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Isolation of Gamma Ray Induced Urd Bean [*Vigna mungo* (L.) hepper] Mutants with Improved Batter Quality

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ABSTRACT

Background: The urd bean variety MDU 1 has a duration of 70-75 days, bushy in nature and is susceptible to yellow mosaic virus. In order to develop a determinate type and improved batter quality, MDU 1 and VBN (Bg) 4 blackgram seeds were treated with different doses (100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy) of gamma rays.

Methods: Uniform sized seeds treated with different mutagenic doses were raised in randomised block design which constitute M_1 generation. Each plant was harvested individually and forwarded to M_2 generation following plant to progeny row method. The determinate types were selected from M_2 generation and forwarded to further generations. After attaining homozygosity in the advanced generation, the mutants were checked for its biochemical characters (Total solubule protein content analysed by kjeldahl method, albumin and globulin analysed by Lowery's method, arabinose content analysed by Bial method.

Result: The mutants ACM - 16 -011, ACM - 16 -015, ACM - 16 -018 were found to have an arabinose content of 8.28%, 8.98% and 8.14% respectively. All these mutants recorded more batter volume over the variety MDU 1. The albumin (%) and globulin (%) contents were also found at remarkably increased levels in the mutants. These mutants have the potential to develop a high quality variety of urd bean and therefore are very useful in breeding programme.

Key words: Arabinose, Battering quality, Blackgram, Gamma rays, Idli.

INTRODUCTION

Pulses are seeds of leguminous plants and are used as food. They are one of the most important commodities in Indian diet as they are richer in protein content than cereals. Pulses are valuable for the cropping system to maintain and improve the productivity of soil due to their nitrogen fixation ability. Therefore, the only practical means of solving the protein malnutrition problem is to greatly increase the production of the pulse crop. India is the largest producer and consumer of pulses in the world accounting for 37 percent of world area and 28 percent of the production. The area under the cultivation of pulses in India is around 29.99 million hectares with a production of 25.23 million tonnes (2017-18) Mohiuddin *et al*., 2018. (Annual report 2019-20, DAC&FW).

Black gram (*Vigna mungo* L.) is reported to have originated in India. It is an important grain legume with easily digestible protein and low flattulence content (Srivastava *et al*., 2011). It is an important pulse crop in South India especially in states such as Tamil Nadu, Karnataka and Andhra Pradesh as fermented food in the form of idli, dosa and vada. The speciality of blackgram in idli preparation is owing to the presence of mucilaginous material which is absent in other edible legumes. The mucilaginous material is a complex carbohydrate containing galactose and arabinose together with a small amount of rhamnose and galacturonic acid (Umadevi *et al*., 2008). In recent years, there has been increasing interest in the functional potential of plant proteins. In view of the increasing utilization of grain legumes in composite flours for various food formulations, the functional properties of legume flours are assuming greater significance as food ingredients on account of the functional characteristics and sensory qualities they impart to the end product. The functional properties of legume flours are provided not only by proteins, but also by the carbohydrates and other components such as pectins and mucilage (Kaur and Singh, 2007).

Moreover, the battering quality of blackgram is found to be deteriorating within 2-3 months after the harvest. This is a major problem faced by the farmers, traders and consumers. Bio-chemically, the soft spongy texture observed in the leavened steamed idli made out of blackgram is due to the presence of two components; surface active protein (globulin) and an arabinogalactan (polysaccharide) in blackgram. The mucilaginous principle of blackgram is identified as arabinogalactan. Arabinogalactan has a strong safety profile. It is believed that this mucilaginous principle helps in the retention of carbon dioxide during the

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fermentation of the thick batter prepared from rice and urdbean in the proportion 3:1 or 4:1 and is thus responsible for the soft spongy honeycomb texture of the idli (Susheelamma and Rao, 1978). Fermentation brings about physical and chemical changes in the idli batter. With the progress of fermentation there is an increase in the batter volume, acidity and non protein nitrogen.

A few studies have pointed out that improvement in the battering quality is imparted by the polysaccharides like galactoses, arabinogalactans *etc*., collectively called Pentosans (Baiying Cao *et al* 2016). Though many varieties and local types are in cultivation, the knowledge about the influence of genotypes in battering quality is scanty. MDU 1 (Madurai 1) blackgram variety was released from Tamil Nadu Agricultural University, Madurai, India during 2014. It gives a higher yield and possesses a high arabinose content of 7.5%. The volume of batter increased in the variety MDU 1 (Madurai 1) compared to the other existing varieties. However, this variety is susceptible to yellow mosaic virus (YMV) disease. The present study was carried out to find out urd bean mutants of MDU 1 and VBN (Bg) 4 with high yield and good battering quality.

MATERIALS AND METHODS

Two popular urd bean varieties MDU1 and VBN (Bg) 4 Were released from Tamil Nadu Agricultural University, Coimbatore. The seeds were obtained from Agricultural College and Research Institute, Madurai and National Pulse Research Centre, Vamban respectively. The blackgram seeds of MDU1 and VBN (Bg) 4 were irradiated with different doses (100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy) of gamma rays using Gamma chamber installed at Tamil Nadu Agricultural University, Coimbatore.

After mutagenic treatment, the seeds were sown in the field in a randomized block design along with control (the untreated seeds) during 2013-2014. The number of seeds that germinated on the 7th day was counted, the germination percent was calculated and the plants those survived from germination to maturity were counted and recorded. The shoot length and root length of 25 seedlings from each treatment was recorded on the 14th day from the day of soaking in paper cups and compared with the control. Pollen sterility was tested for each treatment by using two percent freshly prepared potassium iodide solution and examined under stereomicroscope. Dark stained and normal size pollen grains were considered as fertile while those of irregular shape and size with light or no stain were considered as sterile. The number of plants that survived till maturity were scored from each treatment and recorded as % survival and compared with the control.

For each treatment, well filled 500 seeds with uniform moisture content was used. For, gamma rays the seeds of MDU1 and VBN (Bg) 4 were treated with 100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy doses by utilizing the gamma rays facility available at Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. After completion of the treatment with gamma rays and their respective control seeds were sown immediately to raise the M_1 generation in a randomized block design (RBD) with three replications. All the survived plants in M_1 generation were forwarded to M_2 generation. The chlorophyll mutants and their segregation were recorded in M_2 generation. Two hundred high yielding mutants irrespective of the treatments were identified in M_2 generation and forwarded to M_3 generation. Thirty high yielding determinate mutants were selected and raised as M_4 generation. These mutant lines showed homozygosity with terminal pod and high yield.

These mutants were tested at three different environments; Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai, National Pulse Research Centre, Tamil Nadu Agricultural University, Vamban and Agricultural Research Station, Tamil Nadu Agricultural University, Vaigai Dam to test the stability of the seed yield over environments during 2015 along with two check varieties MDU1 and VBN (Bg) 4. During the summer 2016, the stable high yielding mutants were selected and tested in the preliminary yield trial at Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai. Four and one best high yielding mutants ACM 16 - 011, ACM16-015, ACM 16-018, ACM 16 -017, ACM 16-025 were identified from MDU-1 and VBN4 respectively and were tested in the Comparative Yield Trial and Advanced Yield Trials at Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai, India.

These five mutants were subjected to various biochemical tests like arabinose (%), albumin (%), globulin (%), total soluble protein (%), methionine (mg/g) and batter volume.

Seed protein content

Protein content was analysed by micro kjeldahl method. Both digestion and distillation was done by the use of the ''KELPLUS '' machine. Protein was analyzed by the amount of nitrogen available in the sample by micro kjeldahl method. One hundred mg of powdered seed sample was transferred into a 250 ml digestion flask along with three grams of catalyst mixture and 10 ml of concentrated sulphuric acid. The catalyst mixture consists of sodium sulphate and copper sulphate (5:1 ratio). The sample was digested until the solution became colourless.

The digested sample was placed in the distillation unit for ammonia recovery. The solution was distilled and the ammonia was collected in the receiver solution. The solution was titrated against 0.1N hydrochloric acid for the end point, until the colour changed. The same procedure was repeated to get the blank titrate value and the nitrogen content of the sample was calculated. The nitrogen value was multiplied by a factor of 6.25 to arrive at the crude protein content of the sample in percent.

Albumin and Globulin analysis

Albumin and Globulins were extracted from urd bean flour (250 mg) using 10 ml of distilled water at room temperature for 30 min and the extract was centrifuged at 3000 rpm for

30 minutes.The supernatant was used for the determination of a water soluble albumin by Lowry *et al.,* (1951) method. The residue was then extracted successively in a similar manner with 1.0 (% or N) NaCl. The supernatant of each extract was collected separately and used to estimate the salt soluble globulin.

Arabinose Analysis

Arabinose analysis was carried as per the protocol given by Bial (1902). The method depends on conversion of the pentose, arabinose in the presence of hot acid to furfural which then reacts with orcinal to yield a green colour.

Seed samples were powdered and 500 mg was transferred into a boiling tube and hydrolysed for 3 hrs. 2.5 N HCL was added to this and cooled to room temperature. It was neutralised by increasing the volume upto 50 ml with solid sodium carbonate. 1 ml of the working standard was pipetted out in a test tube and increased up to 3ml with water. 6ml of orcinal acid reagent and 0.4ml of 6% alcoholic orcinol was added to each tube, mixed well by shaking the tubes and kept in boiling water for 20 minutes. The tubes were cooled and absorbance was read at 660nm against the blank.

RESULTS AND DISCUSSION

The M_2 generation of MDU 1 and VBN 4 were raised on a single plant basis. Frequency of different types of chlorophyll mutants were counted and analysed and the same are provided in Table 1. The results revealed that chlorophyll mutations occurred in large numbers in almost all the doses studied. Five different types of chlorophyll mutants were observed *viz*., albino, xantha, chlorina, viridis and xanthaviridis. More number of albino (white color leaf) mutants was noticed in both the varieties. All the five types of mutants appeared in 100 Gy, 200 Gy and 500 Gy of MDU1 and 100 Gy, 300 Gy of VBN (Bg) 4. Among the different chlorophyll mutants, a high percentage (40%) of chorina mutants was observed in 300 Gy gamma rays. This observation in our study indicated that gamma ray irradiation was effective in producing a large proportion of chlorophyll mutants in blackgram varieties. The percentage of seed germination reduced progressively with the increasing dose of mutagen. The stimulating effect of gamma rays in germination may be attributed to the activation of RNA or protein synthesis and it may occur during the early stage of germination after the seeds are irradiated (Abdel - Hady *et al*., 2008). The frequency of chlorophyll mutation in M_2 generation is used as an index for mutation. According to Chopra (2005), the high frequency of chlorophyll mutations obtained with mutagen is due to the selective action of chemical and physical mutagens on genes (C-G rich chloroplast genome) responsible for chlorophyll development.

Chlorophyll mutation are considered to be the most dependable indices for evaluating the efficiency of different mutagens in inducing the genetic variability for crop improvement and are also used as genetic marker in basic and applied research Wani and Anis (2004). Types of chlorophyll mutants and their frequencies vary according to mutagens, genotype and method of treatment. The frequency of chlorophyll mutant was dose dependent in this present study.

Biochemical analysis

The top high yielding mutants ACM 16-011, ACM 16-015, ACM 16-018, ACM-16-025 and ACM 16-017 were subjected to biochemical analysis and their results are provided in Table 3. All the five mutants selected for high yield (> 10%) also recorded higher arabinose content than that of the parental varieties. Similar genetic relationship between yield and battering quality was reported in blackgram (Veni *et al*., 2016). The arabinose content was found to be higher in ACM-16-025. Albumin and globulin content were the highest in ACM-16-015. The mutants were tested for its batter volume which is responsible for idli making.

The increased batter volume was found in the mutants ACM-16-011, ACM-16-015, ACM-16-018 and ACM-16-017. Biochemically, the soft spongy texture observed in the

Table 1: Frequency of different types of chlorophyll mutants in M_2 generation of MDU 1 and VBN (Bg)4.

Mutagen	Total chlorophyll mutants in M_2 generation	Relative percentage of chlorophyll mutants (%)				
MDU1 - Gamma rays(Gy)						
		Albino	Xantha	Chlorina	Viridis	Xanthaviridis
100	310	26.45	19.68	27.87	11.67	14.33
200	260	26.54	34.27	18.15	8.94	12.10
300	125	24.00	27.04	40.00	3.24	5.72
400	110	59.18	19.64	21.18	-	-
500	104	42.88	33.75	13.75	9.62	-
VBN (Bg) 4 – Gamma rays (Gy)						
100	299	24.78	22.81	21.44	18.13	12.84
200	248	29.03	22.98	27.82	20.16	-
300	108	34.26	18.98	22.50	13.89	10.37
400	87	35.86	17.24	22.07		-
500	74	32.43	24.32	28.38	14.86	-

leavened batter in the steamed idli made out of blackgram is due to the presence of two components, namely surface active protein (globulin) and an arabinogalactan (polysaccharides) in blackgram. The mucilaginous principle of blackgram is a complex carbohydrate containing galactose and arabinose together with a small amount of rhamnose and galacturonic acid (Umadevi *et al*., 2008). Increased batter volume is a desirable property for idli preparation with soft texture. Arabinose is an aldopentose – a monosaccharide containing five carbon atoms and including an aldehyde (CHO) functional group. (Bhat *et al*., 1986) recorded presence of arabinose in blackgram husk and endosperm. The compositional data of blackgram seed is apparent that the cold-water soluble fraction of the husk is predominantly a hexosan – type polysaccharides, whereas the hot water soluble fraction is pentosans type. The hot-water soluble fraction of the endosperm was essentially glucan-type polysaccharides. The pectic fraction from both husk and endosperm contained arabinose which is responsible for the mucilage characteristic of blackgram flour (or) seed.

In the present study, arabinose, globulin and batter volume of the mutants ACM-16-011, ACM-16-015, ACM-16-017 and ACM-16-018 were increased over the parent MDU1 blackgram variety in Table 2. The increase in batter volume could lead to an increase in the number of idli, dosa and vada which are common foods of India. The results obtained in the present investigation are in accordance with that of Steinkraus *et al*. (1967). With a limited quantity of blackgram, it is possible to produce more batter volume by using these blackgram mutants. MDU1 variety is superior in batter volume and more globulin content compared to other blackgram varieties available in the country. This finding is in accordance with Umadevi and Meenakshi Ganeshan (2008), Veni (2012) and Sangeetha (2014) in black gram. The lactobacillus activity is higher in before and after fermentation of the idli batter present in the blackgram mutants than that of the control (Vanniarajan *et al.,* 2019). The blackgram mutants of MDU1 surpassed the parent and recorded a breakthrough. These mutants were raised during April 2017 and subjected to YMV screening. These four mutants were moderately resistant when compared to MDU 1 was moderately susceptible. These mutant again have to be raised in the hotspot areas of Yellow Mosaic virus for further confirmation.These mutants will be further utilized for crossing programm or as such promoted for various trials to release as a variety (Table 4). The mutants with improved yield and battering quality will be useful for further breeding work in urdbbean.

Table 2: Biochemical analysis in urd bean mutants.

Biochemical analysis	Genotypes								
	ACM 16 -011	ACM 16-015	ACM 16-018	ACM 16-017	MDU 1	Mean	SE	CV %	CD (%)
Arabinose (%)	8.28	8.98	8.14	8.18	8.08	8.92	0.50	13.75	5.61
Albumin (%)	8.14	8.94	6.85	6.81	6.98	7.75	0.37	11.78	4.77
Globulin (%)	11.69	12.23	11.97	10.05	9.94	11.18	0.44	9.72	3.94
Total soluble protein (%)	23.1	24.2	23.5	25.1	24.13	23.73	0.38	3.97	1.60
Methionine (mg/g)	3.60	2.00	3.60	2.0	3.60	3.04	0.39	31.69	12.83

Biochemical analysis	Genotypes					
	ACM 16 -025	VBN 4	Mean	SE	CV %	CD (%)
Arabinose (%)	11.03	7.28	8.92	0.50	13.75	5.61
Albumin (%)	8.02	10.25	7.75	0.37	11.78	4.77
Globulin (%)	9.97	9.94	11.18	0.44	9.72	3.94
Total soluble protein (%)	22.73	23.54	23.73	0.38	3.97	1.60
Methionine (mg/g)	4.00	4.00	3.04	0.39	31.69	12.83

Table 3: Comparison of batter volume prepared from mutants for Idli preparation.

Mutant	Initial batter volume for Idili (ml)	Final batter volume for idli (ml)	Increased batter volume (ml)	Batter volume over MDU 1 in %
ACM-16-011 (Mutant of MDU1)	50	160	110	12.24
ACM-16-015(Mutant of MDU1)	50	164	114	16.33
ACM-16-018(Mutant of MDU1)	50	155	105	7.14
ACM-16-017 (Mutant of MDU1)	50	158	108	10.20
ACM-16-025 (Mutant of VBN4)	50	136	86	-12.24
MDU 1	50	148	98	
Mean	50	153.5	103.5	
SE	-	4.13	4.13	
CV%	-	6.59	9.77	

Table 4: Yield performance of the selected mutants.

Name of the trial	Genotypes (Mutants of MDU 1)										
	ACM 16-011		ACM 16-015		ACM 16-018		ACM 16-017		MDU 1		
	Days to maturity	Grain yield kg/ha	Days to maturity	Grain yield kg/ha	Days to maturity	Grain yield kg/ha	Days to maturity	Grain yield kg/ha	Days to maturity	Grain yield kg/ha	CV (%)
IET (Rabi, 2015)	71	1102	70	1028	71	1089	69	1029	73	945	7.18
PYT (Jan, 2016)	73	1063	68	952	70	1008	66	1085	75	932	6.48
CYT (Sep, 2016)	73	1006	69	955	70	945	70	1035	75	915	8.00
AYT (Jan, 2017)	70	1095	71	1008	69	995	69	1153	73	956	7.54
Mean	71.75	1066.5	69.5	985.75	70	1009.25	68.5	1075.5	74	937	
% increase MDU 1		19.34		11.69		15.63		20.12			

Name of the trial	Genotypes				
	ACM 16-025 (mutant of VBN (Bg) 4		VBN 4 (Bg) 4		CV (%)
	Days to maturity	Grain yield kg/ha	Days to maturity	Grain yield kg/ha	
IET (Rabi, 2015)	71	999	76	825	7.18
PYT(Jan, 2016)	72	917	76	863	6.48
CYT(Sep, 2016)	70	898	76	875	8.00
AYT(Jan, 2017)	71	864	75	812	7.54
Mean	71	919.5	75.75	843.75	
% increase VBN 4		15.85			

IET- Initial evaluation trial, PYT- Preliminary yield trial, CYT – Comparative Yield Trial, AYT – Advanced Yield Trial.

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