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Original Research Article

Marker Trait Association Studies in Pigeonpea F_2 Population

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A B S T R A C T

Pigeonpea is one of the important grain legume crops, in India and World (Singh, 2003). It is a major source of protein for about one fifth of population, especially for poor people. The present study involved a set of 255 F_2 individuals of cross between TAT-10 and ICPL 87119 which constitute the contrasting characters such as days to 50 percent flowering, days to maturity etc., were screened by 48 SSR primers. Using simple linear regression a total of nine primers were identified which showed significant association with earliness, i.e. days to 50 percent flowering, days to maturity. Therefore markers identified during present study need to be subjected to validation or functional analysis for respective trait.

Keywords

Pigeonpea, Molecular markers, Traits, Marker trait association

Introduction

Pigeonpea (*Cajanus cajan* L. Millsp.) is one of the important grain legume (pulse) crop in India which holds prestigious position among all legume crops (Singh, 2003). It is used as diverse source of food, fodder and feed (Rao *et al.*, 2002), fuel wood and for rearing lac insects (Zenghong *et al.*, 2001), as hedges and windbreaks, also it is used for soil conservation, green manuring and house roofing (Thu *et al.*, 2003). As a native, India is the largest pigeonpea producer followed by Myanmar and Kenya (Anonymous, 2006a), it is grown throughout the tropics especially in Africa, West Indies, Ceylon, Australia and Malaya on 4.92 million ha area with production 3.65 million tons (ICRISAT, 2010).

Knowledge of genetic inheritance of yield related traits plays an important role in deciding breeding strategies and methodologies for crop improvement. In comparison to other economically important crops, relatively less effort has been made to understand the genetics of important traits in pigeonpea. Both additive effects and dominant non-additive effects have been reported as being important in determining yield, plant height, and other traits. Pleiotropic effects of genes, physiological changes, and highly sensitive nature of pigeonpea towards the environmental changes makes it difficult to interpret the inheritance of yield and associated characters (Varshney *et al.*, 2009). Although pigeonpea improvement through conventional breeding and hybrid technology is ongoing, molecular breeding should accelerate utilization of the substantial variability among the pigeonpea landraces and germplasm lines for various morphological, physiological, and

agronomic traits. In above context to address the need for genomic tools in pigeonpea the work is focused on molecular markers and their association with different traits across the F_2 mapping population of cross between parent TAT-10 and ICPL-87119 (Asha).

Materials and Methods

The experimental material comprising of genotypes TAT-10, ICPL 87119 and 255 F_2 population derived from cross of above genotypes was used for study. Different morphological observations were recorded on 255 F_2 labelled plants derived in field. Recommended package of practices were adopted for the good growth of crop. Morphological descriptors used in the present study are branching pattern, plant height at maturity, 50 % flowering, 100 seed weight, no. of pods per plant, no. of branches per plant, seeds per pod, days to maturity and yield which were recorded at particular stage of crop.

Leaves from two weeks old seedlings were taken for DNA extraction. DNA was extracted using C-TAB method (Doyle and Doyle 1990) and (Anonymous, 2006b). For parental polymorphism study 48 SSR primers were screened (Table 1). Bulk segregant analysis as suggested by Michelmore *et al.,* (1991) was used for rapid identification of markers linked to different agronomic trait. Based on phenotypic observations, two bulks; early maturity bulk (B_1) comprised of ten F_2 plants and late maturity bulk (B_2) comprised of ten F_2 plants were made. A pooled DNA sample was prepared for each bulk by mixing in equal quantity the DNA of ten respective component F_2 plants. The parents and the bulks were screened by polymorphic primers identified by parental polymorphism to identify appropriately polymorphic markers. These markers were then used to know the genotyping of randomly selected 14 F_2 individuals. The molecular data and phenotypic data obtained were analysed were by simple linear regression method to know the association between the markers and agronomic component traits.

Results and Discussion

Two parents viz. TAT – 10 and ICPL 87119 showed significant variation for all agronomic traits recorded, F_2 individuals under study also showed segregation for all agronomic traits recorded. The F_2 population revealed plant height varied from 107 cm to 210 cm. Erect type of branching was observed in 102 F_2 individuals, while 117 individuals were recorded semi spreading and 32 individuals recorded spreading type of branching habit. Number of branches ranged from 2 to 7 branches per plant. Pods per plant ranged from 24 to 486 pods/plant. 100 seed weight was found between 4 gm to 11.3 gm while seeds per pod ranged from 1.1 to 4.5. Days to 50 percent flowering ranged from 85 to 135 while days to maturity ranged between 114 and 205. Yield was recorded between 11.5 gm to 188 gm. Simple correlation coefficients between agronomic traits components were estimated based on F_2 plants (Table 2). This helps to understand the varying degree of association and contribution of each character in building up total genetic architecture of agronomic trait considering the varying degree of phenotypic values.

For parental polymorphism study 48 SSR primers were screened out of which 22 SSR primers revealed polymorphism. These polymorphic primers further carried over for bulk segregant analysis. Bulk segregant analysis was used for rapid identification of marker linked to earliness. Based on phenotypic observations two bulks were made.

Table.1 Sequences of SSR primers

S. N.	Primer	Sequence text	S. N.	Primer	Sequence text
1	1FP 1RP	aaggcaagatacttggttctgg tctctccctgaaggtttccatt	25	25FP 25RP	tcacagaggaccacacgaag tggactagacattgcgtgaag
2	2FP 2RP	gtgggataccatgttccagg ccgaatacatgcttttgggg	26	26FP 26RP	gcgctaagggaaaacaaaa aactcccttgttgtcatatggtg
3	3FP 3RP	ccccctttagcatgtattggg tttaaacgaattcgcccttg	27	27FP 27RP	agagggaaagggaagagaaga tcaagcaactccaagaaattca
4	4FP 4RP	gcatagagttaggaacattcattgc agcgttcaacccaaacaaaa	28	28FP 28RP	aaggcttttcaacaaataggg agaagagaaaaagcataaaacttca
5	5FP 5RP	tcctcatgtagcctattgggtt catgtgaatattccattcgatgc	29	29FP 29RP	catttatttctctctggcattcac cgagctgcaagcataaacg
6	6FP 6RP	aaatttttagcacaatggccg aattatcacaaatggcacgc	30	30FP 30RP	tgcacagattcgaaggttc cctcaagattcctctttctctca
7	7FP 7RP	ggattaaccaattgtgagtgaacc tgcactttataagcatttaccaaca	31	31FP 31RP	tcaggggtaaatgcggtatc gaattgctttttgcttcctca
8	8FP 8RP	tttttatggaatatttatgagttggc aagagtttcccaaccctgct	32	32FP 32RP	gagaaatatgagagggcagagagaga aagataattcattagggggtgga
9	9FP 9RP	agaacaacaagggagcgaga ccatgacatcattgcatgataaa	33	33FP 33RP	taaggaaatggctggggttg cacataaatttgggggttcg
10	10FP 10RP	atccccacccttgtgtcata tctttccatttacaccccgt	34	34FP 34RP	ggacttgttactggggcact aattcccatggtcattcg
11	11FP 11RP	atcaccaacatccccatgat caccaacgatgaattgtgaa	35	35FP 35RP	tgggcatggtagaggaagtt cgtcatgaagcaacaggaga
12	12FP 12RP	gctccaatttttcatttcgg atcaaacaatgcacccatga	36	36FP 36RP	taatcccattccgttgtcgt cccaggaagagatgagacca
13	13FP 13RP	gcaagtgttccctacgttgc ctccaacggccatagtagga	37	37FP 37RP	aggctttctcccttcaatcc gccttttcaaacttttctcaca
14	14FP 14RP	cccgaactgcattcaaaaat gcgtaggtggaagaagatcg	38	38FP 38RP	acatgtgtggcgtagtgtga gcaaaaccgttccataaaaa
15	15FP 15RP	gaggctgaggggtgaaaaat cctctggattccctcttcc	39	39FP 39RP	gaggattgcaccaagcaact gcactgctggccttaccata
16	16FP 16RP	agtttgaaattgcttttggct gaattgggagagcccgcata	40	40FP 40RP	tgggctgtgatcgatgaat cgacaacaacaacaccgact
17	17FP 17RP	gcgggattctcttgcttac tcacaaaacaatttggcaca	41	41FP 41RP	tgttccgtttcaagtggtca cgacatttacccactcgttca
18	18FP 18RP	acaccaccatgctaaagaacaag ccaagcaagacacgagtaatcata	42	42FP 42RP	tagagcgttgtccctttctg tcgaaggacaactcaagcatt
19	19FP 19RP	catcgcctacaatcatacaaaga tcttgtccttttttcagtcatcgt	43	43FP 43RP	tcgtgggaatgctctacaac aaccacaagtacacccacacc
20	20FP 20RP	atcgctttgcatccttatc cttcacgtacattttcgttt	44	44FP 44RP	atgggcatggtagaggaggt cgctcatcatcgtcatcaaa
21	21FP 21RP	tgcttcaagttgcctaccag tcaagggaggtggactacaaa	45	45FP 45RP	gggaaactcacctatattaccaa cactaccgtctacagccatctc
22	22FP 22RP	gtagaggaggttccaaatgacata atctgtctggtgttttagtgtgct	46	46FP 46RP	gttcttcttgttgttgttgttg aattcgtggagttcattgg
23	23FP 23RP	ctcttgcttacgcgtggact cttttgcttttgcgtgctt	47	47FP 47RP	gatagcacacacacacacaaca taccttagggtcaccaacga
24	24FP 24RP	tcttagcatgtcctctattttcgt agtacatttcaaatccacacatcc	48	48FP 48RP	ctttgttcagagcggagcat tttttaggacattgggaagca

Table.2 Phenotypic correlation among components of agronomic traits in pigeonpea F_2 individuals from cross between TAT-10 and ICPL 87119

	Plant height	Branching pattern	No of branches	Pods per plant	100 Seed weight	Seeds/pod	Days to 50 percent flowering	Days to maturity
Branching pattern	0.040							
No of branches	0.312**	-0.034						
Pods/plant	0.372**	-0.096	0.223**					
100 Seed weight	0.140**	-0.019	-0.008	-0.031				
Seeds/Pod	-0.174	0.046	-0.023	-0.325	-0.235			
Days to 50 percent flowering	-0.243	-0.017	-0.170	-0.075	0.026	0.237**		
Days to maturity	-0.231	-0.003	-0.152	-0.060	0.011	0.216**	0.952**	
Yield	0.283**	-0.053	0.228**	0.583**	0.108*	0.557**	0.193**	0.183**

(* significant at p = 0.1, ** Significant at p = 0.05, Degrees of freedom n-2)

Table.3 Simple linear regression analysis for marker trait association

Primers → Traits ↓	13	31	32	33	35	36	37	38	39	40	41	43	45	46	47
Plant height	0.47	0.39	0.35	1.91*	0.85	0.69	-0.24	0.39	0.99	0.85	1.43	-1.42	0.49	0.99	0.18
Branching pattern	0.63	-0.42	-0.75	0.29	0.93	1.51	-1.53	-0.68	0.57	0.93	-6.79	0.70	1.34	1.08	0.61
No. of branches	1.54	0.52	1.13	1.24	0.95	0.77	0.21	0.63	1.05	0.95	0.13	-0.53	0.39	1.03	1.05
Pod/ plant	0.21	1.07	1.81*	0.57	-0.29	-0.59	0.73	0.14	0.05	-0.29	1.58	0.52	-0.81	0.04	-0.15
100 seed weight	1.44	1.25	-0.76	1.23	1.91*	2.11*	-0.64	-0.62	0.53	1.91*	0.59	-2.22	2.08*	1.04	1.97
Seeds / pod	-1.07	-1.07	1.48	0.28	-0.63	-0.79	0.47	0.04	-0.25	-0.63	0.61	1.42	-0.64	-0.62	-0.85
50 percent flowering	4.54***	2.33**	0.11	0.84	5.83***	5.02***	-1.32	-0.73	3.22***	5.83***	1.23	-2.16	3.91***	3.88***	3.30***
Days to Maturity	5.98***	2.55**	-0.04	1.11	8.45***	6.55***	-1.49	-0.64	3.61***	8.45***	1.23	-2.52	4.78***	4.66***	4.00***
Yield	0.30	0.49	1.37	1.27	0.56	0.50	-1.32	0.19	-0.07	0.56	2.00*	-0.25	0.69	0.85	-0.27

(* significant at p = 0.1, ** Significant at p = 0.05, *** Significant at p = 0.01, Degrees of freedom n-2)

Bulk segregant analysis was done for the two bulks along with parents. A pooled DNA sample was prepared for each bulk by mixing equal quantity DNA of ten respective components F_2 individuals. The parents and bulks were screened for 22 SSR primers to identify the linked polymorphic markers. From the BSA out of 22 nearly 68% i.e., 15 SSR markers were identified to be polymorphic and associated with earliness. These 15 SSR markers were than used to know the genotyping of randomly selected 14 F_2 individuals.

The molecular data and phenotypic data obtained were analysed were by simple linear regression method to know the association between the markers and the recorded traits (Table 3). The polymorphic SSR primers 13, 31, 32, 33, 35, 37, 36, 38, 39, 40, 41, 43, 44, 46, and 47 was significantly associated branching pattern, number of branches/ plant, pods /plant, 100 seed weight, yield, plant height, grains /pod, days to 50% flowering and Days to maturity. Most of the primers i.e., 13, 31, 35, 36, 39, 40, 45, 46 and 47 were found significantly associated with days to 50% flowering and Days to maturity. While primer number 37, 38 and 43 were found non-significant.

Kumar *et al.,* (2011) stated that successful pyramiding of genes and QTL could have been possible only because tight marker trait association. Further he stated that to use MAS in conventional breeding programmes, makers tightly linked to genes controlling target trail must be identified first. Genetic linkage mapping, QTL mapping and association mapping and comparative genomics are commonly used approaches in crop plants. In pulses several genes and QTL controlling target traits (qualitative and quantitative) have been mapped using molecular marker (Kumar *et al.,* 2011). On basis of the results obtained from the present investigation, it is concluded that the markers identified to be significantly associated with different traits can be effectively utilized in marker assisted selection programme and aimed towards improvement in pigeonpea. Therefore markers identified during present study need to be subjected to validation or functional analysis for respective trait.

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