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USE OF SDS-PAGE MARKERS FOR DETERMINING QUANTITATIVE TRAITS LOCI IN BLACKGRAM [*VIGNA MUNGO* (L.) HEPPER] GERMPLASM

ABDUL GHAFOOR, ZAHOOR AHMAD AND MUHAMMAD AFZAL

Plant Genetic Resources Institute (PGRI),
National Agricultural Research Centre (NARC), Islamabad, Pakistan.
Email: ghafoor59@hotmail.com
Phone 92-51-9255203, Fax 92-51-9255201

Abstract

One hundred and five genotypes of blackgram from diverse origin were evaluated for agronomic traits for 2 years and seed proteins were analyzed using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) through vertical slab type unit. Screening analysis for markers to quantitative traits revealed its significance in determining quantitative trait loci (QTL) in blackgram through SDS-PAGE markers. The factors affecting quantitative traits may occur as individual genes or gene clusters scattered throughout the genome, therefore, quantitative traits were expected differently at several loci. Variation in seven quantitative traits out of ten was significantly associated with 9 protein sub-units, however, the actual number of QTLs might be fewer because several of these traits were correlated. Variation at protein peptides in the vicinity of QTL in blackgram may be an indication of genetic variation potentially available to breeding programmes. Expansion of genetic base for blackgram breeding might be accomplished by systematic use of germplasm that differ from common banding pattern and known to be associated with variation in quantitative traits.

Introduction

Among biochemical techniques, Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is most widely used due to its validity and simplicity for describing genetic structure of crop germplasm. The SDS-PAGE is a practical reliable method because seed storage proteins are largely independent of environmental fluctuation (Gepts 1989 and 1990, Murphy *et al.,* 1990). Genetic markers are useful in screening germplasm in minimum time and labour (Nakajima *et al.,* 1994). Seed protein patterns obtained by electrophoresis have been successfully used to resolve the taxonomic and evolutionary problems of crop species (Khan 1992; Rao *et al.,* 1992). One of the approaches to use biochemical data in germplasm is to assess its association with quantitative traits that helps in screening crop germplasm for identified markers (Ghafoor 2000).

Detection of Quantitative Trait Loci (QTL) into individual genetic components by use of biochemical markers has been demonstrated in tomato (Tanksley *et al.,* 1982), garden pea (Kneen *et al.,* 1984) and lentil (Hoffman *et al.,* 1986; Tahir and Muehlbauer 1995). The researchers can use genetic similarity information to make decision regarding the choice for selecting superior genotypes for improvement or to be utilized as parents for the development of future cultivars through hybridization. For blackgram, this type of study has not yet been carried out, although it is an important summer pulse crop of many

South Asian countries including Pakistan, India, Nepal, Bangladesh, Thailand and Korea. Blackgram has been identified as a potential crop in many countries of the world and has received attention by the researchers (Ghafoor *et al.,* 2001). In Pakistan it is cultivated under a wide range of agro-ecological zones particularly under rainfed conditions. The present study was initiated to investigate association between SDS-PAGE and quantitative traits.

Materials and Methods

A set of 105 genotypes was evaluated for quantitative traits during summer seasons (mid July to end October) of 1999 and 2000 in an augmented design. Prior to experimentation, genotypes were self pollinated for two years to establish homozygosity. Two rows of 4 meter length for each genotype were planted with 10 and 75 cm intra and inter-row spacing. Approved variety, Mash 1 was repeated as check after every 10 rows. Pesticides were sprayed to protect the crop from pests especially white fly (*Bemisia tabaci* Genn.), a vector for Mungbean Yellow Mosaic Virus (MYMV). Data were recorded following IPGRI descriptors for *Vigna mungo* and *V. radiata* (IBPGR 1985). Days to flowering and to maturity were recorded at 50% of flowering and 90% maturity and these variables were represented by a single value for each genotype. Branches/plant, pods/plant, grain yield (g) and biomass yield (g) were recorded on 10 plants sampled randomly. Pod length (cm) and seeds per pod were recorded on ten pods sampled at random within each genotype. The 100-seed weight was recorded in grams and harvest index was determined as economic yield expressed in percentage over total biomass.

For extraction of proteins, single seed was ground to fine powder with mortar and pestle. Sample buffer (400 µl) was added to 0.01 g of seed flour as extraction liquid and mixed thoroughly in Eppendorf tube with a small glass rod. The extraction buffer contained the following final concentrations: 0.5 M Tris-HCl (pH 6.8), 2.5% SDS, 10% glycerol and 5% 2-mercaptoethanol. Bromophenol Blue (BPB) was added to the sample buffer as tracking dye to watch the movement of protein in the gel. Seed protein was analyzed through slab type SDS-PAGE using 11.25% Polyacrylamide gel. In order to check the reproducibility of the method two separate gels were run under similar electrophoretic conditions. The molecular weights of the dissociated polypeptides were determined by using molecular weight protein standards "MW-SDS-70 kit" from Sigma Chemical Company, USA. The SDS-PAGE of total seed protein was carried out in the discontinuous buffer system according to the method of Laemmli (1970).

From the perspective of statistical genetic analysis, genetic-marker data fall into two broad categories; 1) quantitative traits (e.g. many agronomical features) with continuous variation governed by several to many genes; and 2) biochemical or morphological data e.g., with discrete phenotypes governed by one to several genes. Importantly, these two types of traits may simply be variants of a single genetic theme, distinguishable only by the magnitude of allelic substitution effects (Comstock 1978, Robertson 1989). The quantitative data and SDS-PAGE data were analyzed for association of biochemical markers with genes affecting the variation of QTL. The group means of quantitative characters were calculated, and *t-test* was applied to compute the probabilities that two group means were equal.

Table 1. Summary of protein peptides observed in blackgram

	MW	Band No.	Total	Polymorphic	Significant for QTLs
Region I	>66.0kd	B1, B2, B3, B3a, B3b, B3c	6	5 (B1, B2, B3, B3a, B3b)	3
Region II	34-66kd	B4, B4a, B5, B5a, B6, B7, B8, B9, B9a, B9b	10	5 (B4a, B5, B5a, B9a, B9b)	3
Region III	24-34kd	B9c, B10, B10a, B10b, B11, B11a, B12, B12a, 12b, B13, B13a	11	8 (B10, B10a, B10b, B11a, B12a, B12b, B13, B13a)	3
Total			**27**	**18**	**9**

Results

Seed protein profiles for majority of the genotypes were similar and only 46 exhibited differences for protein banding pattern based on SDS-PAGE. In total, 27 protein bands were recorded, whereas protein subunits with lower MW were not considered due to inconsistency in reproducibility. Occasionally, variation was also observed in the density or sharpness of a few bands but this variation was not taken in consideration. Out of 27 protein subunits, 18 were polymorphic and 9 were monomorphic (Table 1). On the basis of banding pattern, gel was divided into four regions, I, II, III and IV (Fig. 1). In region I, among six protein bands having more than 66 kd, MW, three protein peptides were found with significant differences for QTL and 2 bands (3a and 3b) gave significant differences for two QTLs, hence could be used for screening blackgram germplasm. Region II (34 to 66 kd) consisted of ten protein peptides and out of these 5 were polymorphic. Out of 5 polymorphic protein peptides, 3 were observed significant for QTL detection. Protein peptides of region II proved their importance for detection of QTL and the band 9a was significant for seeds/pod and 100-seed weight.

Out of 18 polymorphic bands, 9 exhibited significance in detecting one or the other QTL. To detect QTL association between SDS-PAGE and agronomic traits, correlation among protein peptides and transformed data for quantitative characters were also conducted using computer software SPSS for Windows. Seventeen significant differences were recorded from a total of 162 mean comparisons were observed (Table 2). The Fig. 2 presents the presence (+) or absence (-) of polymorphic protein peptides in relation to days to maturity, 100-seed weight, biomass and grain yield. The presence of two bands (3b and 5a) increased the biomass and grain yield. The presence of band 3a increased the maturity duration, whereas the genotypes with positive signal for band 5a were early maturing, thus these two bands could be used for screening blackgram germplasm for maturity duration.

In total, two protein peptides revealed significant differences for days to maturity, 100-seed weight and grain yield, each one for branches/plant and days to maturity, four for seeds/pod and five for biomass. Presence of band 12b increased 100 seed weight (5.30 g) and the genotypes lacking this band produced low seed weight (4.62 g). Seventeen cases were recorded with significant mean differences which revealed that about 10.5% of total polymorphic bands showed importance in detecting QTLs on the basis of protein peptide in blackgram. On the basis of two statistics and two years, 14 markers were observed significant in detecting QTLs during 1999 and 16 during 2000. These ranged from 8.6 to 9.8 percent of the markers significant for detecting QTLs.

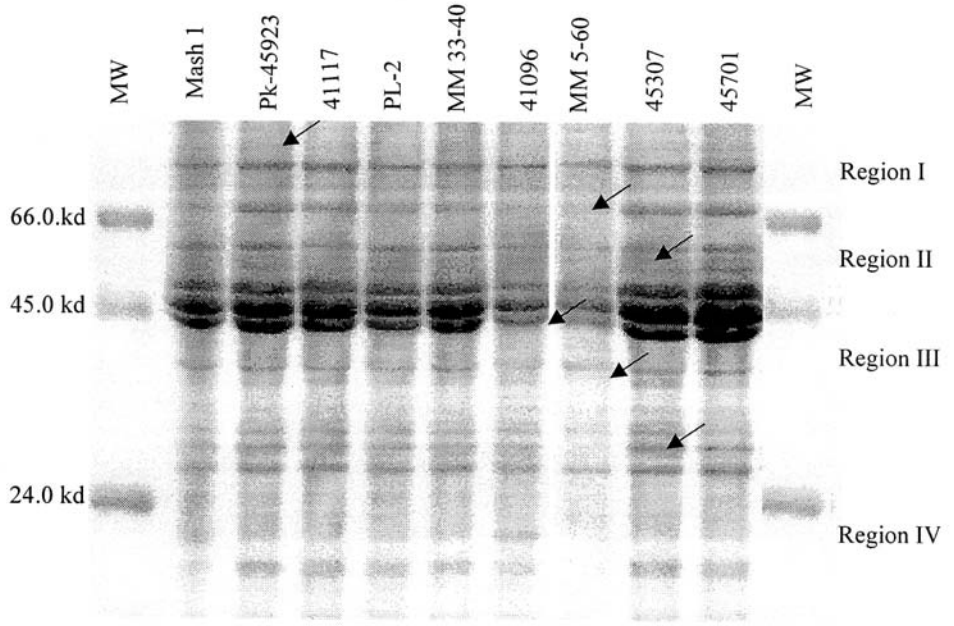

Fig. 1. Variation in seed proteins of *Vigna mungo* (L.) Hepper. The molecular marker used in this gel was SDS-70 KIT from SIGMA chemical company. The arrows represents the variation in the protein profile.

Table 2. Significance of protein subunits for detecting QTLs in blackgram.

		1999		2000	
QTL	**Band**	***t* statistics**	***r* statistics**	***t* statistics**	***r* statistics**
Days to flowering	B5a	10.3±1.97**	0.1624*	9.2±1.08**	0.1731**
Days to maturity	B3a	7.6±2.96**	0.1420	8.5±2.07**	0.1534*
	B5a	8.2±2.08***	0.0136	8.6±1.99***	0.1012
Branches per plant	B3a	4.79±2.06*	0.1887*	3.99±1.26*	0.1771*
Seeds per pod	B3	0.34±0.16*	0.2008*	0.31±0.12*	0.1918*
	B5a	0.75±0.19**	0.1951*	0.81±0.21**	0.2125*
	B9a	0.29±0.11**	0.2135*	0.21±0.07**	0.2016*
	B9b	0.31±0.13*	0.1839*	0.35±0.09*	0.1659*
100-seed weight	B9a	0.25±0.13*	0.1964*	0.31±0.11*	0.2159*
	B12b	0.68±0.09***	0.1551*	0.58±0.04***	0.1719*
Biological yield/plant	B3b	25.98±5.78**	0.1440	24.21±7.17**	0.1551*

	B5a	27.01±5.56**	0.1496*	24.21±6.26**	0.1749*
	B9b	12.65±7.25*	0.1595*	13.42±5.19*	0.1499*
	B10a	20.42±9.55*	0.2172*	23.25±8.94*	0.2271**
	B10b	24.51±10.12*	0.2505***	20.41±8.94*	0.3109***
Grain yield per plant	B3b	10.11±1.09***	0.1874*	11.51±1.71***	0.1791*
	B5a	11.32±1.73***	0.2097*	9.42±2.42***	0.1965*

*, ** and *** significant at $p<0.05$, 0.01 and 0.001

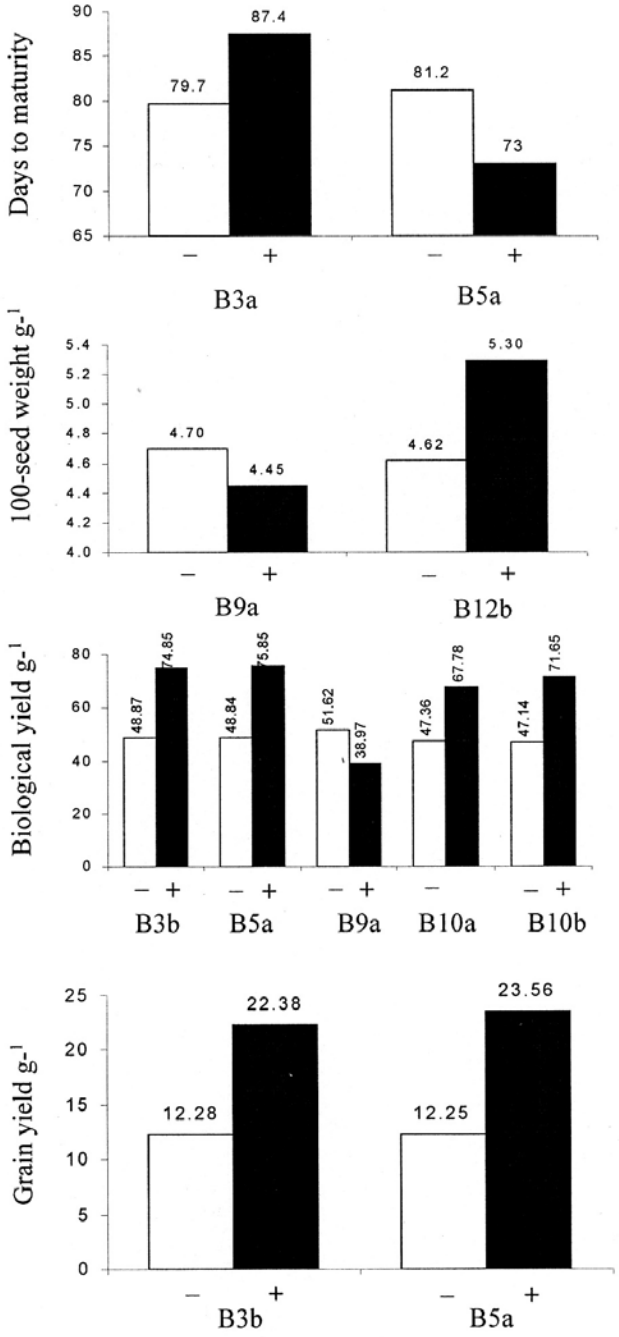

Fig. 2. Effect of SDS-PAGE marker on days to maturity, 100-seed weight, biological yield and grain yield in blackgram germplasm. The sign "—" denotes absence and "+" presence of markers.

Discussion

Although variation in storage protein banding pattern was revealed by SDS-PAGE that exists in the present material, but the magnitude was low because out of 105 genotypes tested for SDS-PAGE, only 46 exhibited variation which were about 44 % of the total material under investigation. It also needs to be broaden through collections and acquisition of germplasm from centres of origin. The variation on the basis of protein peptides has been reported by Rao *et al.,* (1992) and Jha and Ohri (1996). The seed protein obtained several sequences where pattern differences enabled a discrimination between various genotypes. In all three regions investigated, variation was observed with major differences in the region III where eleven bands were recorded and 8 were polymorphic in nature. Among these, 3 were found significant in detecting QTL with mean comparison for various characters. Moller and Spoor (1993) suggested 5 regions in *Lolium* spp., and observed major differences in the regions B, C and D. Previously Ferguson and Grabe (1986) and Murphy *et al.,* (1990) indicated potential power of electrophoresis techniques for determining genetic variation in crop germplasm.

Screening analysis for marker bands to QTLs were carried out and significance of protein peptides was observed in determining QTLs in blackgram. The factors or loci affecting variation in quantitative traits may occur as individual genes or gene clusters scattered throughout the genome, therefore, same quantitative trait may be expressed differently at several loci (Tahir and Muehlbauer 1995). Variation in seven quantitative traits out of ten was significantly associated with 9 protein peptides, however, the actual number of QTL might be fewer because several of these traits were correlated. The association of QTL with easily identifiable markers could permit the rapid and precise identification and transfer of QTL into superior crop cultivars (Tanksley *et al.,* 1989). Variation at protein peptides in the vicinity of QTL in blackgram germplasm may be an indication of genetic variation potentially available to breeding programmes. Expansion of genetic base for blackgram breeding might be accomplished by systematic use of germplasm that differ from common banding pattern and known to be associated with variation in quantitative traits.

The link of protein pattern has already been reported by Murphy *et al*., (1990), whereas, Moller and Spoor (1993) could not detect any link for days to maturity, winter hardiness and disease. The frequency of these markers based of protein peptides for QTL are not very commonly observed since these protein subunit bands would tend to be simply inherited whereas, agriculturally important traits are usually polygenic in nature. The initial results are encouraging for locating factors that influence the expression of quantitative traits. However, the conclusions are specific to the sample investigated, and the environment in which the measurable traits were recorded. Although variation was observed for total seed protein but the level was lower that needs to be extended by the use of other biochemical markers in blackgram. The association of biochemical variation with QTL was also observed and can be used for germplasm screening and further exploitation for blackgram improvement.

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