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ORIGINAL RESEARCH

Induction and Inheritance of Determinate Growth Habit in Blackgram (*Vigna mungo* (L.) Hepper)

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ABSTRACT

The purpose of this study is to evaluate the effects of DT induction and inheritance in black gram (*Vigna mungo* L. Hepper) using mutagenesis. Since black gram is mostly a self-pollinating species, hybridization is not an option; therefore, mutant breeding is a good way to get desirable traits. Two black gram cultivars, AKU-15 and Black Gold, were subjected to treatments of gamma irradiation (100-400 Gy) and electromagnetic fields (0.1-0.4%) in order to promote determinate development. With a primary emphasis on the 300-400 Gy gamma and 0.3-0.4% EMS treatments, the study investigated DT mutants with terminal and axillary inflorescences. The evidence of a recessive digenic epistatic model for DT development in the segregating generations (F1 to F4), with a ratio of 3:13. Studies have shown that by breeding mutants, a consistent growth habit may be achieved, which could be used as a genetic resource for better black gram cultivation.

KEY WORDS: Black gram, Determinate growth, Inheritance, Mutation breeding, *Vigna mungo*

INTRODUCTION

One of the most important pulse crops is Black gram, scientifically known as *Vigna mungo* (L.) Hepper., belongs to the family Leguminosae and subfamily Papilionaceae. The crop under study has a chromosomal number of 22 (Bhatnagar *et al*., 1974). It primarily displays cleistogamous traits and is self-pollinating. Black grams are best grown on loam or sandy loam soils that have good drainage from the inside (Goyal *et al*., 2019). Black gram was grown on 3.24 million hectares in India during 2015 and 2016, yielding 1.95 million tons.

Black gram is difficult to hybridize since it is autogamous and self-pollinating. Plant breeders rely on mutation induction as a key tool to increase diversity in crop breeding materials. According to Toker (2007), traditional plant breeding methods can be made more effective through mutation breeding. By improving a specific attribute while maintaining the basic genetic structure of the cultivar, mutation breeding provides numerous benefits in plant improvement (Chopra, 2005). It would be useful to induce favourable macromutations to increase genetic diversity and then use trait-specific DNA for improving crops in the future. Terminal blooming and improved vegetative development in black gram cultivars are achieved only by induced mutation. Certain genetic material accessions show terminal flowering behaviour but have poor vegetative growth, which results in less-than-ideal plant shapes and poorer yields when contrasted with the indeterminate type. This study set out to understand how the (DT) trait is passed down through generations by using mutagenesis to enhance DT growth in a plant with desirable agronomic traits.

MATERIAL AND METHOD

Two varieties of black gram, AKU-15 and Black Gold, used as plant components. They are adaptable but produce less overall. The experimental seeds for both varieties obtained from Plant Breeding Section of Dr. Panjabrao Krishi Vidyapeth Akola, Maharashtra.

Raising M1 generation

The uniform, healthy, and dried seeds of each variety (with a moisture content of 10 -12%) were selected for radiation. The gamma radiation doses of 100, 200, 300, and 400 Gy using Gamma Chamber (Model-900), was at the

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Determinate Type in 400 Gy

Control of *Vigna mungo*

Fig. 1: Determinate and control Type, *Vigna mungo*

Department of Chemistry, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, Maharashtra, India. Consistently healthy seeds of each variety were soaked in distilled water for nine hours prior to being treated with varying concentrations of EMS (0.1, 0.2, 0.3, and 0.4 %) for six hours, with periodic shaking at a temperature of 22± 1°C, in order to conduct chemical mutagenesis. To cultivate the M1 generation, treated seeds from each dosage and a control were sowed the following morning during the Kharif season.

Conditions during the growing season ranged from 30 to 35°C on average, with humidity levels between 50 and 57% and daylight durations between 12 and 13 hours. Treated and control varieties were seeded three times with 100 seeds each at the Agriculture Farm in Nandura, District Buldhana, Maharashtra using a randomized complete block design (RCBD). Within each row, the inter-seed spacing was always 30 cm and 60 cm. The field was prepared, seeds were sown, and the black gram density was managed using agronomic techniques. Harvested in October, the crop's seeds were saved for the following year and planted in the field to produce M2 generation.

Raising M2 generation

During the kharif season's M2 generation, 27 seeds were sowed in plant progeny rows from each M1 plant that appeared normal across all treatments, along with their corresponding controls in each category. The progeny count was 50 in both the treatment and control groups. The seed spacing 30 cm and the row spacing between 60 cm were maintained and the experiments (trials) replicated thrice for each therapy.

Three hundred and seventy IDT F2 plants were hand-picked, and the following generation grew offspring from those plants. The number of DT and IDT plants, with in each progeny, as well as the number of segregating and non-segregating progeny involving more than fifteen plants were counted. While the F3 progenies were separated into DT and IDT plants, and large number of IDT plants were taken from the F4 population. The pollen fertility of both DT and IDT plants was determined by staining their pollen grains with a 2% acetocarmine solution. Under a light microscope, the pollen grains were visible as they displayed consistent shape and dark

Fig. 2: Flower of *Vigna mungo*

staining. The results showed that the M2 group that received the 400 Gy treatment produced a plant with a definite growth habit. Pods could not be autonomously produced by the plant, despite the abundance of flowers. In terms of pollen and plant morphology, the DT plant stood out from the control group with its unusual shape, stronger growth pattern, and fewer leaflets per leaf. The flowers were deformed; they did not have a gynoecium and had extra sepals and petals. After staining to the anthers with acetocarmine stain and observe under the light microscope, the pollen grains were considered as fertile if they were darkly stained. In contrast to IDT, DT pollen was more spherical, smaller, and less reticulate.

RESULTS AND DISCUSSION

The results of the present study suggest that a plant with DT growth habit in the M2 population of the 400 Gy treatment, plant flowered profusely, but failed to form pods naturally.

Plant and pollen morphology

The DT plant was morphologically determinate, had a bushy growth habit and a reduced number of leaflets per leaf compared with control. The flowers were malformed with repeats of sepal- and petal-like structures and lacked a gynoecium. The anthers contained pollen that appeared almost normal when stained with acetocarmine and examined under the light microscope. It revealed the pollen from DT plants to be more rounded, smaller, and less reticulated than pollen from IDT.

The software LINKAGE-1, developed by Suiter (1983), was used to examine the F2 data for trait inheritance. LINKAGE-1 uses chi-square analysis, which was developed by (Subha Srinivasan, 2006), to evaluate the goodness-of-fit to anticipated ratios.

All other mutagenic treatments were outdone by 200 Gy of gamma radiation in terms of seedling damage (Khursheed, 2018). The AKU-15 variety was shown to be most susceptible to pollen sterility when exposed to 100 Gy gamma rays, while other mutagen treatments, including 0.2% EMS, were less effective. It lowered mortality and produced pollen sterility for the JS-335 variety, the EMS treatment (0.05%) was the most effective.

Satpute (2012) reported that gamma rays (20Kr and 30Kr) and EMS treatment (0.15% mortality) had the lowest efficiency levels. The similar results have been observed in other studies reported earlier; Sassi Kumar *et al*. (2003), in limabean, Sharma *et al*. (2006), in urdbean, Badere and Choudhary, (2014) in linseed, Dhanavel *et al.* (2008), Girija & Dhanvel (2009) and Ashok Kumar *et al.* (2009), in cowpea, Bhosle & Kothekar (2010), in cluster bean, Giri & Apparao (2011) in pigeon pea, and many more studies have shown that the effectiveness of higher doses of mutagens decreases as their concentrations or dosages increase. In Satpute *et al.* (2012), study showed that that pollen sterility is becoming more common in DT development. High mutation rate with minimal deleterious effects is a hallmark of a mutant breeding program. Significant developmental damage, infertility, and other negative effects are common outcomes of mutagens that increase the mutation rate (Blixt, 1964).

The results of the present study showed that mutants showed terminal and axillary inflorescence as part of their determinate growth. All gamma ray treatments and 0.4% EMS of Black-gold as well as AKU-15 treated to 300 and 400 Gy gamma irradiation contained this mutation. About 25 mutants isolated were determinate type and promoted 10 that produced the highest yield to the next generation.

Inheritance of the determinate character

Since the F1, plants showed both normality and IDT, we could conclude that DT development is recessive. With 379 IDT plants and 81 DT plants, the DT: IDT ratio was 1:4.68 in the F2 generation. Instead of supporting the idea of DT growth, characteristic monogenic inheritance, these points to a 3:13 ratio of digenic epistatic inheritance, is still not confirmed. Data sets F3 and F4 show the overall number of DT and IDT plants, the number of DT and IDT

Table 1: Segregation of determinate and indeterminate

Genotype	Observed numbers			Expected ratio			X^2	P %
	DT	IDT	Seg	DT	IDT	Seg		
Control	-	+	-	-	+	-		
AKU-15	-	+	-	-	+	-		
Black gold	-	+	-	-	+	-		
F1 plants	-	+	-	-	+	-		
F2 plants	81	379	-	3	13	-	0.394	0.50 - 0.60
F3	230	792	-	5	19	-	1 .732	0.15 - 0.20

1. - = none; + = all
2. Segregating progenies with 15 or more plants.

plants within each progeny, and the quantity of segregated progeny, respectively, lending credence to the notion. When dealing with plant traits, it is acceptable to use the approved notation cd for the recessive allele that determines the phenotype and Dt for the allele that controls it (Muehlbauer & Singh, 1987).

A soybean mutant that is male-sterile and female-sterile (w4-m sterile) was found in a gene-tagging study of germinal revertants. It is important to learn everything about the w4-m sterile's cytology (microsporogenesis and microgametogenesis) and genetics (inheritance, allelism, and linkage). There was just one recessive nuclear gene that had the mutation, and it was not related to the st2, st3, st4, st5, and st6 mutants that are known to be male-and female-sterile. The w4-m sterile mutant did not show any association with the w4w4, y10, y11, y20, fr1, and fr2 alleles (Palmer & Horner, 2000).

Limiting excessive vegetative development and lodging is the goal of the DT growth habit. In the present study it was found that the DT plants tested were sterile in females. A single (Cd cd Dt Dt) or double (Cd cd Dt dt) heterozygous gene arrangement is required by the cd cd Dt allelic combination and to do this, the seeds from Cd cd and Cd cd specimens need to be utilized.

CONCLUSION

This study used gamma irradiation and ethylmethane sulphonate (EMS) to successfully establish a determinate (DT) growth habit in black gram (*Vigna mungo*) plants. A terminal inflorescence followed by axillary flowering improved vegetative development in the DT mutants. The complex genetic regulation of DT development has been validated by genetic studies, which have shown that it follows a recessive digenic epistatic inheritance pattern (3:13 ratio). These findings demonstrate that mutant breeding is a powerful tool for creating novel genetic variants in crops without compromising their underlying genetic makeup. Future black gram breeding efforts can benefit greatly from the identified DT mutants with high yield potential, which can improve crop structure and productivity.

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