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INHERITANCE OF QUALITATIVE TRAITS AND THEIR LINKAGE IN BLACKGRAM [*VIGNA MUNGO* (L.) HEPPER]

MUHAMMAD ARSHAD, ABDUL GHAFOOR AND AFSARI SHARIF QURESHI[1]

National Agricultural Research Center, Islamabad, Pakistan.
E. mail: marshadnarc@hotmail.com

Abstract

Four parents (Mash 1, Mash 3, MM 33-40, and 45726) were used to study inheritance of four qualitative characters, i.e., pubescence, seed coat colour, presence of spots on the seed coat and pod colour. All the four traits revealed monogenic nature of inheritance segregating in Mandelian ratio (3:1). The hairiness pattern was observed dominant over non-hairiness; brown seed coat colour dominant over green seed coat colour. Presence of spots on seed coat was dominant to absence of spots and black pods were dominant over brown pods in blackgram. Out of three hybrids, two (Mash 1/MM33-40 and 45726/MM33-40) revealed linkage between pod colour vs presence of spots on seed coat and pod colour vs seed coat colour that is suggested to be used for preliminary mapping in blackgram.

Introduction

Polymorphic monogenic traits were some of the earliest genetic markers employed in scientific investigations and they may still be optimal for genetic, breeding and plant germplasm management. Although morphological markers are limited in nature but their assays neither require sophisticated equipments nor complicated procedures (Singh & Singh, 1992). Monogenic or oligogenic morphological markers are generally simple, rapid and inexpensive to score (Ghafoor, 1999). Until recently scientific plant classification was based exclusively on morphological traits (Stuessy, 1990), some of which may serve as genetic markers suitable for plant germplasm management (Gottlieb, 1984; Hilu, 1984; Stanton *et al*., 1994). The amount of information provided by marker based approach would depend on the type and number of markers and their linkage relationship (Singh *et al*., 1991; Tahir *et al*., 1995).

Koenig & Gepts (1989) in *Phaseolus* and Ghafoor *et al*., (2003) in blackgram studied morphological markers inheritance and reported monogenic inheritance for traits. Sirohi *et al*., (1998) reported that non-hairy character of pods in blackgram was mono-factorial recessive in nature. Monogenic inheritance of seed coat texture in cowpea was reported by Kehinde & Ayo-Vaughan (1999). Chen *et al*., 2001 reported that the inheritance of black and green seed colour in mungbean was controlled by one gene and seed luster possibly controlled by more than two genes. The objectives of the present study were to investigate the genetic basis of qualitative characters (plant pubescence, seed coat colour, pod colour and presence of spot on the seed) and their validity in determining linkage for utilization in plant breeding.

Materials and Methods

The genotypes used for this study included approved varieties (Mash 1 and Mash 3); advance breeding lines (45726) and pure-lines originally obtained from AVRDC (MM 33-40). The botanical descriptions of parents are given in Table 1. For present study a set of crosses were made during 1998 using the techniques reported by Ghafoor *et al*., (1999).

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[1]Department of Biological Sciences, Quaid-i-Azam, University, Islamabad, Pakistan.

Table 1. Botanical descriptors of parents used in hybridization of blackgram.

Genotype	Source	Plant pubescence	Seed colour	Presence of spot on seed coat	Pod colour
Mash 1	Pakistan	Glabrous	Brown	Present	Black
Mash 3	Pakistan	Pubescent	Brown	Present	Black
MM 33-40	AVRDC	Pubescent	Green	Absent	Brown
45726	Pakistan	Pubescent	Brown	Present	Black

Table 2. Segregation for four qualitative traits in F_2 population in blackgram.

Plant character/Crosses	Observation				
Plant pubescence	**HH, Hh**	**Hh**	**Expected atio**	**χ^2**	**P at 5%**
Mash 1/MM 33-40	77	23	3:1	0.213	0.644
Mash 3 /Mash 1	83	24	3:1	0.376	0.539
Pod colour	BB, Bb	Bb			
Mash 1/MM 33-40	72	28	3:1	0.480	0.488
45726 /MM 33-40	54	18	3:1	0.665	0.412
Seed coat colour	CC, Cc	Cc			
Mash 1/MM 33-40	72	28	3:1	0.480	0.488
45726/MM 33-40	86	21	3:1	1.648	0.200
Presence of spot on seed coat	SS, Ss	Ss			
Mash1 /MM 33-40	78	22	3:1	0.480	0.488
45726 /MM 33-40	53	19	3:1	1.251	0.174

HH= Dominant homozygous hairy, Hh= heterozygous hairy; BB= Homozygous black, Bb= Heterozygous black; CC= Homozygous brown, Cc= Heterozygous brown; SS= Homozygous spots present, Ss= Heterozygous spots present

The seeds obtained from three crosses (Mash 1/MM33-40, Mash 3/Mash 1 and 45726/MM33-40) were divided equally into two parts. Hybrid seeds were planted under field conditions at NARC along with parents during next year in summer season to raise F_1 population. The remaining hybrid seeds were preserved in the gene bank. During summer 2000, the remaining hybrid seeds were planted along with the harvested seed from F_1 populations and parents. Plants were allowed to grow in an insect and weed free natural field conditions at 30-42°C. Data for plant pubescence was recorded at the 50% flowering stage. The spots on seed coat and pod colour were recorded after harvesting individual plants at maturity. Data thus recorded were analysed with the help of chi-square (X^2) most commonly used to test hypothesis concerning the frequency distribution of one or more populations. In this study, we used X^2 for a fixed ratio hypothesis using data from F_2 segregating population of each cross as described by Gomez & Gomez (1984).

Results

Pubescence: Two hybrids, i.e., Mash 1/MM33-40 and Mash 3/Mash 1 were investigated for pubescence. Out of parents used in these crosses Mash 1 was glabrous, whereas others (MM 33-40 and Mash 3) were having hairs on plants. The allelic notion for this character was assigned as *HH* (dominant homozygous hairy); *Hh* (heterozygous hairy) and *hh* (homozygous recessive non-hairy). The F_1 plant were all having hairs for both the hybrids either female parent was kept pubescence or glabrous, suggesting *hh* alleles recessive to *HH*, *Hh* types. The F_2 segregation for these crosses showed 3:1 ratio that fit for goodness by χ^2 method (Table 2). This 3:1 ratio indicated the monogenic nature of this character.

Pod colour: Two crosses (Mash 1MM33-40 and 45726/MM33-40) gave segregation for pod colour. Two types of pod colour have been observed in blackgram germplasm, i.e., black and brown and these were assigned allelic notion *BB* (homozygous black); *Bb* (heterozygous black) and *bb* (recessive brown). Pod colour was recorded in F_1 and F_2 on the basis of black or brown colour and the data were analyzed for inheritance. Among parents used in the study, MM 33-40 has brown pod, whereas others had black pod colour. All the F_1 plants were observed as black pod colour for each hybrid which revealed the presence of dominance for black pod colour, whereas brown pods being recessive in blackgram. The F_2 population segregation in a 3:1 ratio for all the crosses which fit for goodness by χ^2 method with slight variation in probability (Table 2). This ratio indicated the monogenic nature of this character.

Seed coat colour: The observation on seed coat colour was taken at maturity in the crosses (Mash 1/MM33-40 and 45726/MM33-40). One parent (MM 33-40) produced green seed coat colour and hence it was used for hybridization with other contrasting parents. The allelic notion was given as *CC* (homozygous brown); *Cc* (Heterozygous brown) and *cc* (recessive green). The F_1 having brown seed coat colour with slightly diffused black spots for both the hybrid, either female parent was kept brown or green, suggesting the dominance of brown seed coat in nature, whereas green being recessive. The F_2 population segregated in a 3:1 ratio for both the crosses in which χ^2 did not fit well for 3:1 ratio (Table 2). This 3:1 ratio indicated the monogenic nature of seed coat colour in crop.

Spots on seed coat: Presence or absence of spots on seed coat colour were observed on freshly harvested seeds and analysed for inheritance (Table 2). Allelic forms were assigned as *SS* (homozygous spots present); *Ss* (heterozygous spots present) and *ss* (recessive spots absent). The parental line MM 33-40 was without spots and green seeded. All the F_1 plants of two crosses (Mash 1/MM33-40 and 45726/MM33-40) were observed with spots for both either female parent was kept spotted or un-spotted which indicated the dominant nature of this character, whereas the un-spotted nature was recessive. The F_2 population segregated in a ratio of 3:1 for all the crosses which fit for goodness by χ^2 method with slight variation in probability. This 3:1 ratio revealed the presence of monogenic gene action for phenotypic expression on seed coat of blackgram.

Linkage analysis: Inheritance of qualitative characters revealed that single dominance gene was involved for pubescence (*HH, Hh*), seed coat colour (*GG, Gg*), seed spots (*SS, Ss*) and pod colour (*BB, Bb*). Further, analysis of linkage among these characters was carried out and the results regarding linkage loci are presented in Table 3. Three hybrids (Mash 1/MM33-40, Mash 1/45702 and 45726/MM33-40) were investigated for the linkage analysis among genetic markers in F_2 generation using the computer programme “LINKAGE 1” of Suiter *et al*., (1983). This joint segregation of independent assortment revealed linkage for various characters pairs.

The hybrid Mash 1/MM33-40 revealed linkage of three characters pairs i.e., black pod colour vs seed coat colour producing 92% parental types, pod colour vs presence of spots on seed coat with 88% parental types and seed coat colour vs spots on seed that produced 94% parental type (Table 3).

Table 3. Joint segregation for four morphological markers in three F_2 populations in blackgram.

Hybrid	Number of plants/observations								
	Loci	**-/-**	**-/+**	**+/-**	**+/+**	**χ^2**	**P**	**r**	**Parental type (%)**
Mash1 /MM 33-40	HH:BB	6	17	14	63	0.692	0.406	0.43±0.08	69
	HH:GG	7	16	21	56	0.087	0.767	0.48±0.07	63
	HH:SS	7	16	15	62	1.238	0.266	0.42±0.08	69
	BB:GG	20	0	8	72	64.286	0.000	0.85±0.10	92
	BB:SS	15	5	7	73	40.924	0.000	0.14±0.09	88
	GG:SS	22	6	0	72	72.527	0.000	0.06±0.09	94
Mash 1/45702	HH:BB	3	75	19	111	5.977	0.014	0.25±0.06	54
45726/MM 33-40	BB:GG	21	0	33	18	9.882	0.002	0.34±0.10	54
	BB:SS	18	3	5	46	39.43	0.000	0.11±0.12	88
	GG:SS	19	35	4	14	1.043	0.307	0.41±0.10	45

-/- and +/+ are homologous recessive and homologous dominant, whereas, -/+ and +/- are heterozygous dominant. HH- denotes plant hairiness; GG- seed coat colour; SS- the spots present on seed coat and BB- black pod colour.

Discussion

In the present study, all the four qualitative traits revealed monogenic segregation in 3:1 ratio. Hairiness was dominant over non-hairiness or glabrous; brown seed coat colour was dominant to green; presence of spots on seed coat was observed dominant over absences of spots and black pod colour was dominant to brown. Monogenic markers are useful in estimating the rate of crossing in predominantly self pollinated crops like blackgram (Senapati & Roy (1990). They also help in identification of F_1 hybrids in the breeding programme. Heterozygous are not possible to detect in case of complete dominance for morphological markers. Sen & Jana (1963) and Ghafoor *et al*., (2003) reported inheritance of pod colour and found black colour dominant over brown colour in blackgram.

Joint segregation of character pairs revealed normal distribution of independent assortment (9:3:3:1) for most of the character pairs but some distorted segregation were also observed that indicated linkage for these characters pair. The character pairs *HH* vs *BB*, *HH* vs *GG*, and *HH* vs *SS*, segregated in a normal independent assortment while the remaining pairs i.e. *BB* vs *SS, BB* vs *GG* and *GG* vs *SS* were linked in all cases. Deviation from normal assortment might be due to linkage for some alleles, and this type of distorted ratios have been observed by Kazan *et al*., (1993) in chickpea; Zamir & Tadmor (1984); Muehlbauer *et al*. (1989) in lentil; Weeden & Marx (1987) in pea; Koenig & Gepts (1989) in *Phaseolus* and Ghafoor *et al*., (2003) in blackgram.

As linkage between black pod colour and presence of spots on seed coat in two different hybrids may be due to genes for these two characters from same origin. Ghafoor (2000) observed similar result between these two traits in blackgram. Morphological markers are limited in plants especially, blackgram because limited genetic work has been conducted on this crop. Five morphological loci have been reported by Kazan *et al*., 1993 in chickpea. The identified linkage in the present study is suggested to be used for initial mapping of genome as there is no information of this type in blackgram. The arrangements proposed were based on linkage observed between genetically diverse cultivated blackgram in the present study. The usefulness of the mapped marker loci should be realized when loci affecting QTLs including diseases and other economically important genes are added to the linkage group. The use of closely linked markers should facilitate breeding by giving a unique identity by tagging the genes of economic importance and by providing a mean of selection in the absence of nurseries and screening procedures that can be costly and time consuming. It is suggested to utilize diverse parents for both qualitative and quantitative traits for planning experiments for inheritance and mapping. Further, enhancement of markers (morphological, protein and DNA) is suggested to have a precise understanding of linkage groups in blackgram.

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