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Evaluation of morpho-molecular diversity and pod borer resistance in country bean

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ABSTRACT

Country bean is a nutritious vegetable with commercial importance. Pod borer is a major pest of country beans in Bangladesh. Assessment of genetic variation among germplasm is a prerequisite for improvement of any crop. The present study was conducted to evaluate the yield potential, pod borer resistance and genetic diversity of 20 country bean varieties. At first, a field experiment was conducted with twenty cultivars to evaluate yield contributing characters and pod borer resistance. The data revealed that the variety Kaloputi showed the highest yield (2111.09 g) of pods plant^{-1}. This variety (Kaloputi) had the highest number of pods plant^{-1} (271), lowest percentage (3.08%) of infestation, highest number (1086.0) of seeds plant^{-1} and highest dry seed yield over the other varieties. The variety BARI seem 5 showed the least performance in most of the parameters. Another experiment was conducted to find the genetic diversity among the 20 cultivars using SSR markers. The cluster analysis showed that the genotypes were divided into four groups. The cluster 1 was composed of seven BARI released varieties, cluster 2 was composed of two modern (IPSA seem 2 and BARI seem 8) and three local varieties (Khisamoti, Rifa and Goalgada), cluster 3 was constructed by six local and one modern variety (BARI seem 9) and Cluster 4 consisted of only cultivar Mostofa. The wide variations in phenotypic and genotypic level and pod borer resistance observed among the genotypes can be potentially used for future improvement of country bean.

Introduction

Country bean is a high-value vegetable crop. It is nutritious and commercially important. It is a versatile multipurpose plant that offers food, fodder, green manure and traditional home remedies. It is an important vegetable-cum-pulse crop grown everywhere in Bangladesh. It is commonly known as Seem, Hyacinth bean, Indian bean, Egyptian kidney bean and Bovanist bean (Rashid, 1999). The tender pods and green seeds of this bean are used as a vegetable, while dried seeds are popular as pulse. It is well grown in winter, but a few modern varieties (BARI seem 3, IPSA seem 2 etc.) are nowadays cultivated in summer also. In Bangladesh, about 40,992 metric tons of country beans are produced per year from 88,581 hectares of land. Country bean cultivation faces various problems, including disease and pest infestation (Rashid, 1999) and unavailability of quality seed. Due to attack by diseases and pests and lack of high yielding variety, the yield is low. Among the insect pests, the pod borer (*Maruca vitrata*) is considered as one of the major pests of beans in Bangladesh and 100% yield reduction in pigeon pea has been reported by bean pod borer infestation (Rahman, 1987). The farmers in Bangladesh are highly dependent on chemical insecticides to control the pest infestation on country beans (Rahman and Rahman, 1989; Begum, 1993). Insecticides commonly used, however, are not specific and they frequently kill beneficial insects and may cause upset and the resurgence of other pest populations (Debach and Rosen, 1991; Pedigo, 1999). In addition, the development of insect biotypes resistant to the commonly used insecticides has been found (Debach and Rosen, 1991; Pedigo, 1999). Thus as an alternative to sole reliance on insecticide(s), the use of resistant cultivars and other non-chemical methods would provide avenues towards safer pest control practices.

The existence of genetic diversity is a prerequisite for the development of new variety with desired traits. Assessment of diversity based solely on phenotypic traits is traditionally long being practiced. Assessment of genetic diversity in accordance with phenotype has limitations, as long as most morphological characters are greatly influenced by environmental factors and the developmental stage of the plant (Konstantinos *et al*.,

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2008). In opposition to, molecular markers based on DNA sequences, are independent of environmental conditions and show a higher level of polymorphism (Rai *et al*., 2010). The advances in molecular biology techniques have made it easy to develop large numbers of highly informative DNA markers for the identification of genetic polymorphism (Fevzi, 2001). In recent days, diversity analysis by a combination of both morphological and molecular markers has become popular.

While DNA markers are rarely developed, mapping within the legume species provides evidence that the DNA markers available from related legume species can be applied in this neglected species, *L. purpureus* (Venkatesha *et al*., 2007; Zhu *et al*., 2005). Assessment of genetic variation in germplasm is an integral part of any crop improvement programme (Somta *et al*., 2009). *L. Purpureus* has been neglected in research and development; thus information on its genetic diversity is very scanty and consequently, our understanding of its diversity is also limited (Angessa *et al*., 2008). Moreover, reports on screening for pod borer resistance in country bean have been scarce. The present study was, therefore, planned to evaluate the diversity of country bean at a phenotypic level using yield contributing traits and pod borer resistance and at the DNA level using SSR markers.

Materials and Methods

Plant materials

Twenty genotypes were originally collected from Plant Genetic Resource Centre (PGRC), Bangladesh Agricultural Research Institute (BARI), Gazipur, Dhaka and local cultivars were collected from different parts of Bangladesh (Table 1).

Morphological characterization

The experiment on morphological characterization was carried out at the Entomology Experimental Field, Bangladesh Agricultural University, Mymensingh, during the rabi season. The land was ploughed and cross ploughed several times with power tiller to obtain good tilth. The Experimental plots were fertilized with cow dung @10 t/ha, Urea @50 kg/ha, TSP @150 kg/ha and MoP @150 kg/ha (Rashid, 1999). The experiment was laid out in a randomized complete block design with three replications. The unit plot size was 1.3 x 1.3 m maintaining 0.25 m distance between the plots. Bamboo sticks were used to facilitate the climbing of the plants upwards. Data were recorded on vein and leaf colour, length, width and shape of leaf, leaflet length, growth habit, number of primary and secondary branches plant^{-1}, flower buds raceme^{-1} and raceme plant^{-1}, days to 50% flowering, length, width and colour of pod, number of healthy and infested pods plant^{-1}, infestation percentage, yield of green pods plant^{-1} and yield of dry seeds plant^{-1} from each plant of each plot.

Table 1. List of the selected SSR primers

Primer Name	Sequence	Annealing Temperature
AB176566	TATGCTGTGGGTGTGG AGAGCTTTTGAACTTGTAAAAGGG	56
AF0674171	TACAGTGCTTCCTGAATGGG ACAAACAACATACAAGTACTAACTGC	48
PV-ag004	TTGATGACGTGGATGCATTGC AAAGGGCTAGGGAGAGTAAGTTGG	59
BMd-46	GGCTGACAACAACTCTGCAC CTGGCATAGGTTGCTCCTTC	57
Y583516	CGTGTGTTGAGAGGGAGGG AGAACAAGCTCGTGGGAAGTC	59
Hbp-001	GACAACGACAGGGATAA GAGTAAGAAGGGAAGTGA	49
Hbp-002	TTCCGCAAAGACAAGTT CGTCAGCGAGAAGGGTA	51
Hbp-003	CCAAATCCGAATCAGCA CTAAGCAGGAAAGCAAA	49
Hbp-006	GGTTCTCCTGCTGTTTG CCATCTTGCTGCCCTCA	52

Statistical analysis

The data were subjected to analysis of variance (ANOVA) and the treatment means were separated by Duncan's Multiple Range Test using MSTAT-C software.

Molecular diversity analysis

Total DNA was extracted from the leaves by Cetyl Trimethyl Ammonium Bromide (CTAB) method (Saghai-Maroof *et al*., 1984). Thirteen microsatellite primers were selected for the present study based on genetic diversity experiment (Shivachi *et al*., 2012). Out of thirteen primers, nine primers showed clear amplified bands. The details of the selected primers are given in Table 2. The PCR cocktail for SSR analysis had a total volume of 10 µl/reaction, including DNA. It was composed of 1.0 µl genomic DNA, 5 µl PCR master mix (Go-taq green master mix, Promega corporation, U.S.A), 1 µl forward primer, 1 µl reverse primer, and 3 µl nuclease-free water. Samples were subjected to the following thermal profile for amplification in a thermocycler: The reaction mix was preheated at 94ºC for 3 min followed by 35 cycles of 3 min, denaturation at 94ºC, 1 min annealing at 55-65 ºC (based on the annealing temperature of the individual primer) and elongation at 72ºC for 1 min. After the last cycle, a final step was maintained at 72ºC for 5 min to allow complete extension of all amplified fragments followed by holding at 4ºC until electrophoresis.

Visualization of amplification products was accomplished on a 40% Polyacrylamide gel in 1 X TAE buffer. The Polyacrylamide gel was stained with ethidium bromide solution for 40 min. The stained Polyacrylamide gel was illuminated by UV-trans-illuminator and photographed for assessing the DNA profiles. The allelic variations at the DNA level have been represented by nine gel pictures Fig. 1 (a-e). The genetic analysis software POWER MARKER (version 3.23) was used to determine the summary statistics,

including the number of alleles per locus, major allele frequency, gene diversity and Polymorphism Information Content (PIC) values (Liu and Muse, 2005).

SSR data analysis

Molecular weights for SSR products were estimated with AlphaEase 4C software. The individual fragments were assigned as alleles of the appropriate microsatellite loci. Molecular weight data of alleles were used to determine the genetic distance for phylogeny reconstruction based on the neighbor-joining method (Saitou and Nei, 1986). The 20 genotypes were clustered based on the matrix of genetic similarities using the Unweighted Pair Group Method with arithmetic averages (UPGMA). The cluster analysis and dendrogram construction were performed using NTSYS-PC (version 2.1).

Results

Morphological diversity

All the genotypes showed significant variations on the length and width of the leaf (Table 2). The local varieties Goalgada and Mostafa possessed identical longest leaf (36.0 and 35.50 cm, respectively) followed by BARI seem 9. Ali produced the shortest leaf. In the case of leaf width, variety Nodi produced the highest width (33.50 cm) of leaf followed by BARI seem 9 while the leaf width was the lowest (18.0 cm) in varieties BARI seem 5, BARI seem 7 and Kaloputi.

Statistically significant genotypic diversity on the length of leaflet was found. Leaflet length ranged from 8.00 to 16.00 cm. It was revealed that the plants of BARI seem 9 and BARI seem 6 produced the longest leaflet while the shortest leaflet was obtained from the variety BARI seem 5 (Table 2). The shape of leaf was ovate–lanceolate in 55% of the varieties (11 varieties), ovate in 35% varieties and the rest two (10%) varieties produced round shaped leaf (data not presented). All the varieties had an indeterminate type of growth habit except BARI seem 5 and Rifa that had determinate growth habit (data not presented). A variation on raceme length was observed depending on the type of varieties. The length of raceme ranged from 7.16 to 60 cm in this study (Table 2). Among the varieties, BARI seem 3 had the longest raceme. The raceme of Noldoc was the shortest (Table 2). The number of raceme plant^{-1} varied among the country bean varieties studied (Table 2). Kaloputi was highly potential for producing the higher number of raceme which was statistically different from all other varieties. The plants of BARI seem 5 produced the lowest number of raceme in this study (Table 2). The number of flower buds raceme^{-1} varied from 7.00 to 37.00 among the varieties. Variety Mostofa produced a higher number of flower buds as compared to that of other varieties. Chonchol was the least performing variety (Table 2).

The length and width of pod varied from 7.76 to 21.13 cm and 1.90 to 3.80 cm, respectively among the varieties (Table 3). Variety BARI seem 6 had the longest pod, which was statistically identical to that of Noldoc (20.66 cm). Similarly, the variety Mostafa had the shortest pod followed by IPSA seem 2 (8.26 cm). On the other hand, the variety Goalgada showed the highest pod (3.80 cm wide) followed by BARI seem 3 (3.50 cm) and Khisamoti (3.46 cm) while the same shortest pods (1.90 cm) were recorded from the variety BARI seem 2 and Chonchol (Table 3).

The number of total pods plant^{-1} and the green pod yield were significantly different among the varieties (Table 3). Number of pods plant^{-1} widely ranged from 19 to 271. The highest number of pods plant^{-1} (271) was found in local variety, kaloputi and the lowest number of total pods plant^{-1} (19) was in BARI seem 5. Variation in individual pod weight was significant and ranged from 4.39 to 22.47g. Among the varieties, Goalgada showed the highest and Rifa had the lowest weight of single pod (Table 3). Pod yield plant^{-1} ranged from 124.26 to 2111.09 g. The local variety kaloputi showed the highest amount of pod yield plant^{-1} (2111.09 g) and BARI seem 5 showed the lowest amount of pod yield plant^{-1} (124.26) in the present study (Table 3).

Data on length and width of seeds were subjected to analysis of variance. It was found that both the data significantly varied among the varieties. The highest length (1.60 cm) of seed was recorded in HYV BARI seem 4 while the highest width (1.20 cm) of seed was recorded with HYV check BARIseem9. In contrast, HYV BARI seem 5 and local Rifa showed same lowest (1.10 cm) length of seed while same width of seed (0.70 cm) was also obtained from the HYV BARI seem 2 and local Rifa (Table 4).

Number of seeds pod^{-1} and plant^{-1} was counted where HYV BARI seem 6 produced the highest number of seeds (7.00) pod^{-1} while local Kaloputi exhibited the highest number of seeds (1086.0) plant^{-1}. On the contrary, the same lowest number of seeds pod^{-1} was recorded from four varieties. Again, number of seeds plant^{-1} was found lowest (76.0) in HYV BARI seem 5 (Table 4). A significant variation in hundred seed weight of the studied twenty varieties was observed which ranged from 19.80 to 68.95g (Table 4). Among the varieties, HYV BARIseem9 showed the highest and local Rifa gave the lowest weight of 100 seed in this study (Table 4).

Seed yield variation among the studied twenty country bean varieties was statistically significant. The local Kaloputi produced the highest amount of seed yield (595.70g plant^{-1}) followed by HYV BARI seem 8 (378.10g plan^{-1}). HYV BARI seem 5 showed the lowest yield of dry seeds (19.38g plant^{-1}) followed by local Rifa (Table 4).

Pod borer resistance

In the present study, pod borer resistance was evaluated by estimating the degree of pod infestation by pod borer. A wide variation was observed among the twenty varieties. Pod borer infestation ranged from 3.08 % to 15.71 %. The infestation percentage of pod borer was lowest in local variety Kaloputi and highest in Noldoc (Table 3). Kaloputi was closely followed by Mostafa with 4.07 % infestation. These two varieties can be considered as tolerant variety. BARI 2, BARI seem 3, BARI seem 5, BARI seem 7 and BARI seem 8 had a considerable lower percentage of infestation and can be considered as moderately resistant variety. The rest of the varieties had a higher level of pod borer infestation.

Table 2. Genotypic performance on morphological characteristics of country bean

Variety	Leaf length (cm)	Leaf width (cm)	Leaflet length (cm)	Raceme length (cm)	Raceme plant^{-1}	Flower bud raceme^{-1}
BARI seem 1	26.50 g	29.00 d	12.00 d	35.20 i	22.00 e	30.00 c
BARI seem 2	20.00 i	21.50 h	11.00 e	30.00 k	18.00 h	26.00 e
BARI seem 3	27.00 f-g	19.00 i-k	10.50 e	60.00 a	16.00 j	32.00 b
BARI seem 4	30.00 d	27.00 e-f	13.00 c	36.50 h	26.00 c	26.00 e
BARI seem 5	22.00 h	18.00 k	8.000 g	41.12 f	8.000 n	26.00 e
BARIseem 6	32.50 b-c	30.50 c	16.00 a	7.750 no	19.00 g	22.00 g
BARI seem 7	22.00 h	18.00 k	11.00 e	42.97 e	19.00 g	22.00 g
BARI seem 8	28.00 e-f	26.60 e-f	14.00 b	44.66 d	21.00 f	28.00 d
IPSA Seem 2	26.50 g	27.00 e-f	13.00 c	31.27 j	28.00 b	36.00 a
Khisamoti	27.50 f-g	20.00 i	9.500 f	39.37 g	17.00 i	24.00 f
Rifa	21.50 h	19.50 i-j	10.50 e	36.50 h	11.00 m	18.00 h
Goal gada	36.00 a	26.00 f	14.00 b	8.120 no	15.00 k	14.00 i
Nodi	31.50 c	33.50 a	12.00 d	9.870 m	18.00 h	13.00 i
Noldoc	28.00 e-f	27.50 e	12.50 cd	7.160 o	18.00 h	9.000 j
Ali	21.00 hi	18.50 j-k	10.80 e	23.25 l	23.00 d	8.000 jk
Laluri	29.50 d	27.50 e	14.00 b	49.80 c	23.00 d	32.00 b
Mostofa	35.50 a	22.00 h	13.00 c	58.37 b	17.00 i	37.00 a
Kaloputi	29.00 de	18.00 k	11.00 e	31.16 j	33.00 a	21.00 g
Chonchol	29.50 d	23.30 g	12.00 d	8.550 n	21.00 f	7.000 k
BARI –9	33.00 b	32.00 b	16.00 a	42.00 ef	12.00 l	36.00 a
Level of sig.	**	**	**	**	**	**
CV (%)	2.8	3.17	2.57	2.14	2.46	2.8

In a column figures having same letters do not differed significantly at 5% level whereas figures with dissimilar letter(s) differed significantly as per DMRT at same level; **= Significant at 1% level of probability.

Table 3. Genotypic performance on yield contributing characteristics of country bean

Varieties	Pod length (cm)	Pod width (cm)	No of pod plant^{-1}	Infestation (%)	Weight of single pod (g)	Green pod yield (g plant^{-1})
BARI seem 1	10.50 e-f	2.35 c-f	140.0 b	5.57 g	10.14 f	1420.60 d
BARI seem 2	11.00 e	1.90 f	58.0 h	6.90 g	7.560 h	438.48 q
BARI seem 3	9.800 f-g	3.50 a-b	116.0 d	6.03 g	6.170 i	715.72 k
BARI seem 4	14.80 c	2.03 e-f	67.0 g	8.45 f	12.08 de	809.36 j
BARI seem 5	10.90 e	2.73 c	19.0 m	14.04 b	6.540 i	124.26 s
BARI seem 6	21.13 a	2.43 c-e	111.0 e	11.23 cd	14.90 b	1653.90 c
BARI seem 7	9.460 g-h	3.25 b	115.0 de	6.74 g	7.860 h	903.90 g
BARI seem 8	14.56 c	2.23 d-f	128.0 c	6.41 g	14.26 b	1826.28 b
IPSA seem 2	8.260 i-j	2.06 ef	131.0 c	9.31 ef	6.790 i	889.49 h
Khisamoti	11.20 e	3.46 a-b	39.0 k	8.97 f	14.15 b	544.77 n
Rifa	8.730 h-i	2.43 c-e	34.0 l	11.76 cd	4.390 j	149.26 r
Goal gada	17.06 b	3.80 a	62.0 h	8.39 f	22.47 a	1393.14 e
Nodi	17.33 b	2.13 d-f	47.0 i	11.91 c	13.22 c	621.34 l
Noldoc	20.66 a	2.33 c-f	42.0 jk	15.71 a	11.44 e	475.90 p
Ali	9.850 f-g	2.55 c-d	41.0 k	9.15 ef	12.21 de	500.61 o
Laluri	8.760 h-i	2.26 d-f	93.0 f	8.39 f	9.250 g	860.25 i
Mostofa	7.760 j	2.53 c-d	126.7 c	4.07 h	9.230 g	1172.21 f
Kaloputi	9.200 g-h	2.26 d-f	267.7 a	3.08 h	7.790 h	2111.09 a
Chonchol	13.16 d	1.90 f	70.0 g	10.48 de	8.990 g	629.30 l
BARI seem 9	9.500 g-h	2.15 d-f	46.0 ij	11.96 c	12.32 d	566.72 m
Level of sig.	**	**	**	**	**	**
CV (%)	3.74	9.25	3.24	8.72	4.31	15.58

In a column figures having same letter(s) do not differed significantly at 5% level whereas figures with dissimilar letter(s) differed significantly as per DMRT at same level; **= Significant at 1% level of probability.

Table 4. Genotypic performance on seed quality characters of country bean

Variety	Length of seed (cm)	Width of seed (cm)	Number of seeds pod^{-1}	Hundred seed weight (g)	Dry seed yield (g plant^{-1})
BARI seem1	1.30 d	0.90 d	5.00 d	40.65 g	284.5 e
BARI seem 2	1.20 e	0.70 f	5.00 d	31.65 i	91.15 o
BAR Iseem 3	1.40 c	1.00 c	4.00 e	47.75 e	220.6 h
BARI seem 4	1.60 a	0.90 d	5.00 d	57.75 b	201.4 i
BARI seem 5	1.10 f	0.80 e	4.00 e	25.50 k	19.38 s
BARI seem 6	1.40 c	1.10 b	7.00 a	43.75 f	340.8 c
BARI seem 7	1.20 e	0.90 d	4.50 d	27.20 j	140.9 m
BARI seem 8	1.60 a	1.00 c	5.50 c	53.85 c	378.1 b
IPSA seem 2	1.30 d	0.90 d	5.00 d	34.60 h	226.6 g
Khisamoti	1.40 c	0.90 d	5.50 c	40.50 g	85.75 p
Rifa	1.10 f	0.70 f	4.50 d	19.80 l	30.49 r
Goal gada	1.50 b	1.00 c	6.00 b	48.75 e	181.8 j
Nodi	1.30 d	0.90 d	6.00 b	40.20 g	113.4 n
Noldoc	1.40 c	1.00 c	5.50 c	39.95 g	91.48 o
Ali	1.30 d	1.00 c	5.00 d	34.25 h	70.21 q
Laluri	1.40 c	1.00 c	5.00 d	53.55 c	249.5 f
Mostofa	1.40 c	1.00 c	4.00 e	58.75 b	298.5 d
Kaloputi	1.30 d	1.00 c	4.00 e	54.95 c	595.7 a
Chonchol	1.20 e	0.90 d	4.50 d	50.55 d	159.2 k
BARI –9	1.50 b	1.20 a	4.50 d	68.95 a	153.8 l
Level of sig.	**	**	**	**	**
CV (%)	2.59	3.71	5.41	1.94	0.83

In a column figures having same letter(s) do not differed significantly at 5% level whereas figures with dissimilarly letter(s) differed significantly as per DMRT at same level; **= Significant at 1% level of probability.

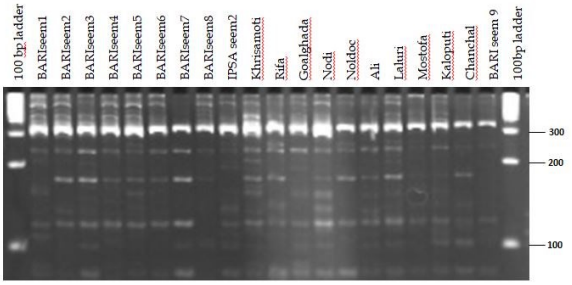

Fig. 1(a). SSR profile of 20 country bean genotypes at Locus BMd 46

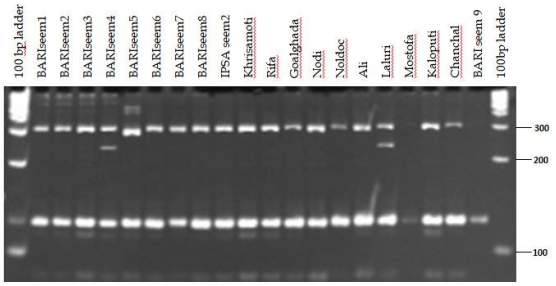

Fig. 1(b). SSR profile of 20 country bean genotypes at Locus Hbp001

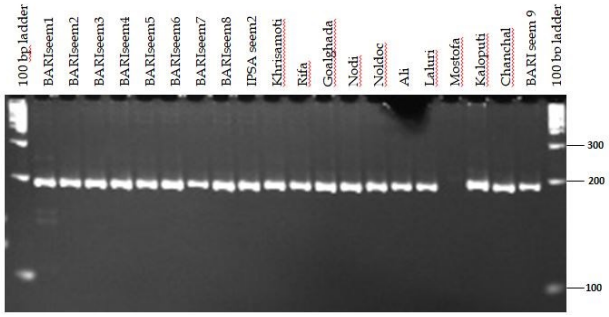

Fig. 1(c). SSR profile of 20 country bean genotypes at Locus Hbp006

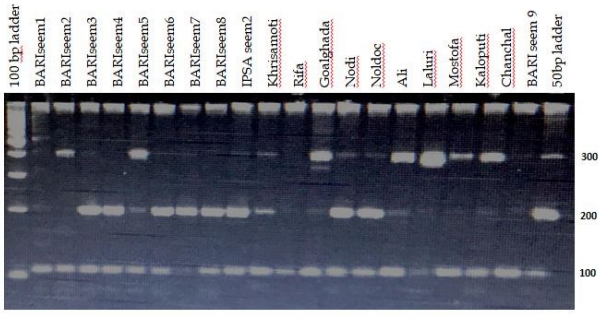

Fig. 1(d). SSR profile of 20 country bean genotypes at Locus Y583516

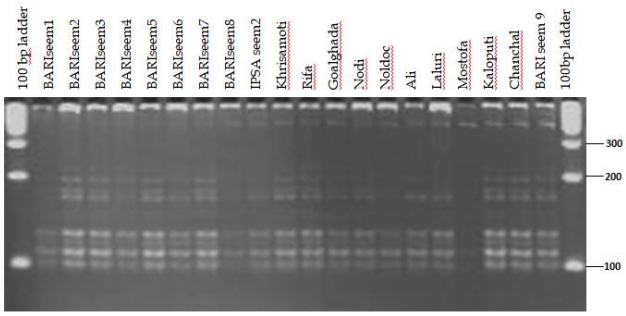

Fig. 1(e). SSR profile of 20 country bean genotypes at Locus AB176566

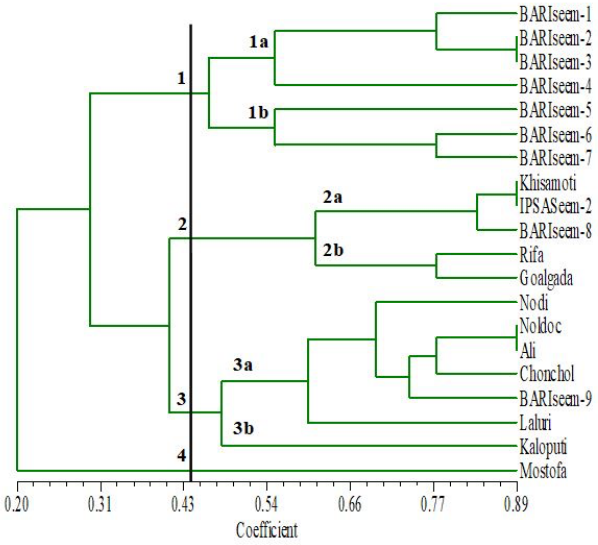

Fig. 2. UPGMA dendrogram for 20 country bean genotypes showing the genetic similarity

Molecular diversity

The nine SSR markers (AB176566, AF067471, BMd46, Hbp001, Hbp002, Hbp003, Hbp006, Pvag004 and Y583516) were used to evaluate diversity among the country bean genotypes. A total of 37 alleles were detected at 9 loci. A wide range of allelic variants was observed for each locus. The lowest number of alleles (2) was found for marker AB176566 and the highest number of alleles (7) was detected for marker Y583516 with an average of 4.11 (Table 5). A genotype was assigned a null allele for an SSR locus whenever an amplified product (s) was not detected for the particular genotype and marker combination. Of the 9 SSR loci used in this study, the locus that showed the highest frequency of null alleles were resulted by two loci HBP006 and Y583516 (nulls detected in six genotypes) and lowest frequency of null allele was detected by five loci AB176566, AF067471, Hbp003, Hbp006 and Pvag004 (nulls detected in one genotypes) with an average of 1.44 (Table 5). In the case of null alleles, PCR amplifications were repeated to exclude the possibility that a failed PCR reaction could be the cause of the null allele. Null alleles can arise from point mutation(s) in one or both of the primer binding sites and thereby inhibiting primer annealing (Shikazono *et al*., 2005). Major allele is the allele with the highest frequency and also known as most common allele at each locus. The frequency of the most common allele at each locus ranged from 0.9500 in AB176566to 0.3500 in HBP002 with a mean frequency of 0.5444 (Table 5). The size of the different major alleles at different loci ranged from 127 bp for AB176566 to 122bp for HBBP002 (Table 5).

Gene diversity, often referred to expected heterozygosity, is the probability that two randomly chosen allele from the population are different. The highest gene diversity (0.7600) was observed in loci Y583516 and the lowest gene diversity (0.0950) was observed in loci AB176566 with the mean diversity of 0.5744 as estimated following the formula of Nei's, (1973) (Table 5). It was observed that markers detecting the lower number of alleles showed lower gene diversity than those detecting the higher number of alleles revealed higher gene diversity. As a measure of the informative nature of microsatellites, the PIC values ranged from 0.0905 in AB176566 to 0.7302 in Y583516 with the average value of 0.5198 (Table 5). PIC values in this study showed a significant, positive correlation with the number of alleles and allele size range for

microsatellites evaluated. The allele size range and the number of allele themselves were also highly correlated. The molecular experiment was conducted to find the genetic diversity and relatedness among the 20 cultivars through molecular marker (SSR). A dendrogram based on the Nei's (1973) genetic distance calculated from the 37 alleles (by 9 pod borer linked SSR markers) generated from 20 country bean genotypes. All country bean genotypes (20) could be easily distinguished.

Table 5. Summary statistics of 9 SSR markers found among 20 country bean genotypes

Marker name	Obtained allele range	Allele no.	Null allele	Major allele		Gene diversity	PIC
				Frequency	Size (bp)		
AB176566	127	2	1	0.950	127	0.095	0.0905
AF067471	258-267	3	1	0.550	158	0.535	0.4359
BMd 46	297-313	3	0	0.500	302	0.605	0.5270
Hbp001	252-294	5	2	0.600	294	0.595	0.5613
Hbp002	122-126	4	3	0.350	122	0.725	0.6746
Hbp003	150-164	6	1	0.500	156	0.685	0.6501
Hbp006	198-201	3	1	0.550	201	0.535	0.4359
Pvag004	131-135	4	1	0.500	133	0.635	0.5729
Y583516	197-391	7	3	0.400	194	0.760	0.7302
Mean	-	4.11	1.44	0.544	-	0.574	0.5198

The UPGMA cluster analysis showed significant genetic variation among the country bean genotype studied, with a similarity coefficient varying between 0.20 and 0.89 (Fig. 2). The UPGMA cluster analysis led to the grouping of the 20 germplasm into four major clusters formed at 0.45 cut off similarity coefficient below which the similarity values narrowed conspicuously. Cluster 1 was consisted of two sub clusters (sub cluster 1a and sub cluster 1b); sub cluster 1a with BARI seem 1, BARI seem 2, BARI seem 3, BARI seem 4, another sub cluster 1b contains BARI seem 5, BARI seem-6, BARI seem-7. Cluster 2 was divided into two sub clusters. The sub cluster 2a was constituted with Khisamoti, IPSA seem 2 and BARI seem 8 whereas sub cluster 2b contained Rifa and Goalgada. Cluster 3, was constructed by two sub-clusters. The sub cluster 3a comprised of Nodi, Noldoc, Ali, Chancol, BARI seem 9, Laluri. Whereas, the other sub cluster 3b contained only Kalaputi. Cluster 4, was consisted only of Mostofa.

Discussion

Results obtained with different traits related to morphology, inflorescence and pod borer infestation of country bean revealed significant variations among the varieties. Similar views were reported by Singh and Taylor (1978), Rahman (1987) and Rahman and Rahman (1989). The variation in colour of vein, leaf, flower or pod among the varieties might be due to the variation in their genetic characters. Jagadeesh *et al*. (2008) reported that the different genotypes of mung bean showed variation in colour of plant parts. Similar variations were reflected in the present findings. Chakravarthy (1977) reported that the pod colour and pubescence were closely associated with borer resistance. However, Regupathy *et al*. (1970) did not find any effect of seed colour on the preference of borers. We also could not establish any clear relationship between pod or seed colour with pod borer resistance.

The local variety, Kaloputi performed best in pod and seed production which might be due to the variation in adaptability with the regional condition of the study area. The local environment might be favourable for the adaptive capacity of this variety, which ultimately influenced its growth and yield of country bean and reduced the pod borer infestation because the larval population build up of pod borer is influenced by various climatic factors of the area (Roshan and Raju, 2018). The present findings agree well with the findings of Sarder and Kundu (1987) who reported that the borers caused up to 7% yield reduction of different varieties in Bangladesh while Kabir *et al*. (1983) found that the insect caused up to 17% damage to country bean pods and yield loss up to 32%. Besides, Deshmukh *et al*. (2010) reported that the genotypes *H. armigera*, BG-372, HC-1, SAKI- 9516, Vijay and Avarodhi gave higher grain yield with lower larval population and also had lower pod damage. Genotypes Dahood Yellow and BG-256 also gave good grain yields despite having high larval numbers and high pod damage. The findings of the present study also were consistent well with Dawoodi *et al*. (2010); Umbarkar *et al*. (2011). The great variability among the varieties in yield contributing characters found in the present study indicates that different varieties have different unique characters favourable for higher performance on the growth and yield of country bean.

Deshmukh *et al*. (2010) found that the genotypes Dahood yellow and BG-256 gave good grain yields despite having high larval numbers and high pod damage among the 15 chickpea genotype might be due to the variation in genetic characters and also the variation in adaptability with the studied region (Raut *et al*., 1993). These findings supported the results of the present research. Similar results were also reported by (Dawoodi *et al*., 2010; Umbarkar *et al*., 2011). Data obtained from the present study were analyzed to evaluate the usefulness of the microsatellites for genetic diversity of the 20 country bean genotypes. Simple sequence repeats analysis revealed that number of alleles ranged from 2 to 7 with an average of 4.11 alleles per marker. The allele numbers observed in this study are significantly higher than the allele number reported from other studies by

Shivachi *et al*. (2012), indicating a higher degree of diversity among the genotypes studied. The more alleles at a locus, the higher the degree of diversity that can be revealed, and the more efficiently closely related genotypes can be distinguished (Nagy *et al*., 2012). SSR markers are locus-specific and generally amplify one locus. Genetic diversity evaluation within a population is indispensable for the characterization of germplasm and offers insight into the evolutionary characteristic, management, exploitation and establishment of breeding approaches for breeders (Li *et al*., 2011). Using RAPDs and SRRs, mean amplifications of 5.69 and 3.6 alleles per primer, respectively were reported in lablab bean previously (Rai *et al*., 2010; Wang *et al*., 2007). Similar studies also found mean amplifications of over 7.0 alleles per primer in *Phaseolus vulgaris* (Jose *et al*., 2009; Maras *et al*., 2008) and 12.2 alleles per primer in soybean (Wang *et al*., 2006) which were much higher than that of ours.

The genetic diversity was measured by the polymorphic information content (PIC). PIC provides an estimate of the discriminatory power of a locus by taking into account not only the number of alleles expressed, but also the relative frequency of those alleles (Nagy *et al*., 2012). In the current study, PIC values ranged from 0.0905 to 0.7302 with the average value of 0.5198 per marker. The highest PIC value of 0.7302 was observed in SSR primers Y583516 while the lowest PIC value of 0.0905 was observed in primer AB176566. The PIC values recorded in this study are significantly higher than the PIC values reported from other studies. Polymorphic information content values range from 0 (indicates monomorphism) to 1.0 (very high discriminative power with many alleles in equal frequencies) and the higher the PIC value, the more informative is the SSR marker (Nagy *et al*., 2012). Uddin and Boerner (2008) found similar observations. Hence, primers BMd 46, Hbp001, Hbp002, Hbp003, Hbp006 and Y583516 were found to be highly informative in revealing the genetic diversity among the Lablab populations as they had PIC value above 0.5 and can be efficiently used in future genetic diversity analysis. The markers showed an average PIC value of which confirm that SSR markers used in this study were highly informative because PIC values higher than 0.50 indicate high polymorphism (Vaiman *et al*., 1994). The PIC can be looked as the measurement of usefulness of each marker in distinguishing one individual from another.

Some country bean genotypes were genetically similar probably partly due to duplication of genotypes among farmers, while accessions obtained from the gene bank could be cultivated forms sourced from farmers. Another possibility is that these genotypes were mainly composed of cultivated forms that tend to have low levels of genetic diversity. As a result, the computed genetic distance showed low variability. Most genotypes were in the third cluster, an indicator of similarity among genotypes from the two sources, resulting in low genetic distances (0.111 to 0.556). However, Rai *et al*. (2010) using RAPDs and Maass *et al*. (2005) using AFLP obtained fairly large genetic distances of 0.38 to 0.96 and 0.217 to 0.915 while evaluating country bean germplasm after inclusion of wild relatives for their studies.

There were four clusters found in the dendrogram. Cluster 1 contains most of the high yielding varieties (BARI seem 1, BARI seem 2, BARI seem 3, BARI seem 4, BARI seem 5, BARI seem-7), whereas Cluster 3 contains most of the local varieties (Nodi, Noldoc, Ali, Chanchal, Laluri, Kalaputi) except BARI seem 9. Cluster 2 composed of both high yielding and local varieties Khrishamoti, Rifa, Goalghada, IPSA seem 2 and BARI seem 8, whereas Mostofa alone belongs to the Cluster 4. It indicates that the modern varieties are dissimilar with the local varieties at the molecular level. However, no distinct grouping of the varieties could be possible on the basis of infestation percentage and molecular marker analysis. Further studies with more number of genotypes and markers are necessary and under progress.

Conclusion

The wide range of dissimilarity values found in the molecular study suggests that the germplasm collection represents a genetically diverse population. The results show that rich diversity exists between the germplasm collections from different geographical regions of the country. The morphological study confirmed that the local cultivar Kaloputi showed higher plant growth and yield. This variety showed higher resistance to pod borer infestation. Similar performance was shown by the cultivar Mostafa. The findings suggest that some of these genotypes, especially two local varieties, kaloputi and Mostafa, are promising for using as parents for the development of new variety. Further studies are necessary for the development of pod borer resistant variety with a higher yield.

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