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Short Communication

Segregation distortion in recombinant inbred lines of blackgram [*Vigna mungo* (L.) Hepper] detected by inter simple sequence repeat (ISSR) markers

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Mapping technologies have made it possible to study the quantitatively inherited traits and to dissect quantitative variation into individual Mendelian factors. QTL (quantitative trait loci) mapping is based.on the principle of linkage disequilibrium between the marker and a genomic region influencing a quantitative trait inferred by statistical analysis [1]. The construction of linkage map is of fundamental importance for efficient exploitation of crop genetic resources.

Different mapping populations like RIL and doubled haploid (DH) are being employed for QTL mapping using a variety of molecular markers. The critical step in the construction of a linkage map is the selection of diverse parents so that DNA markers can reveal maximum polymorphism between the parents. In self pollinated crops parents that are distantly related are required [2]. The expected segregation ratio for codominant and dominant markers for RILs and DH populations is 1:1. Generally, markers segregate in Mendelian fashion although distorted segregation may be encountered [3, 4]. Segregation distortion has been well documented in a wide range of organisms and can be detected with almost any kind of genetic marker, including morphological mutant markers, isozymes and DNA markers [5]. The development of high density molecular linkage map provides a chance to survey the whole genome for loci showing distorted segregation [6]. The objective of this study was to measure the level of distortion in a RIL population derived from the cross between blackgram cv. TU 94-2 and *Vigna mungo* var. *silvestris* using ISSR markers.

Vigna mungo cv. TU 94-2 was crossed with *Vigna mungo* var. *silvestris*. One hundred four F_2 plants were advanced to F_8 generation by single seed descent method. DNA extraction, PCR amplification and visualization of bands were as described earlier [7]. The segregation ratio at each marker locus was statistically analyzed for deviation from the expected Mendelian segregation ratio by χ^2 test using Excel software.

Of the 100 ISSR primers used to detect polymorphism between the parents, TU 94-2 and *Vigna mungo* var. *silvestris*, 36 primers showed polymorphism. The primers produced varying numbers of amplicons depending on their SSR motifs. The average number of bands amplified per primer was higher (11) for the 5'-anchored primers as compared to 3'-anchored primers (8). The highest number of polymorphic bands was produced by dinucleotide repeat primers as compared to tri- and tetra-nucleotide repeat primers. The average number of bands amplified by primers based on $(AG)_n$ and $(GA)_n$ repeat motif was higher (10.5 and 8.7 respectively) followed by $(AC)_n$ repeats (7.7).

In this study, ISSR markers were used to detect polymorphism as they have been successfully utilized for genetic relationships in the genus *Vigna* [8] and genetic diversity studies in blackgram [7]. About 36% polymorphism was observed between the parents using the ISSR approach. Of the 36 polymorphic primers a subset of 17 ISSR primers were surveyed in 104 individual RILs to establish their segregation pattern. Thirty three polymorphic markers were scored unambiguously. Of the 33 marker loci, only 21 marker loci (64 %) fit the expected segregation ratio of 1:1 based on χ^2 test at 0.05 probability (Table 1). Deviation from the expected ratio was significant for 12 loci (36 %) at $P \leq 0.05$ and 9 loci (27.3 %) at $P \leq 0.01$. Figure 1 shows the distorted segregation of a marker amplified by ISSR UBC 807 primer.

Segregation distortion is a common occurrence for populations derived from wide hybridization and has been reported previously from many other crop species like rice [4] and *Vigna* sp. [9, 10]. The reasons for the segregation distortion remain unclear, although many theories have been put forward. Since the distorted

Table 1. Segregation pattern of ISSR markers in RIL population derived from the cross *V. mungo* cv. TU 94-2 × *V. mungo* var. *silvestris*

S. No.	Marker	Observed values of ISSR marker		χ^2	P value
		+	–		
1.	ISSR807$_{675}$	65	39	6.51	0.01
2.	ISSR807$_{1100}$	63	41	4.65	0.03
3.	ISSR808$_{1500}$	35	69	11.11	0.00
4.	ISSR808$_{950}$	69	35	11.11	0.00
5.	ISSR811$_{1450}$	57	47	0.96	0.32
6.	ISSR11$_{1000}$	62	42	3.84	0.04
7.	ISSR11$_{850}$	39	65	6.50	0.01
8.	ISSR834$_{875}$	51	53	0.03	0.84
9.	ISSR834$_{550}$	52	52	0.00	1.00
10.	ISSR836$_{1950}$	45	59	1.88	0.16
11.	ISSR842$_{1200}$	69	39	11.11	0.00
12.	ISSR842$_{1100}$	32	72	15.38	0.00
13.	ISSR881$_{1500}$	42	62	3.84	0.04
14.	ISSR881$_{1300}$	54	50	0.15	0.69
15.	ISSR881$_{700}$	9	95	71.11	0.00
16.	ISSR842$_{550}$	43	61	3.11	0.07
17.	ISSR855$_{1350}$	42	62	3.84	0.04
18.	ISSR856$_{2150}$	42	62	3.84	0.04
19.	ISSR856$_{1825}$	49	55	0.34	0.55
20.	ISSR857$_{920}$	47	57	0.96	0.32
21.	ISSR857$_{850}$	58	46	1.38	0.23
22.	ISSR857$_{775}$	34	70	12.40	0.00
23.	ISSR864$_{2220}$	67	37	8.65	0.00
24.	ISSR880$_{2275}$	66	37	8.16	0.00
25.	ISSR880$_{600}$	43	61	3.11	0.07
26.	ISSR886$_{1800}$	48	55	0.47	0.49
27.	ISSR886$_{500}$	44	60	2.46	0.11
28.	ISSR886$_{400}$	59	45	1.88	0.16
29.	ISSR887$_{1820}$	48	54	0.35	0.55
30.	ISSR887$_{375}$	61	43	3.11	0.07
31.	ISSR889$_{2200}$	37	66	8.16	0.00
32.	ISSR891$_{1365}$	44	60	2.46	0.11
33.	ISSR891$_{920}$	59	44	2.18	0.13

(Table χ^2 value: 3.84 at $P \leq 0.05$ and 6.63 at $P \leq 0.01$)

markers in our study were not restricted to any one linkage group it can be ruled out as due to loss of chromosome segment (unpublished data). The distortion cannot be due to sample size as the phenomenon has been observed by different workers irrespective of the population size. Another reason put forward is as due to inbreeding depression. This does not apply to blackgram or *Vigna* sp. in general as they are self pollinated. The reason for the observed segregation distortion could be due to genetic isolation between

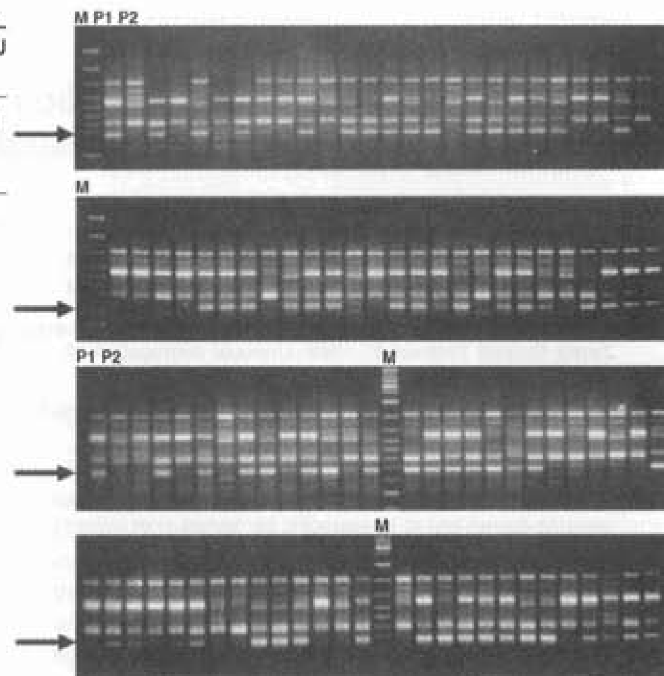

Fig. 1. Segregation of distorted allele (indicated by arrow) amplified by the ISSR primer UBC807 in recombinant inbred lines. Lane M: 100-bp DNA ladder, P1: TU 94-2, P2: *V. mungo* var. *silvestris*, rest of the lanes are RILs

the two parents used in the study. In our earlier work we have observed that irrespective of the markers used for study, *V. silvestris* forms a separate operational taxonomical unit (OTU) in the cluster analysis [7].

With the development of molecular linkage maps numerous examples of segregation distortion have been reported in a wide range of plant species. For the development of high density molecular linkage maps which provide a chance to survey the whole genome attempts are made utilizing inter specific crosses. The selection of diverse parent is important in developing linkage maps and the extent of diversity needs to be addressed to overcome segregation distortion. In wide crosses where wild alleles are found to be disproportionately lost, the frequency of rare alleles can be enhanced by adjusting the type of selection and population structure used in accordance with genetic information about segregation distortion providing more opportunities for a favorable recombination in later generations.

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