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SHORT COMMUNICATION

A Report on Gamma Radiation-Induced Variation in Seed Characters of *Cicer arietinum* L.

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Abstract

Cicer arietinum L. (chickpea) is one of most popular and cheap source of plant protein and minerals worldwide. The present study was directed to induce variations in seed characters of chickpea "Vijay", especially with reference to increase in its protein and mineral content using gamma radiations as mutagenic agent. M_2 population of *Cicer arietinum* L. "Vijay" after post-harvest analysis revealed that 300 Gy dose of gamma radiations induced significant variations in seed characters including seed size, surface texture and seed coat color. Total nine mutants were identified differing from control in seed coat colors and categorized into four groups on the basis of seed size as normal, small, bold and extra bold; two groups on the basis of surface texture and wrinkled or smooth surface. The seed yield of all mutants was noted as 100-seed weight, which was corresponding with seed size. The biochemical analysis of the seed mutants in the form of protein, iron and zinc content indicates that, seeds with dark colored seed coat have higher level of protein and minerals as compare to control.

Keywords: *Cicer arietinum*, gamma radiation, post-harvest, seed coat color, biochemical analysis.

Introduction

Cicer arietinum L. (chickpea) is the second largest pulse crop in the world. It is one of most popular and cheap source of plant protein and minerals like iron and zinc. Apart from this, chickpea plants have some medicinal properties; especially, seeds with dark seed coat are reported to have more medicinal potential than normal light color (Balasundaram et al., 2006). On the other hand, the chickpea grains have significant level of phytic acid, which usually inhibit the actual available content of proteins and minerals at consumers end.

Genetic diversity is an important resource of genes for breeding programs for new quality products. Genetic diversity in 25 chickpea genotypes have been reported (Sharifi et al., 2018). Genetic bottlenecks and subsequent founder effects during domestication resulted in narrow genetic base, especially in crops like chickpea (Abbo et al., 2003). Chickpea variety "Vijay" is one of the most preferred varieties by the farmers of Maharashtra state for its resistance to bacterial wilt and comparatively high yield (Mandhare et al., 2011). The present study was directed to induce variations in seed characters of chickpea "Vijay", especially with reference to increase in its protein and mineral content using gamma radiations as mutagenic agent.

Materials and Methods

Germplasm of *Cicer arietinum* (L.) "Vijay" was procured from Pulse Research Center, Mahatma Phule Krishi Vidyapith Rahuri (MS). The seed material was first screened for any damage and seeds with about 10 to 12% moisture content were treated by gamma radiation doses (300 Gy, 400 Gy and 500 Gy) using Cobalt 60 as source. The irradiation was conducted at Bhabha Atomic Research Center (BARC) Mumbai (MS). Two hundred irradiated seeds per dose were sown in the field to rise M_1 population (October 2015). The M_1 progeny was harvested as individual plants. These seeds then sown on plant to row basis to generate M_2 progeny (October 2016). The M_2 population was monitored closely for morphological and economical traits (Khan et al., 2005) and then harvested. After harvest and threshing, plant having seeds with variation in seed coats color, seed surface texture, and seed size were identified and kept separate for further study. Later 100-seed weight of each isolate was recorded and compared with control.

Nutritional content of the seeds including protein (Bradford, 1976), iron, and zinc was also analyzed and correlated with seed coat coloration. For iron and zinc analysis the methods developed by Velu et al. (2006 and 2008) were employed. Both methods are colorimetric; interaction of sample with reagent material resulted in colored solution and the color intensity was compared with the scale given by authors for iron and zinc analysis. For the iron detection, about 10 mL of Prussian blue (2%) was mixed with one gram of whole grain flour and shaken vigorously. After 10 minutes incubation, the mixture developed blue coloration. The intensity of color was visually scored on a 1 to 3 scale, where 1 = less intense blue color; 2 = medium blue color; and 3 = more intense blue color. The intensity of the red color developed was compared with the standard scale provided.

For zinc detection DTZ (1,5-diphenyl thiocarbazone) (Sigma Aldrich) were used as reagent. One gram of the whole grain flour was taken in the test tube and mixed with 5 ml DTZ (300 $mg.L^{-1}$ in pure methanol) and shaken vigorously using Vortex for 2 minutes. The samples were then incubated for 15 minutes for maximum color development. The intensity of color was visually scored on a 1 to 3 scale, where 1 = less intense red color; 2 = medium red color; and 3 = more intense red color. The intensity of the red color developed was compared with the standard scale provided.

Results and Discussion

After maturity, or about 95 to 100 days after germination, the selected plant types from M_2 population were harvested during February 2017. The post-harvest analysis of some selected isolates revealed significant variation in seed coat color, seed size, surface texture and 100-seed-weight when compared with control variety “Vijay”. In all total nine mutant plant types were isolated (Table 1). All these seed mutants were from 300Gy gamma irradiated population; where the frequency of mutants for seed characters was 0.434% described in Figure 1. Of these, mutant 1 showed black seed coat color, normal seed size with wrinkled surface, mutant 2 showed brown seed coat color with black strips, extra bold in size and wrinkled surface Mutant 3 was with unique green colored seed coat, normal seed size with wrinkled surface, mutant 4 showed golden yellow color with normal seed size and wrinkled surface. Mutant 5 revealed yellow seed coat, normal seed size with wrinkled surface while mutant 6 was with brown seed coat, bold seed size and wrinkled surface. Mutant 7 and 8 were with light brown and dark brown seed coat, bold seed size with smooth surface respectively while mutant 9 was with brown seed coat color with small seed size having wrinkled seed surface texture (Table 1, Figure 1). The 100-seed weight of control variety was noted as 17.53 ± 0.62 g. The 100-seed weight of mutant 1, 3, 4 and 5 were more or less in the range of control while that of mutant 9 was least (16.22 g) and those with bold and extra bold seeds have 19.86 to 24.78 g (Table 1). Thus the seed size corresponds with 100-seed

Table 1. Physical characterization and biochemical analysis of chickpea seed mutants isolated in M_2 population of 300 Gy dose

Plant Types	Seed coat color	Seed size	Surface texture	100-seed weight (g)	Protein ($mg.g^{-1}$)	Iron (mg/ 100g)	Zinc (mg/ 100 g)
Control	Dark Brown	Normal	Wrinkled	17.53 ± 0.62	17.72 ± 0.86	30.8-35.9	<37.0
Mutant-1	Black	Normal	Wrinkled	17.42	20.42	> 60.0	50.0- 65.0
Mutant-2	Brown with black strips	Extra bold	Wrinkled	24.28	23.78	50.0- 59.9	38.0- 49.0
Mutant-3	Green	Normal	Wrinkled	17.58	19.65	> 60.0	50.0- 65.0
Mutant-4	Golden yellow	Normal	Wrinkled	17.56	19.88	36.0- 49.9	38.0- 49.0
Mutant-5	Yellow	Normal	Wrinkled	17.22	17.52	36.0- 49.9	<37.0
Mutant-6	Brown	Bold	Wrinkled	19.86	19.02	36.0- 49.9	38.0- 49.0
Mutant-7	Dark brown	Bold	Smooth	21.33	19.56	> 60.0	50.0- 65.0
Mutant-8	Light brown	Bold	Smooth	21.85	19.83	50.0- 59.9	38.0- 49.0
Mutant-9	Brown	Small	wrinkled	16.22	17.26	30.8-35.9	38.0- 49.0

Note: All mutant types were isolated from 300Gy irradiated M2 population of chickpea “Vijay”

Figure 1. Seed coat color variation in mutant plant type chickpea “Vijay” isolated from M_2 generation

weight of mutant plant types isolated from M_2.

The seed protein content of control variety was 17.72 ± 0.86 mg per g. The highest protein content was noted in mutant 2 while the least was found in mutant 9 (Table 1). All the mutants except number 5 and 9 showed higher protein content than that of control (Table 1). Further, it could be stated that the seeds having dark seed coat colors had more protein and mineral content. The iron and zinc content of control variety seeds noted as 30.8 – 35.9 mg per 100g and <37.0 mg per 100g. The highest amount of iron and zinc was found in seeds of mutant 1, 3 and 7 (> 60.0 and 50.0-65.0 mg per 100 g, respectively). The seeds with lowest iron content of 30.8-35.9 mg per 100 g was observed in mutant 9 which is slightly higher than control, whereas seeds with the lowest zinc content was in mutant 5 (<37.0 mg per 100g). The rest of the mutants have significantly higher zinc content (Table 1). It was noted that more level of iron and zinc was present in seeds of the mutants with darker seed coats as compared to the control.

Inducing genetic variability in crop plant to get altered plant types with desirable characters using various mutagens are a traditional but reliable method of crop improvement. From the present study it is cleared that, 300 Gy dose of gamma radiations is more effective in inducing mutagenic effects in seed characters of chickpea. No apparent change was noted in the seed characters of 400 Gy and 500 Gy chickpea population. Further, both these doses revealed less germination, survival and less frequency of viable morphological mutants. The plant types isolated from 300 Gy, M_2 progeny of chickpea are presented here with their changed physical characters like seed coat colors, seed size and surface texture. These variations might be due to structural rearrangements in DNA upon exposure of lower doses of gamma radiations. The bold seed grains usually preferred by consumers (Magalhaes et al., 2017) and if it has color variation, it will likely be more economically beneficial. Secondly, the seeds of mutants with dark colored seed coat have enhanced level of protein, iron and zinc; it supports the fact that colored chickpea seeds have healing potentials (Balasundarm et al., 2006; Segev et al., 2010 Jukanti et al., 2012 and Bhagyawant et al., 2015). The observed variations in seed characters could be used further to identify the genes responsible these changes at molecular level. Thus, these selected mutant plant types have promising potential. Further, the stabilization of these characters could lead the development of new varieties with some novel characters specially to be used as functional food.

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