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Research Article

Studies of mineral constituents in viable mutants of Pigeonpea seeds

Sangle S M

Department of Botany, Dada Patil Mahavidhyalaya, Karjat Ahmednagar-414402 (M.S.)
Email: sunilsangle10@gmail.com

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Abstract

Objective of the present investigation has been to induce genetic variability in pigeonpea especially in its quantitative and qualitative characters. This would help in improving the yield of existing, locally adapted varieties of pigeonpea (BDN-708 and BSMR-853) through mutation breeding by employing two chemical mutagens like, EMS (Ethyl methanesulphonate) and SA (Sodium azide) and physical mutagen like Gamma rays which have been used in the present programme. Biochemical study is a recent trend in support of the traditional method of crop breeding, so as to obtain better estimates of the breeding values of a variety. By keeping this object in mind, minerals constituents of viable mutants was studied in both the varieties of pigeonpea.The different mineral constituents comprised percentage of nitrogen, crude protein content, total ash content, calcium content and phosphorus content. Mean content of nitrogen ranged from 1.95% to 3.33% and 2.24% to 3.17%, calcium content ranged from 0.25% to 0.37% and 0.26% to 0.51% and phosphorus content of viable mutants ranged from 0.56 % to 0.72 % and 0.58% to 0.80% in varieties BDN 708 and BSMR 853 of pigeonpea, respectively.

INTRODUCTION

Pigeonpea is grown in almost all the states of India, but the major concentration is in the state of Uttar Pradesh in northern India and the states of Gujarat (eastern), Maharashtra (eastern), and Karnataka (north-east) in western India, and Madhya Pradesh (western) in central India. Pigeonpea has several advantages over other leguminous crops for broad scale agricultural production. These include drought tolerance, lodging and shattering resistance.

Pigeonpea cotyledons contribute about 85%, the embryo about 1%, and the seed coat 14% to the total seed mass (Faris and Singh, 1990). The chemical constituents of pigeonpea seed govern its nutritive value. There is a wide variability exists in chemical composition of pigeonpea seeds due to genotype, growth conditions and duration/condition of storage (Amaefule and Onwudike, 2000).

Green seeds of pigeonpea are consumed as vegetable. The green seed is more nutritious than the dry seed because it contains more protein, sugar and fat than the mature seed. Biscuits produced from millet flour (MF) and pigeon pea flour (PPF) blends in various ratios of MF to PPF used were 100:0, 75:25, 65:35, and 50:50 and analyzed for their nutritional composition. They all contained high proportions of protein (7.5–15.2%), fat (17.1–18.1%) and digestible carbohydrate (60.2–66.5%). The moisture content was in the range 5.0 to 6.6%, ash 1.5–2.3% and crude fiber 0–0.1%. (Eneche, 1999).

The seed proteins of wild species have a poor solubility than that of cultivated pigeonpea, and this indicates an increase in solubility under domestication, and perhaps improved nutritional quality in this grain legume (Ladizinsky and Hamel, 1980).

The mutation breeding can play an efficient role in developing an ideal plant type having high yield potential (Sarwar *et al*., 2006).Mutation breeding is one of the plant breeding techniques used for creating genetic variability in yield contributing traits and to improve the yield of crop plants (Ahloowalia *et al.*, 2004).Mutation breeding is a powerful and effective tool in the hands of plant breeders especially for autogamous crops having narrow genetic base (Micke, 1988). Variability in the population creates the chance of selection for desirable improvement. Induced mutagenesis can be used to create variability as the rate of spontaneous mutation is very low. The EMS was more effective than Gamma radiation. The decrease in survival of plant at maturity is due to rapid injection of chemical mutagen and their ability to produce chromosomal abstractions (Sharma *et al.*, 2005). Similar results were also obtained by Bashir *et al.*, (2013) in Fenugreek. Kulkarni and Mogle, (2013) in Horse gram and Sangle *et al.,* (2011) in Pigeonpea.

MATERIALS AND METHODS

Seeds of two pigeonpea varieties Amol (BDN-708) and Vaishali (BSMR- 853) were obtained from the Agricultural Research Station, Badnapur, Dist. Jalna. (Under Marathwada agriculture university Parbhani, M.S. India). Dry, healthy and uniform seeds were presoaked in distilled water for 6 hours as a measure of pre-treatment .The presoaking enhances the rate of uptake of the mutagen through increase in cell permeability and also initiates metabolism in the seeds for treatment. Seeds soaked in distilled water for 12 hours served as control. The different concentrations used for chemical mutagenic treatment were 0.05%, 0.10%, 0.15% for EMS and 0.010%, 0.015%, 0.020% for SA, respectively. Immediately after the completion of treatment, the seeds were washed meticulously under running tap water to leach out residual chemical. Later on they were subjected to post-soaking in distilled water for one hour.

Healthy and dry seeds of the both varieties were packed in small polythene covers and were treated with 5kR, 10kR and 15kR doses of gamma rays.

The lot of treated seeds of each treatment was sown in the field following randomized block design (RBD) with three replications along with control forraising M_1 generation fallowed by M_2 and M_3generation.Three plants from each mutant line were selected and biochemically analyzed pertaining to following characters. All the characters were compared with control. Mature seeds were washed with water, dried and ground to make fine powder.

Estimation of total nitrogen from seeds of control and viable mutants were carried out by Micro-Kjeldahl distillation method.Total crude protein of all viable mutants and control was estimated by Micro-Kjeldahl method (AOAC, 1995).On average, most proteins have 16% nitrogen in their composition. In other words, 1mg nitrogen equals 6.25mg protein. Thus the amount of N content, when multiplied by 6.25, gives the crude protein (CP) content of the sample.

The residue after incineration of sample at 550-600^{o}C is known as ash. For this purpose the sample was subjected to a high temperature up to 600^{o}C and then the ash content was determined. The amount of phosphorus present in sample and was measured by using colorimeter method of Fiske and Subba Rau (1925) described by Oser (1979).

Acid soluble ash fraction of seed powder was used for determination of calcium.Ca in an aliquot is precipitated as calcium oxalate. The precipitate was then dissolved in acid and the content of oxalate ions determined titrimetrically with potassium permanganate **(**AOAC, 1995).

RESULTS AND DISCUSSION

In the present study eleven viable mutants were biochemically analyzed regarding the parameters of percentage of nitrogen content, crude protein content, total ash content, calcium content and phosphorus content.

Total nitrogen and crude protein content (%): (Table 1 and 2)

Nitrogen content of the viable mutants of the varieties BDN 708 and BSMR 853 of pigeonpea ranged from 1.95% to 3.33% and 2.24% to 3.17%,in different mutants. Increase in nitrogen content was observed in the erect and high yielding mutant (3.33%) than the control (2.34%) in variety BDN 708 and (3.17%) in small pod mutant than the control (2.42%) in variety BSMR 853.

The crude protein content in the mutants ranged from 13.50% to 20.79% % as compared to control 14.61% in variety BDN 708.

In variety BSMR 853, highest crude protein percentage could be recorded in small pod mutant (19.82%) and lowest in early flowering mutant (14.02%) as compared to control plant (15.13%). Nitrogen content was correlated with protein content of several legume crops. Sadasivam and Manickam (2008) determined N content by Micro-Kjeldahl method and a conversion factor of 6.25 was used for calculating the crude protein content.The observations of the present study conclusively indicate that the genetic improvement of protein quality and quantity is indeed possible through mutation breeding.

Total ash content (%) :-(Table 1 and 2)

In variety BDN 708 total ash content decreased except light green pod mutant (4.42%) as comparedwith control plant (4.36%).While highest value was recorded in branched mutant (5.37%) in variety BSMR 853 as compared to control plant (3.90%). Okpala and Okoli (2011), Smartt (1976) reportedash 3.8 % and Akande *et al.,* (2010) estimated 3.76 % ash from the row seed of pigeonpea.

Calcium and Phosphorus content (%):(Table 1 and 2)

The mean calcium content of viable mutants ranged from 0.25% to 0.37% and 0.26% to 0.51% in varieties BDN 708 and BSMR 853, respectively. While the mean phosphorus content of viable mutants ranged from 0.56 % to 0.72 % and 0.58% to 0.80% in varieties BDN 708 and BSMR 853 of pigeonpea, respectively. Higher contents of calcium and phosphorus were found in high yielding mutant (0.37%) and small compact leaves mutant (0.72%) in BDN 708 variety of pigeonpea, respectively. The highest percentage of calcium and phosphorus was recorded in early flowering branched mutant (0.51%) and high yielding mutant (0.80%). Lowest valuein high yielding mutant (0.26%) for calcium and (0.58%) for phosphorus could be recorded in five seeded mutant in variety BSMR 853 of pigeonpea. Reduced phosphorus content in seeds reduces the phytate level (anti nutritional factor).Similar results have been reported by Nguyen Thi Lang *et al.,* (2007) in rice, Zhao *et al.,* (2008) in wheat, Shen *et al.*, (2008) in rice, Rasmussen (2008) and Tambe (2009) in soybean.

Mohar *et al*., (2014) were also identified by using mutation breeding, resulted in the release of five mutant cultivars for commercial cultivation in India. About 65 Pigeon pea varieties have been released in India and 57 selections provided by ICRISAT to various Pigeon pea growing countries for their commercial cultivation. Genomic resources and genome sequence analysis predicted about 48,680 genes which may be exploited for further improvement of Pigeon pea. Native PAGE gel analysis of viable mutants in variety BDN 708 showed considerable variation in protein profiling. Maximum bands could be found in early maturing mutant. Highest bands could be observed in xantha mutant followed by three seeded mutants in BSMR 853. SDS-PAGE analysis of viable mutants of pigeonpea revealed a wide range of protein polymorphism and variability with respect to number and mobility of polypeptide bands (Sangle, 2015).

Legumes also contain several nutritional factors whose beneficial effects on human health need to be fully exploited (Gowada *et al.,* 2007). Horse gram is the important source of protein, iron and molybdenum and minerals including Na, K, Ca, Mg, S, P, and Cl as well as the micro-minerals, Fe, Zn, Cu, Mn, I, F, Se, Mo, Co and essential for human life (Bouis and Welch, 2010). Bolbhat and Dhumal (2014) investigated the seeds of eight mutants of horse gram for minerals constituents such as N, P, K, Mg, Zn, Cu, Fe, Mn. The results have revealed changes in the concentrations of macro and micro mineral elements in the mutant seed material. These changes in concentrations are attributed to the altered genetic structure, due to mutations. Gopinath and Pavadai (2015) conclude, the soybean variety co-1 responded more and more number of viable and economic mutants for higher frequency 50 KR gamma rays, 0.5 per cent EMS and 0.4 per cent DES treatments for more effective than the other mutagenic treatments. The isolation of early maturity, high yield, protein content and oil content is possible in 50 KR gamma rays, 0.5 percent of EMS and 0.4 per cent of DES treatments.

Kandhare (2015) concluded that all common and dominant seed-borne fungi of pulses caused reduction in protein content of all test pulses in more or less quantity. In all test pulses, *Fusarium moniliforme* caused maximum reduction in protein content followed by *Aspergillus niger*, *A. flavus*. Minimum loss in protein was reported in Green gram, Chickpea, and Pigeon pea by *Rhizopus stolonifer*, in Black gram by *Aspergillus fumigatus* and *Drechslera tetramera*. This reduction in protein content of test pulses by the seed-borne fungi proves their proteolysis efficacy.

Table 1: Effect of mutagen on Nitrogen (%), Crude protein content (%) Total ash %, Calcium and Phosphorus content (%) in different viable mutants of pigeonpea variety BDN-708.

Sr. No.	Name of mutant	Nitrogen (%)	Crude Protein (%)	Total ash (%)	Calcium (%)	Phosphorus (%)
1	Control	2.34	14.61	4.36	0.34	0.60
2	Tall	2.26	14.13	3.37	0.35	0.65
3	Dwarf	2.49	15.55	4.13	0.26	0.60
4	Branched	2.47	15.44	3.72	0.27	0.61
5	Early flowering	2.53	15.83	3.95	0.25	0.56
6	Early maturing	1.95	12.18	4.22	0.32	0.69
7	Light green pod	2.78	17.35	4.42	0.36	0.70
8	Dark black pod	2.16	13.50	3.82	0.32	0.66
9	Small compact leaves	2.59	16.17	3.73	0.32	0.72
10	High yielding	2.75	17.18	3.68	0.37	0.62
11	Erect and high yielding	3.33	20.79	4.18	0.31	0.71
	SD	0.37	2.28	0.33	0.04	0.05
	SE	0.11	0.69	0.1	0.01	0.02
	CD(p=0.05)	0.25	1.53	0.22	0.03	0.04
	CD(p=0.01)	**0.35**	**2.18**	**0.31**	**0.04**	**0.05**

Table 2: Effect of mutagen on Nitrogen (%), Crude protein content (%) Total ash %, Calcium and Phosphorus content (%) in different viable mutants of pigeonpea variety BSMR-853.

Sr. No.	Name of mutant	Nitrogen (%)	Crude Protein (%)	Total ash (%)	Calcium (%)	Phosphorus (%)
1	Control	2.42	15.13	3.90	0.33	0.67
2	High yielding	2.81	17.56	3.72	0.26	0.80
3	Tall	2.77	17.31	4.22	0.28	0.67
4	Dwarf	2.56	16.00	4.02	0.31	0.74
5	Branched	2.68	16.76	5.37	0.48	0.67
6	Early flowering	2.24	14.02	4.82	0.31	0.66
7	Early flowering with branched	3.14	19.61	4.75	0.51	0.62
8	Three seeded	2.68	16.73	4.14	0.42	0.77
9	*Xantha*	2.84	17.74	3.53	0.48	0.65
10	Five seeded	2.34	14.61	3.77	0.48	0.58
11	Small pod	3.17	19.82	4.08	0.43	0.71
	SD	0.30	1.86	0.55	0.09	0.06
	SE	0.09	0.56	0.17	0.03	0.02
	CD(p=0.05)	**0.20**	**1.25**	**0.37**	**0.06**	**0.04**
	CD(p=0.01)	**0.29**	**1.78**	**0.53**	**0.09**	**0.06**

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