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Biochemical studies in promising mutants of pigeonpea

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Abstract
In the present study two varieties of pigeonpea namely BDN 708 and BSMR 853 were used to induce mutagenesis. For this study a physical mutagen (Gamma rays) and chemical mutagens ethyl methanesulphonate (EMS) and sodium azide (SA) were tried. Studies pertaining to mutation breeding of pigeonpea were spread over three generations. The mutant isolated from M_3 generation subjected to biometrical analysis and screened accordingly. The highest water soluble protein content was recorded in early maturing mutant. Tall mutant, early flowering, light green pod mutant showed significant increase in protein content in BDN 708. Dwarf mutant showed maximum carbohydrate content while in variety BSMR 853, the highest carbohydrate content could be noted in small pod mutant which was statistically significant.

Keywords:EMS, SA, Gamma rays, Proteins.

Introduction
Food legumes comprise good sources of dietary proteins in developing world. They provide nutritious feedstuff for cattle. They also play a key role in maintaining soil fertility. Starch and protein are the principal constituents of pigeonpea seed[1]. showed that the starch content of the cotyledons of several cultivars belonging to different maturity groups ranged between 51.4 and 58.8%. These values were higher than those reported [2].The same report indicated that the cotyledon starch percentage of 22 cultivars had a larger variation, ranging between 39.0 and 55.9% with a mean of 47.7%.
Mature seeds of pigeonpea contain 18.8% proteins, 1.9% fat, 53% carbohydrates, 6.6% crude fibers, soluble sugars 1%, water 1.5% and energy 16-18% [3]. Protein content of the pigeonpea seeds is about 2-3 times more than the cereals. The proteins of pigeonpea are nutritionally superior and important. It contains B-complex vitamins. It also contains minerals like calcium, iron and phosphorus.
Protein quality is of prime importance in the pigeonpea products used for human food **[4]**. The protein quality of pigeonpea seed is a function of the amount of protein, the essential amino acids in that protein, and the proteins digestibility[5]. It is found that the protein content of pigeonpea seed samples ranged between 18.5 and 26.3% with a mean of 21.5% whereas protein content of 43 commonly cultivated varieties of pigeonpea ranged between 17.9 and 24.3% for whole seed, and between 21.1 and 28.1% for dhal samples; indicating only a small variation[6, 7]. High environmental influence on protein content and a negative correlation between yield and percentage of seed protein[8].

Materials and Methods
The experimental plant materials
The experimental plant material used in the present investigation comprised two varieties of pigeonpea(*Cajanuscajan*(L.) Millsp.)namely, Amol (BDN-708) and Vaishali (BSMR-853). The varietal material was obtained from the Agricultural Research Station, Badnapur, Dist. Jalna.

Mutagens Used
The chemical mutagens namely, ethyl methanesulphonate (EMS) and sodium azide (SA) besides physical mutagen gamma rays were used in the present study.
Electromagnetic ionizing radiations were applied from Co^{60}, 1000 curie source of the gamma irradiation unit of the Department of Biophysics, Government Institute of Science, Aurangabad. (M.S.), India. The dose rate was 24,578 rads per hour.

Treatment
Uniform seeds of two pigeonpea varieties Amol (BDN-708) and Vaishali (BSMR- 853) were surface sterilized with 0.1% mercuric chloride solution for about one minute and washed thoroughly with distilled water. They were presoaked in distilled water for 6 hours.The presoaking enhances the rate of uptake of the mutagen through increase in cell permeability and also initiates metabolism in the seeds for treatment. The volume of the chemical mutagenic solution used was five times as that of seeds as to facilitate uniform absorption. Such presoaked seeds were later immersed in the mutagenic solution for 5hours with regular shaking. 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.

Gamma rays treatment
Vigorous and dry seeds of the both aforesaid varieties were packed in small polythene covers and seed samples were exposed to doses like 5kR, 10kR and 15kR of gamma rays.

Biochemical studies
Three plants from each mutant line were selected and biochemically analyzed and were compared with control.

Extraction of water soluble seed proteins
Mature seeds were washed with water, dried and ground to make fine powder. It was defatted with hexane and 100 mg of the powder was kept for extraction in 1 ml of 1:6 proportion of 10 mMTris buffer P^H 8.0 with 1% PVP.

Protein estimation
The protein estimation was carried out by using Lowry's method[9].

Extraction of total carbohydrates
The 100 mg of seed powder was weighed into boiling tubes and 5 ml of 2.5 N HCL was added to them. All the boiling tubes were placed in boiling water bath for 3 hours and thereafter the tubes were cooled at room temperature. The boiled suspension was neutralized with solid sodium carbonate until the effervescence ceased.

Estimation of total carbohydrates
The estimation of total carbohydrates was carried out by phenol-sulphuric acid method [10].

Estimation of total nitrogen (N)
Estimation of total nitrogen from seeds of control and viable mutants were carried out by Micro-Kjeldahl distillation method. The dry sample is digested with concentrated sulphuric acid (H_2SO_4) in the presence of catalyst. The ammonium tetraborate formed is then titrated with 0.035N hydrochloric acid for the determination of nitrogen[11].

Results and Discussions
In the present study eleven viable mutants were biochemically analyzed regarding the parameters of water soluble protein content, carbohydrate content and percentage of nitrogen content from both varieties of pigeonpea.

Water soluble protein content
The results tabulated revealed that viable mutants of both varieties of pigeonpea showed variability in water soluble protein content. The protein content in control was 19.55% in variety BDN 708 and 19.90% in variety BSMR 853. In variety BDN 708 highest value (21.15%) for soluble protein was observed in early maturing mutant, while the lowest (17.00%) was in dwarf mutant. In variety BSMR 853, the highest soluble seed protein value (22.20%) was noticed in three seeded mutant while lowest (17.89%) could be found in branched and five seeded mutant. There was an increase as well as decrease in protein content. Mutation breeding is better for improving the quality of proteins[12].

Carbohydrate content
The carbohydrate content was estimated in the viable mutants of pigeonpea varieties BDN 708 and BSMR 853. Most of the viable mutants exhibited maximum carbohydrate content. Dwarf mutant showed maximum (48.33%) carbohydrate content while the least (36.50%) carbohydrate content has been shown by the small compact leaves mutant. In variety BSMR 853, the highest carbohydrate content could be noted in small pod mutant (46.02%) and lowest in the (39.33%) three seeded mutant and reported an increase in total soluble carbohydrate content in black gram mutants[13]. Experimental results for total carbohydrate content are in agreement with the results obtained by earlier researchers [14, 15].

Total nitrogen content:
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.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. Similar results were also obtained by in pigeonpea[16, 17].
The results obtained decisively demonstrated the usefulness and the effective potential of the induced mutational approach in genetic improvement of pigeonpea for recovering superior mutant plant types having high protein, carbohydrate and nitrogen content.

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Table 1: Water soluble seed protein content (%) in different viable mutants of pigeonpea variety BDN- 708.

Sr. No.	Name of mutant	Water solubleprotein content (%)of defatted seed powder	Total carbohydratecontent (%) ofdefatted seed powder	Nitrogen (%)
1	Control	19.55	44.33	2.34
2	Tall	20.85	46.16	2.26
3	Dwarf	17	48.33	2.49
4	Branched	20.2	46	2.47
5	Early flowering	20.96	44.5	2.53
6	Early maturing	21.15	48.16	1.95
7	Light green pod	21.11	41.18	2.78
8	Dark black pod	19.76	39.4	2.16
9	Small compact leaves	19.93	36.5	2.59
10	High yielding	19.76	39	2.75
11	Erect and high yielding	19.9	38.5	3.33
12	SD	1.1648	4.1561	0.37
13	SE	0.3512	1.2531	0.11
14	CD(p=0.05)	0.7832	2.7944	0.25
15	CD(p=0.01)	1.1133	3.9724	0.35

Table 2: Water soluble seed protein content (%) in different viable mutants of pigeonpea variety BSMR - 853.

Sr. No.	Name of mutant	Water soluble protein content (%) of defatted seedpowder	TotalCarbohydratecontent (%) of defatted seed powder	Nitrogen(%)
1	Control	19.9	43.83	2.42
2	High yielding	19.62	45.33	2.81
3	Tall	18.08	42.03	2.77
4	Dwarf	19.06	40.06	2.56
5	Branched	17.89	39.4	2.68
6	Early flowering	18.66	40	2.24
7	Early flowering with branched	22.04	42.23	3.14
8	Three seeded	22.2	39.33	2.68
9	*Xantha*	19.02	45	2.84
10	Five seeded	17.89	45.33	2.34
11	Small pod	19.48	46.02	3.17
12	SD	1.4902	2.6169	0.30
13	SE	0.4493	0.7890	0.09
14	CD(p=0.05)	1.0020	1.7595	0.20
15	CD(p=0.01)	1.4243	2.5012	0.29

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