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Short Communication

Candidate gene-based characterization of common bean genotypes

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Abstract

A set of 96 common bean genotypes were characterized using 25 candidate gene based SSR markers associated with yield traits. Twenty three SSR markers were found polymorphic and their discriminatory power estimation was performed on the basis of PIC, resolving power and marker index that mainly explain ability of molecular markers to distinguish genotypes. An average of 0.33 PIC was observed where highest value was exhibited by the primer BM184. The primers BM170 and Bmd8 generated four alleles whereas BM170 showed maximum marker index of 1.28 and highest resolving power of 1.89 was found in case of primer BM180. Further, the genotypes were grouped in three major clusters based on DARwin5 software. High level of genetic diversity observed within the genotypes could be used to accelerate genetic improvement of germplasm in common bean targeting yield attributing traits.

Key words: Common bean, candidate genes, yield traits, SSR

Common bean (*Phaseolus vulgaris* L.) is one of the most important and widely cultivated species that represents 50% of the grain legumes consumed worldwide (McClean et al. 2004). It contains substantial amount of quality proteins and other nutritional elements. As such, elucidating the variation among the germplasm is an ongoing concern of researchers for doing improvement in breeding programs. In this regard, the common bean germplasm grown in Himalayan region is most diverse in terms of grain morphology and agro-ecological adaptation (Zargar et al. 2014).

Various attempts have been made to study the variation in the germplasm, characterization, association mapping and construction of linkage maps among the common bean germplasm using different molecular markers (Freyre et al. 1998; Blair et al. 2006; 2009; Kwak et al. 2009; Hegay et al. 2013; Gill-Langarica et al. 2011; Marotti et al. 2007). Besides this, candidate gene based markers or genic markers have been introduced recently that are synthesized mainly from known cDNA/EST sequences. The synthesis of these markers from the sequences within genes has been achieved on account of huge data generated by sequencing technologies. Till now, candidate gene based markers or genic makers have been developed in cereals, grasses, legumes, fiber and oil seed crops, fruits and vegetable crops (Varshney et al. 2007). Keeping in view the above points, a study was conducted to accelerate genetic improvement in common bean targeting yield attributing traits and to characterize a set of 96 genotypes using candidate gene based SSRs.

Ninety-six common bean genotypes were collected from 15 different regions namely, Poonch (P1-P19), Rajouri (R1-R10), Shopian (KS11-KS1), Bandipora (K12, K14, K19 and K3, K16, K18), Baramulla (K3, K17, K19), Qazigand (K20), Bhadarwah (B1-B20), Marmat (MT2, MT5, MT7), Marwah (MR2), Dessa (DE4), Vganda (UG5-UG13), Nishat (N1-N17), Udampur (UD1-UD6) and North and Central Kashmir (KD11-KD17) of Jammu and Kashmir. The material was grown in experimental field of SKUAST, Jammu. The genomic DNA was extracted from 2-3 weeks old tender leaves following CTAB method of Doyle and Doyle (1990). The extracted DNA was spectropho-

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metrically analysed for quantification and quality. DNA of each sample was diluted to 25 ng/µl concentration. Twenty-five SSR markers were selected from published reports (Gaitan-Solis et al. 2002; Leite et al. 2011; Blair et al. 2006) and were synthesized through local vendor. The concentration of the primers was made up to 10 µM and stored at –20°C. The amplification of genomic DNA was performed in 96 well Universal Gradient Thermal Cycler and resolved on 3% Agarose gel. The gel images were then visualized and captured using gel documentation system.

The bands visualized under the gel documentation system were considered as SSR loci. When a PCR product was not amplified, data for the relevant genotype was treated as null allele. In order to determine the efficiency of primers polymorphism information content (PIC) (Powell et al. 1996), polymorphism percentage, resolving power, marker index and major allele frequency were calculated. DARwin5 software was used to calculate dissimilarity matrix using a shared allele index. Pair wise distance matrix was computed for construction of unweighted neighbor joining phylogenetic tree (Perrier and Jacquemoud-Collet, 2006). NEI coefficient (Nei, 1972) with bootstrap procedure of resampling (1000) across markers and individuals from allele frequencies was used to deduce genetic distance between the accessions.

The results of various calculated parameters have been elucidated in Tables 1 and 2. Out of 25 markers, 23 were polymorphic and it produced a total of 59 alleles out of which 57 bands were polymorphic. Primers, Bmd8 and BM170 generated four alleles; three alleles were produced by seven primers and the rest 14 polymorphic markers yielded only two alleles with an average of 2.28 bands per unit assay. PIC ranged from 0.163 to 0.498 with an average value of 0.33. This was comparable to the mean PIC value of 0.28 obtained in another candidate gene based SSRs study in rice (Molla et al. 2015). The values for marker index ranged from 0.391 to 1.28 with an average 0.80 which was less compared to 1.279 reported by Zargar et al. (2016). Average resolving power was 1.12 detected in present study, less value have been observed for Bmd38 and BM172 and that of high for BM184 followed by BM142, BM141, BM98, BM170 and BM161.

The genotypes were grouped into three major clusters (I, II and III) based on Nei coefficient of dissimilarity matrix. Each of the clusters was further divided into two sub clusters (clusters Ia and Ib, clusters IIa and IIb, clusters IIIa and IIIb). A total of 44 genotypes were grouped in cluster I, 40 belong to cluster II and 12 genotypes are classified in cluster III. The genotypes were found well distributed across the different regions in J & K covering 8.3% to 44.4% cultivated area. Dissimilarity matrix revealed genotype R10 and genotype KS11 to be the most distant with a dissimilarity coefficient value of 0.85. Further, genotype K17 (Baramulla) was found to be diverse from that of three Poonch genotypes namely P11, P13 and P18 with a dissimilarity coefficient value of 0.80. Minimum value of the coefficients indicates the similarity between the genotypes. The most similar genotypes were found to be collected from Poonch (P15 and P17, P15 and P18, P17 and P19), Bandipora (K14 and K16), Bhaderwah (B12 and B13) as well as Bandipora (K15) and Baramulla (K19) with minimum dissimilarity coefficient of 0.11. Based on results, we found that most of the genotypes found similar were collected from the same source. This indicates that the geographical origin affects the genetic relatedness among the individuals.

In the present study, it was observed that the average major allele frequency was high which can be attributed to the presence of major allele in most of the genotypes as depicted by functional markers. On an average, less number of alleles were produced per unit assay when compared to the study conducted by Zargar et al. (2016). Comparatively high major allele frequency and less value for other discriminatory parameters observed in our study owes to the origin of candidate gene based markers from highly conserved functional portion of the genome that is involved in phenotypic trait variation. However, using candidate genes the genetic diversity can be examined based on sequences within genome expressing particular traits. Average PIC obtained in the present study was higher as compared to similar studies conducted earlier indifferent crops (Patil et al. 2014). The PIC values in earlier studies ranged from 0.067 to 0.740 with an average of 0.454, 0.30 to 0.89 with an average of 0.67 (Scaranoa et al. 2014); 0.05 to 0.83 in 20 common bean genotypes belonging to the Andean and Mesoamerican gene pools and 0.03 to 0.70 for a set of 60 carioca common beans (Perseguini et al. 2011). Since SSRs used in present study are gene based and highly specific, we detected less effective multiplex ratio i.e. 0.95 compared to that of 11.2 and 11.4 in common bean obtained earlier (Zargar et al.

Table 1. Details of primes with their resolving power in discriminating genotypes

S.No.	Primer	Trait	Linkage group	Expected band size	NPB	MAF	PIC	MI	RP
1	Bmd 1	GY	3	165	3	0.714	0.26	0.79	1.19
2	Bmd 8	GY	4	176	4	0.724	0.16	0.65	1.04
3	Bmd 9	GY	4	135	2	0.729	0.35	0.70	0.94
4	Bmd 12	PH	6	167	3	0.818	0.21	0.64	0.75
5	Bmd 20	GY	5	123	3	0.645	0.35	1.04	1.42
6	Bmd 27	RL	11	109	3	0.656	0.28	0.83	1.17
7	Bmd 32	DF	1	150	0	-	-	-	-
8	Bmd 37	100 SW	6	134	2	0.635	0.45	0.91	1.40
9	Bmd 38	Bng26/R GC	6	178	2	0.851	0.20	0.39	0.45
10	Bmd 39	Bng27/FGC	6	126	2	0.820	0.29	0.58	0.70
11	Bmd 45	GY	1	129	2	0.750	0.32	0.64	0.81
12	Bmd 50	Senescence	5	124	2	0.844	0.24	0.48	0.56
13	BM 98	GY	3	247	2	0.538	0.49	0.97	1.70
14	BM 114	DF	9	234	2	0.708	0.34	0.68	0.88
15	BM 141	GY	9	218	2	0.482	0.49	0.97	1.71
16	BM 142	GY	2	157	3	0.511	0.33	1.00	1.74
17	BM 161	GY	4	185	3	0.484	0.39	1.16	1.65
18	BM 164	PL	2	182	2	0.469	0.37	0.74	1.10
19	BM 170	DF & PH	6	179	4	0.342	0.32	1.28	1.70
20	BM172	GY	3	107	2	0.729	0.23	0.47	0.54
21	BM184	P/P	11	160	2	0.489	0.50	1.00	1.89
22	BM 199	GY	4	304	0	-	-	-	-
23	BM 210	P/P	7	166	3	0.615	0.35	1.04	1.54
24	PVag004	GY	4	201	2	0.724	0.29	0.58	0.71
25	PVttc002	GY	-	200	2	0.394	0.45	0.89	1.37

GY = Grain yield (kg/ha); PH = Pod height; RL = Root length; DF = Days to flowering; SW = Seed weight; GC = Genomic clone; PL = Pod length; P/P = Pods/plant. NPB = No. of polymorphic bands; MAF = Major allele frequency; PIC = Polymorphic Information Content; MI = Marker Index and RP = Resolving Power

Table 2. Elucidation of discriminating power of SSR markers

Indexes	Abbreviation	SSR assay
No. of assay units	U	25
No. of polymorphic bands	n_p	57
No. of monomorphic bands	nn_p	2
Average no. of polymorphic bands/assay unit	n_p/U	2.28
No. of loci	L	25
No. of loci/assay unit	n_u	1
Fraction of polymorphic loci	â	0.95
Effective multiplex ratio	E	0.95
Average polymorphic information content	PIC	0.333
Average marker index	MI	0.80
Average resolving power	RP	1.12

2016). The less average number of alleles in the present study along with high value of major allele frequency indicates that most of the markers used in the present study were not multiple allelic in nature which is expected due to linkage of markers to yield traits. Further, assessment of genetic relatedness showed that the genotypes grown in the same region were genetically more similar than others, which might be because of frequent sharing of alleles among them. The functional markers can be utilized in various breeding programmes such as comparative mapping and marker-assisted selection. Due to their association with a specific trait and ascribed known functions, genic or candidate gene based markers surpass random markers which are originated from non-functional parts of the genome.

Authors' contribution

Conceptualization of research (SMZ); Designing of the experiments (SMZ, RKS); Contribution of experimental materials (SMZ); Execution of field/lab experiments and data collection (NG, RM, SS); Analysis of data and interpretation (NG, RM, FAN, SS, RKS); Preparation of manuscript (NG, SMZ, FAN).

Declaration

The authors declare no conflict of interest.

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