ntonifor *et al*., 1996) [36]. Seed coat colour and texture have important effect on consumer's preference which differs among regions of the world.

Plate: Mutation observed during M$_2$ generation

PGCP-28 (Resistant to thrips)

PL-3 (Black seeded mutant)

PL-2-7-12-1 (Black podded Mutant)

PL-3-51-172-1 (Curved Pods)

PL-3-78-211-1 (High number of pods)

PL-3-97-262-1 (Long peduncle and upright pods)

PL-3-34-61 (Segregation for seed colour)

PL-5-448-145 (Resistant to thrips)

Conclusion

The morphological mutant characters studied can be utilized for identification and characterization of cowpea genotypes. The present investigation revealed that the isolation of flower color and altered size, shape and coat color of seeds is possible in 250 GY of EMS. It provides greater chances for the selection of desired characters. Significant morphological variability was created among mutants using gamma irradiation. The selected mutants show useful agronomic traits capable of conferring on them selection advantage for increased yield and insect resistance. There is therefore, possibility for further cowpea improvement through induced mutation (FAO/IAEA, 2001, 2004) [35, 27].

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