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SCREENING LABLAB BEAN GENOTYPES FOR HIGHER GRAIN YIELD AND RESISTANCE AGAINST BEAN COMMON MOSAIC VIRUS

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SUMMARY

Yield potential of lablab bean is adversely affected by the most significant disease namely Bean common mosaic virus (BCMV). Fifteen genotypes of lablab bean (*Lablab purpureus* L.) were used for genetic analysis and detection of *bc-3* gene conferring resistance to *bean common mosaic virus*. Thirteen yield contributing traits were evaluated for enumerating genetic variability and trait association. Phenotypic variations were greater than the genotypic variations indicating the existence of environmental effect on trait expression. Five pairs of characters showed significant correlation and path analysis revealed both direct positive and negative effects of the yield contributing traits on seed yield. For detection of *bc*-3 resistance gene, two SCAR primers ROC11 and SG6 were used. Nine genotypes were produced expected bands for SG6 primer indicating the partial resistance due to presence of recessive *bc-3* allele. These results could be helpful for further crop improvement program of lablab bean.

Keywords: Lablab bean, genetic analysis, bean common mosaic virus, *bc-3* gene

Key findings: Seed yield is an important trait that should be emphasized during genetic variability studies of lablab bean. Genotypes DS35 and DSN27 performed better considering seed yield with other yield contributing characters and complete resistance to Bean common mosaic virus that can be utilized in future breeding program.

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INTRODUCTION

Lablab bean (*Lablab purpureus*) is a highly nutritious legume vegetable crop enriched with protein, vitamins *viz.* vitamin A**,** vitamin C, riboflavin and minerals like magnesium, calcium, phosphorus, iron, sulfur and sodium (Newaz, 1992). Its green edible pods provide about 25% protein (dry weight basis) and commonly cultivated in tropic and sub-tropic areas including Bangladesh (Kimani *et al*., 2012). It is an important part of commercial agriculture and feeds about 300 million people in tropics and 100 million people in Africa alone (Sofi *et al*., 2014).

Beans are usually cultivated in the winter season. It would be almost impossible to find a homestead in rural areas without a vine of lablab bean (Salim *et al*., 2013). Recently, lablab bean cultivation has become very popular and grown in a significant acreage alongside other vegetables like brinjal, tomato etc. in most of the countries (FSC, 2014). Based on consumption pattern, the lablab bean production is not satisfactory in most of the countries due to their low yield. So, the high yielding lablab bean varieties selection is very important for larger scale cultivation (Hossain *et al*., 2013). The efforts of improving the crop by utilizing indigenous and exotic germplasms have been useful in breaking yield barriers. Great range of variation exists in the plant and pod characters among the cultivars grown worldwide; these differences occurred as to different morphological and biochemical characters (Nasreen *et al*., 2009). Only highly heritable traits should be considered for crop development and the selection of the traits also depends on genetic advance and correlation of the traits. In addition, path coefficient analysis helps in partitioning of the total correlations into direct and indirect contribution thereby indicating the degree of importance of each of the trait towards yield (Rahman *et al*., 2012).

Bean common mosaic virus (BCMV) is one of the most common and destructive viral disease worldwide and causing yield loss significantly (Strausbaugh *et al*., 1999). It generally spreads by aphids, seeds or pollen. Disease is identified by leaf mosaic, leaf distortion, growth retardation, and severe yield reduction (Morales and Castano, 1987). Yield reductions have been reported up to 50% to 80% depending upon cultivar, environment, and infection time (Galvez and Morales, 1989). Recently, several researchers worked on BCMV disease screening in common bean genotypes and identified few resistance genotypes (Wani *et al*., 2017). The management practices are very difficult; the common control measure is to reduce the infestation of aphid, but sometimes it becomes very difficult due to their fast growing habit. The most effective strategy for broad spectrum control of the disease is to use resistant varieties. The genetic control towards the virus is controlled by one dominant *I* gene and a number of recessive genes which are *bc-u*, *bc-1*, *bc-1*^{2}, *bc-2*, *bc-2*^{2} and *bc-3* (Pasev *et al*., 2013). Additionally, the *bc-3* gene confers resistance to all known strains of BCMV with presence of the dominant *I* gene with recessive *i* gene, *bc-3* requires the presence of *bc-u* to be fully expressed while *bc-u* is not required in the presence of the dominant *I* gene (Mukeshimana *et al*., 2005). Therefore, for developing BCMV resistant varieties, *bc-3* gene detection is very crucial and several SCAR markers are already reported for the purpose (Johnson *et al*., 1997; Mukeshimana *et al*., 2005). The present investigation, therefore, shall reveal the different genetic parameters and associations between the traits, as well as resistance status of the genotypes used to BCMV based of detection of *bc-3* gene.

MATERIALS AND METHODS

Study materials and their management

The experiment was conducted at the field laboratory of the Department of Genetics and Plant Breeding, Bangladesh

Agricultural University and at the Biotechnology Laboratory of Bangladesh Institute of Nuclear Agriculture. Fifteen lablab bean genotypes were used for genetic analysis and molecular identification of (BCMV) genotypes. Among these, twelve advanced generation lines were obtained from the Department of Genetics and Plant Breeding, Bangladesh Agricultural University *viz.,* DS11, DS30, DS35, DS57, DS113, DS116, DS173, DSN12, DSN17, DSN25, DSN26 and DSN27 and other three local land races were from different areas of Bangladesh namely; Ashina, Knoldog, and Kartika. The morphological evaluation was done in the open field conditions during the season of 2014-15. The experiment was carried out in RCBD with three replications. The experimental plot was prepared by 2 to 3 times spading and clods were broken into small pieces. Pits were prepared using 25g TSP and 15g MOP and mixed in each of the pits. No urea was given as lablab bean is a leguminous plant. Seeds were sown by making pits and keeping more or less equal distances within the plot. After germination, the seedlings of each genotype in each plot were allowed to grow till maturity with proper care. Plants were spaced at 1.5 m in both plant to plant and row to row. Recommended agronomic management practices were followed as necessary.

Data collection

A total of thirteen traits were studied; namely data on days to first flowering (DFL), days to maturity (DM), numbers of racemes per plant (NRP), raceme length (RL), number of flower buds per raceme (NBR), number of nodes per raceme (NNR), green pod length (PL), green pod yield per plant (GPYP), test fresh weight (TFW), test dry weight (TDW), shelling percentage in fresh pods (S%F), shelling percentage in dry pods (S%D) and dry seed yield per plant (DSYP) were recorded for genetic analysis.

Genetic parameters estimation

Genotypic variance (Vg), phenotypic variance (Vp), heritability in broad sense (h^2b), genetic advance (GA), genetic advance in percentage (GA%), genotypic (Covg) and phenotypic covariance (Covp) were performed according to the given formula as stated by the author of Johnson *et al.* (1955). Genotypic (GCV) and phenotypic coefficient of variation (PCV) were calculated according to the formula suggested by Burton and Devane (1952). Genotypic (r_g) and phenotypic (r_p) correlation coefficient were estimated by the given formula of Weber and Moorthy (1952). Path coefficient analysis was done according to formula given by Dewey and Lu (1959).

Statistical analysis

Data were analyzed by MSTAT-C statistical software. Analysis of variance (ANOVA) was executed for different traits following the General Linear Model. A Pearson correlation analysis was carried out to explore relationship among all traits.

Collection of leaf samples, genomic DNA isolation and PCR analysis

Young, green, fresh leaf samples were collected from two weeks plants of each of the genotypes. Genomic DNA was extracted using the modified CTAB (Cetyl Trimethyl Ammonium Bromide) method (Agbagwa *et al*., 2012). Previously reported two sets of SCAR primers were used in the PCR (Table 1) (Johnson *et al*., 1997; Mukeshimana *et al*., 2005). PCR was carried out with 10 µL reaction mixtures contained 0.5 µL of forward 0.5 µL reverse primers (10 pmol), 5.0 µL Emerald PCR master mix (Takara, Shiga, Japan), 3.0 µL ultra-pure water and 1.0 µL DNA (70 ng). The number of thermal cycle in case of ROC11 primer was 34, where denaturation was done at 94^0C for 10 s, annealing was done at 55^0C for 40 s and elongation was done at 72^0C for 2

Table 1. List of SCAR primers used in this study.

Primer	Size (bp)	Forward Primer (5'-3')	Reverse Primer (3'-5')	Annealing temperature (^oC)
ROC11	420	CCAATTCTCTTTCACTTGTAACC	GCATGTTCCAGCAAACC	55
SG6	595	GTGCCTAACCGAGTTATCTAGAGT	TGCCTAACCCTCCTAAATGACCT	

minutes. In case of SG6 primer the number of thermal cycle was 30 where denaturation was done at 94^0C for 10 s, annealing was done at 55^0C for 1 minute and elongation was done at 72^0C for 1 minute. The amplified PCR products were separated by electrophoresis using 8 µL of PCR products in 1% Agarose gel dissolved in 1x TBE buffer and stained with HIQ Blue Mango (20,000X) (bioD, Seoul, South Korea) and run for 40 min with 100 V before visualizing under UV light (302 nm). Markers were considered amplified upon visual presence in the gel from PCR products.

RESULTS

Genetic analysis of lablab bean genotypes

ANOVA demonstrated significant differences ($P \leq 0.01$) among the genotypes for all the morphological traits (Table 2). Mean performances of the genotypes for the traits considered were presented in Table 3. The genotypes like DS57 and DS116 were accounted for higher mean for number of flower buds per raceme (19.75), shelling percentage in fresh pod (39.94), shelling percentage in dry pod (72.96), green pod yield per plant (888.06) and dry seed yield per plant (262.37); also had the better performance with the number of racemes per plant (24.33), test fresh seed weight (65.62), and test dry seed weight (50.30), compared with other genotypes. Additionally, the genotypes DS35, DSN12 and DS113 had better yield performance with the green pod yield per plant, dry seed yield per plant, days to flowering, green pod length, number of flower buds per raceme, shelling percentage in fresh pod, and shelling percentage in dry pod; but the green and dry yield is not satisfactory for DSN12 genotype (Table 2).

Estimation of genetic parameters

The perusal of data showed variances had inherent genetic differences among the genotypes. Among the traits, green pod and seed yield per plant exhibited high estimates of GCV (42.94% and 49.57%) and PCV (44.41% and 49.58%). However, test fresh seed weight (7.88% and 8.34%) and test dry seed weight (7.14% and 7.41%) showed low GCV and PCV (Table 3). In addition, the traits examined in this study expressed to high heritability estimates ranging from 71.25% to 99.92% with the exception of number of raceme per plant (53.28%). Maximum desirable characters were showed high heritability such as days to maturity (99.24%), raceme length (97.55%), green pod length (98.99%), green pod yield per plant (93.57%), test dry seed weight (93.14%), shelling percentage in fresh pods (98.22%), shelling percentage in dry pods (99.59%), dry seed yield per plant (99.92%), respectively, whereas the number of racemes per plant (53.28%) showed medium or low heritability (Table 3). Furthermore, highest genetic advance was found for green pod yield per plant (760.15) and dry seed yield per plant (267.80); but the lowest was observed for number of nodes per raceme (3.80). The genetic advance as percentage of mean (%) was the highest for dry seed yield per plant (102.07%) followed by green pod yield per plant (85.60%); the lowest was found for test dry seed weight (14.12%) (Table 3).

Table 2. Mean performances of fifteen lablab bean genotypes based on different morphological traits.

Genotypes	DFL	DM	NRP	RL	NBR	NNR	PL	GPYP	FTW	DTW	S%F	S%D	DSYP
DS11	68.67 bcd	128.00 e	25.33 bcde	23.00 c	21.66 bcd	11.33 ab	10.67 f	750.00 g	63.00 ef	49.67 fg	37.40 efg	68.60 h	273.67 f
DS30	62.00 d	125.00 f	32.67 ab	18.00 f	12.00 f	6.67 f	18.42 b	1216.67 bc	64.00 e	51.67 bcde	28.18 i	63.47 j	285.00 e
DS35	75.00 b	130.00 d	26.50 bcde	21.83 cd	22.50 bc	12.50 a	8.75 g	1087.50 cd	61.00 f	45.00 h	38.82 de	76.05 e	345.50 c
DS57	69.67 bcd	119.00 h	37.33 a	21.67 cd	21.67 bcd	9.00 cde	10.83 ef	1483.33 a	70.67 b	54.00 a	42.79 c	79.27 d	488.67 a
DS113	63.50 cd	122.00 g	27.50 bcd	21.50 d	17.50 de	7.50 ef	12.88 c	1015.00 de	65.00 de	50.50 efg	39.80 d	72.05 f	337.00 d
DS116	68.33 bcd	128.00 e	28.33 bc	22.67 cd	28.67 a	13.00 a	12.08 d	1267.00 b	70.00 bc	52.67 abc	51.30 a	89.03 a	487.67 a
DS173	70.67 bc	129.00 de	18.67 ef	22.67 cd	18.67 cde	9.00 cde	10.67 f	800.00 g	65.67 de	52.33 bcd	33.40 h	62.63 j	192.33 i
DSN12	93.67a	154.00 a	20.00 def	29.33 a	24.67 ab	10.00 bc	7.33 hi	216.67 h	71.00 b	54.00 a	51.40 a	88.20 a	88.67 i
DSN17	73.67 b	144.33 b	15.67 f	5.33 g	11.33 f	4.33 g	8.00 h	291.67 h	63.67 ef	51.00 def	48.27 b	85.10 c	93.00 kl
DSN25	74.33 b	135.00 c	19.33 ef	24.67 b	18.67 cde	8.67 cde	12.92 c	993.33def	61.00 f	46.33 h	36.53 fg	62.73 j	209.67 h
DSN26	90.67 a	152.67 a	21.00 cdef	17.33 f	21.33 bcd	9.33 cd	7.10 i	240.00 h	67.67 cd	51.33 cde	51.27 a	87.07 b	98.33k
DSN27	64.67 cd	126.00 f	22.33 cdef	20.00 e	20.00 cd	8.33 def	8.75 g	1258.33 b	53.33 g	41.00 i	38.20 def	70.03 g	382.33b
Ashina	70.00 bc	125.00 f	28.33 bc	25.33 b	20.67 bcd	9.00 cde	11.58 d	1016.67de	71.00 b	53.00 ab	36.70 fg	63.63 j	277.67 f
Knoldog	63.67 cd	122.00 g	23.33 cdef	17.00 f	15.33 ef	8.67 cde	21.67 a	831.67 fg	63.33 ef	49.33 g	28.63 i	60.77 k	152.00j
Kartika	69.67 bcd	123.33 g	18.67 ef	22.67 cd	21.67 bcd	10.33 bcd	11.42 de	853.37 efg	74.00 a	52.67 abc	36.43 g	65.80 i	240.00 g
CV%	6.48	0.73	19.93	4.06	13.58	11.92	3.57	11.26	2.72	1.95	2.57	0.92	1.32
Maximum	93.67	154.00	37.33	29.33	28.67	13.00	21.67	1483.33	74.00	54.00	51.40	89.03	488.7
Minimum	62.00	119.00	15.67	5.33	11.33	4.33	7.10	216.67	53.33	41.00	28.18	60.77	88.67
Mean	71.87	130.88	24.33	20.86	19.75	9.17	11.53	888.06	65.62	50.30	39.94	72.96	262.37
LSD (0.05)	7.79	1.60	8.11	1.42	4.49	1.83	0.69	167.20	2.98	1.63	1.71	1.11	5.81

Legend: DFL = Days to flowering, DM = Days to maturity, NRP = Number of racemes per plant, RL = Raceme length (cm), NBR = Number of flower buds per raceme, NNR = Number nodes per raceme, PL = Green pod length (cm), GPYP = Green pod yield per plant (g), FTW = 100 Fresh seed weight (g), DTW = 100 Dry seed weight(g) , S%F = Shelling percentage (Fresh), S%D = Shelling percentage (Dry), DSYP = Seed yield per plant.

Table 3. Estimation of genetic parameters for all morphological characters in fifteen lablab bean genotypes.

Characters	Mean	Range		Vg (δ^2g)	Vp (δ^2p)	GCV (%)	PCV (%)	h^2b (%)	GA	GA (%)
		Min.	Max.							
DFL	71.87	62	93.67	76.45	98.15	12.17	13.78	77.89	15.90	22.12
DM	130.88	119	154	119.47	120.38	8.35	8.38	99.24	22.43	17.14
NRP	24.33	15.67	37.33	26.83	50.35	21.29	29.16	53.28	7.79	32.01
RL	20.86	5.33	29.33	28.33	29.04	25.52	25.84	97.55	10.83	51.92
NBR	19.75	11.33	28.67	17.85	25.05	21.39	25.34	71.25	7.35	37.19
NNR	9.17	4.33	13	4.33	5.52	22.70	25.63	78.45	3.80	41.42
PL	11.53	7.1	21.67	15.76	15.92	34.43	34.60	98.99	8.14	70.56
GPYP	888.06	216.67	1483.33	145519	155518	42.96	44.41	93.57	760.15	85.60
FTW	65.62	53.33	74	26.77	29.94	7.88	8.34	89.41	10.08	15.36
DTW	50.30	41	54	12.90	13.85	7.14	7.40	93.14	7.14	14.20
S%F	39.94	28.18	51.4	58.23	59.28	19.11	19.28	98.22	15.58	39.01
S%D	72.96	60.77	89.03	107.21	107.65	14.19	14.22	99.59	21.29	29.17
DSYP	262.37	88.67	488.7	16911.5	16923.6	49.57	49.58	99.92	267.80	102.07

Legend: DFL = Days to flowering, DM = Days to maturity, NRP = Number of racemes per plant, RL = Raceme length, NBR = Number of flower buds per raceme, NNR = Number nodes per raceme, PL = Green pod length, GPYP = Green pod yield per plant, FTW = 100 Fresh seed weight, DTW = 100 Dry seed weight, S%F = Shelling percentage (Fresh), S%D = Shelling percentage (Dry), DSYP = Seed yield per plant.

Table 4. Genotypic correlation of the thirteen characters studied in fifteen lablab bean genotypes.

Traits	DM	NRP	RL	NBR	NNR	PL	GPYP	FTW	DTW	S%F	S%D	DSYP
DFL	0.1372	-0.0944	0.0657	0.1528	0.1705	-0.2682	-0.0031	0.1016	0.1061	0.1508	0.1000	-0.0070
DM		-0.1579	-0.0261	0.0302	-0.0418	-0.1858	-0.0029	0.0204	0.0402	0.1134	0.0768	-0.0067
NRP			0.1159	0.0308	0.1165	0.2374	0.0049	0.0799	0.1857	-0.0596	-0.0073	0.0176
RL				0.4495*	0.8580***	-0.0465	0.0016	0.1544	0.0866	-0.0135	-0.0386	0.0050
NBR					0.3695	-0.3936	0.0007	0.2510	0.1251	0.2358	0.1463	0.0106
NNR						-0.3016	0.0031	0.3705	0.0052	0.1731	0.1249	0.0203
PL							0.0035	-0.0448	0.0499	-0.3359	-0.2110	0.0022
GPYP								-0.0012	-0.0023	-0.0021	-0.0013	0.0003
FTW									0.6808***	0.1193	0.0720	-0.0012
DTW										0.1300	0.0952	-0.0044
S%F											0.1688	-0.0007
S%D												0.0002

* indicates significant at 0.1 probability; ***indicates significant at 0.01 probability

Legend: DFL = Days to flowering, DM = Days to maturity, NRP = Number of racemes per plant, RL = Raceme length, NBR = Number of flower buds per raceme, NNR = Number of nodes per raceme, PL = Green pod length, GPYP = Green pod yield per plant, FTW = 100 Fresh seed weight, DTW = 100 Dry seed weight, S%F = Shelling percentage (Fresh), S%D = Shelling percentage (Dry), DSYP = Seed yield per plant.

Table 5. Phenotypic correlation of 12 yield contributing characters on seed yield of fifteen lablab bean genotypes.

Traits	DM	NRP	RL	NBR	NNR	PL	GPYP	FTW	DTW	S%F	S%D	DSYP
DFL	0.1080	-0.1028	0.0414	0.0878	0.1037	-0.2108	-0.0025	0.0708	0.0770	0.1154	0.0776	-0.0055
DM		-0.0851	-0.0265	0.0167	-0.0329	-0.1836	-0.0027	0.0162	0.0399	0.1106	0.0761	-0.0067
NRP			0.0718	0.0988	0.2582	0.1452	0.0039	0.0543	0.0617	-0.0429	-0.0037	0.0096
RL				0.3245	0.6762**	-0.0468	0.0015	0.1306	0.0697	-0.0139	-0.0376	0.0049
NBR					0.0975	-0.2440	0.0013	0.1765	0.0618	0.1650	0.1031	0.0079
NNR						-0.2259	0.0035	0.1298	-0.0793	0.1550	0.1031	0.0161
PL							0.0034	-0.0393	0.0412	-0.3253	-0.2082	0.0022
GPYP								-0.0010	-0.0025	-0.0020	-0.0012	0.0002
FTW									0.6223**	0.0938	0.0607	-0.0010
DTW										0.1242	0.0859	-0.0041
S%F											0.1663	-0.0007
S%D												0.0002

** indicates significant at 0.05 probability; ***indicates significant at 0.01 probability

Legend: DFL = Days to flowering, DM = Days to maturity, NRP = Number of racemes per plant, RL = Raceme length, NBR = Number of flower buds per raceme, NNR = Number of nodes per raceme, PL = Green pod length, GPYP = Green pod yield per plant, FTW = 100 Fresh seed weight, DTW = 100 Dry seed weight, S%F = Shelling percentage (Fresh),S%D = Shelling percentage (Dry),DSYP = Seed yield per plant.

Table 6. Direct and indirect effects of yield contributing characters on seed yield of lablab bean at genotypic level.

Traits	DFL	DM	NRP	RL	NBR	NNR	PL	GPYP	FTW	DTW	S%F	S%D	DSYP
DFL	0.0146	-0.0031	-0.0060	0.0223	-0.0138	-0.0593	0.0267	-0.0002	0.0205	-0.0194	0.0058	0.0047	-0.0070
DM	0.0021	-0.0244	-0.0121	-0.0095	-0.0028	0.0140	0.0188	-0.0002	0.0041	-0.0071	0.0042	0.0038	-0.0067
NRP	-0.0013	0.0040	0.0671	0.0383	-0.0028	-0.0419	-0.0238	0.0003	0.0164	-0.0336	-0.0023	-0.0030	0.0176
RL	0.0010	0.006	0.0081	0.3193	-0.0416	-0.0300	0.0049	0.0001	0.0308	-0.0160	-0.0039	-0.0019	0.0050
NBR	0.0022	-0.006	0.0020	0.1437	-0.0924	-0.1291	0.0386	0.0005	0.0513	-0.0229	0.0093	0.0080	0.0106
NNR	0.0025	0.008	0.0081	0.2746	-0.0342	-0.3488	0.0297	0.0002	0.0759	-0.8E-3	0.0066	0.0057	0.0203
PL	-0.0039	0.0043	0.0161	-0.0159	0.0360	0.1047	-0.0990	0.0003	-0.0082	-0.0088	-0.0131	-0..0099	0.0022
GPYP	-0.0040	0.0060	0.0030	0.0060	-0.0060	-0.0010	-0.0030	0.0007	-0.0020	0.0030	-0.0070	-0.0040	0.0003
FTW	0.0015	-0.004	0.0054	0.0480	-0.0231	-0.1290	0.0039	-0.0007	0.2052	-0.1202	0.0046	0.0033	-0.0012
DTW	0.0016	-0.008	0.0128	0.0287	-0.0120	-0.0017	-0.0049	-0.0001	0.0139	-0.1768	0.0051	0.0047	-0.0044
S%F	0.0022	-0.0024	-0.0040	-0.0032	-0.0222	-0.0593	0.0337	-0.0001	0.0246	-0.0229	0.0386	0.0080	-0.0007
S%D	0.0015	-0.0018	-0.004	-0.0128	-0.0157	-0.0419	0.0208	-0.0007	0.0144	-0.0177	0.0066	0.0472	0.0002
Residual value = 0.999										Bold diagonal values are direct effects			

Legend: DFL = Days to flowering, DM = Days to maturity, NRP = Number of racemes per plant, RL = Raceme length, NBR = Number of flower buds per raceme, NNR = Number of nodes per raceme, PL = Green pod length, GPYP = Green pod yield per plant, FTW = 100 Fresh seed weight, DTW = 100 Dry seed weight, S%F = Shelling percentage (Fresh), S%D = Shelling percentage (Dry), DSYP = Seed yield per plant

Table 7. Direct and indirect effects of yield contributing characters on seed yield of lablab bean at phenotypic level.

Traits	DFL	DM	NRP	RL	NBR	NNR	PL	GPYP	FTW	DTW	S%F	S%D	DSYP
DFL	-0.0081	-0.0040	0.0050	-0.0013	0.0019	0.0048	-0.0023	-0.0001	-0.0007	0.0007	-0.0008	-0.0003	-0.0055
DM	-0.0008	-0.0036	0.0004	0.0009	0.0004	-0.0015	-0.0019	-0.0001	-0.0002	0.0003	-0.0007	-0.0003	-0.0067
NRP	0.0008	0.0003	-0.0055	-0.0023	0.0021	0.0127	0.0017	0.0001	-0.0005	0.0005	0.0002	0.0010	0.0096
RL	-0.0003	0.0001	-0.0003	-0.0331	0.0066	0.0331	-0.0005	0.0007	-0.0013	0.0006	0.0070	0.0001	0.0049
NBR	-0.0007	-0.0070	-0.0005	-0.0118	0.0201	0.0049	-0.0027	0.0003	-0.0018	0.0005	-0.0012	-0.0003	0.0079
NNR	-0.0008	0.0001	-0.0014	-0.0225	0.0021	0.0487	-0.0025	0.0001	-0.0013	-0.0007	-0.0011	-0.0003	0.0161
PL	0.0017	0.0006	-0.0008	0.0016	-0.0049	-0.0112	0.0111	0.0001	0.0004	0.0003	0.0023	0.0008	0.0022
GPYP	0.0020	0.0010	-0.0020	-0.0060	0.0020	0.0001	0.0030	0.0003	0.0010	-0.0020	0.0010	0.0003	0.0002
FTW	-0.0005	-0.0070	-0.0002	-0.0043	0.0037	0.0063	-0.0004	-0.0003	-0.0102	0.0057	-0.0006	-0.0002	-0.0010
DTW	-0.0006	-0.0002	-0.0003	-0.0023	0.0012	-0.0039	0.0004	-0.0001	-0.0063	0.0092	-0.0008	-0.0003	-0.0041
S%F	-0.0009	-0.0004	0.0002	0.0003	0.0035	0.0078	-0.0036	-0.0007	-0.0009	0.0011	-0.0071	-0.0006	-0.0007
S%D	-0.0006	-0.0002	0.0020	0.0013	0.0021	0.0049	-0.0023	-0.0003	-0.0006	0.0008	-0.0012	-0.0038	0.0002
Residual value = 0.994											Bold diagonal values are direct effects		

Legend: DFL = Days to flowering, DM = Days to maturity, NRP = Number of raceme per plant, RL = Raceme length, NBR = Number of flower buds per raceme, NNR = Number of nodes per raceme, PL = Green pod length, GPYP = Green pod yield per plant, FTW = 100 Fresh seed weight, DTW = 100 Dry seed weight, S%F = Shelling percentage (Fresh), S%D = Shelling percentage (Dry), DSYP = Seed yield per plant.

Table 8. Phenotypic and molecular screening results for BCMV resistance in lablab bean genotypes.

Genotypes	Molecular marker		Phenotypic marker	
	ROC11	SG6	Observed BCMV symptoms	Disease reaction
DS11	Nd	nd	Present	S
DS30	Nd	nd	Present	S
DS35	Nd	+	Absent	R
DS57	Nd	nd	Present	S
DS113	Nd	+	Absent	R
DS116	Nd	nd	Present	S
DS173	Nd	nd	Present	S
DSN12	Nd	+	Absent	R
DSN17	nd	+	Absent	R
DSN25	nd	+	Absent	R
DSN26	nd	+	Absent	R
DSN27	nd	+	Absent	R
Ashina	nd	+	Absent	R
Knoldog	nd	+	Absent	R
Kartika	nd	Nd	Present	S

Legend: nd-not detected, +-band present, S-susceptible and R-resistant

Estimation of correlation coefficient

Correlation study indicates the traits which should be given importance to increase yield. Among the associations of the traits studied, three associations showed positive significant correlation, *viz.* number of flower buds per raceme with raceme length, number of nodes per raceme with raceme length and test fresh seed weight with test dry seed weight indicating the significance of these characters in selection for crop improvement program (Tables 4 and 5).

Path coefficient analysis

Estimation of the correlation coefficient value denotes only the nature and extent of association existing between pairs of characters. Among the traits studied, seven characters *viz*. days to flowering, number of racemes per plant, raceme length, green pod yield per plant, test fresh seed weight, shelling percentage in fresh pod and shelling percentage in dry pod, had positive direct effect on seed yield and the remaining five traits such as days to maturity, number of flower buds per raceme, number of nodes per raceme, pod length and test dry seed weight had negative, direct effect on seed yield at genotypic level (Table 6). Alternatively, five characters were number of flower buds per raceme, number of nodes per raceme, pod length, green pod yield per plant and test dry seed weight had positive direct effect on seed yield and the remaining seven characters of days to flowering, days to maturity, number of raceme per plant, raceme length, test fresh seed weight, shelling percentage in fresh pod and shelling percentage in dry pod had negative, direct effect on seed yield at phenotypic level (Table 7).

Molecular detection of *bc-3* gene conferring resistance to *Bean common mosaic virus*

Among the fifteen lablab bean genotypes, no expected band (420 bp) was found from ROC11 marker, indicating the absence of *bc-3* gene, but some unexpected bands (it might be in around 150 bp) were observed among the genotypes (Figure 1A). As well SG6 marker produced nine expected bands at 595 bp position; these genotypes were DS35, DS113, DSN12, DSN17, DSN25, DSN26, DSN27, Ashina and Knoldog; suggesting these genotypes might contain recessive *bc-3* allele in respective locus (Figure 1B). Based on the amplification from markers, nine genotypes might be partially resistant to BCMV because without dominant *I* gene recessive *bc-3* allele will not confer perfect resistance to BCMV and the presence of dominant *I* gene is not confirmed in the genotypes studied. Therefore, these nine genotypes could be used for further investigation for detection of dominant *I* gene in respective locus.

The lablab bean genotypes were also evaluated for BCMV disease resistance through visual observation and novel resistant genotypes were identified. Based on the observed symptoms on the leaves of lablab bean, six genotypes (DS11, DS30, DS27, DS116, DS173 and Kartika) were produced typical mosaic symptoms, however there was no visible symptoms were present on the rest of the nine genotypes (DS35, DS113, DSN12, DSN17, DSN25, DSN26, DSN27, Ashina and Knoldog) based on the disease reaction scoring criteria (Table 8 and Figure 2). Hence, the visual observation and molecular screening results were found consistent for screening out resistant and susceptible genotypes (Figures 1 and 2). Based on the whole analysis of the genotypes studied such as yield traits, phenotypic and molecular marker analysis, the genotypes DS35 and DSN27 could be useful for further crop improvement breeding.

DISCUSSION

All the genotypes displayed considerable amounts of difference in their mean performance that indicated the bean genotypes were genetically diversified

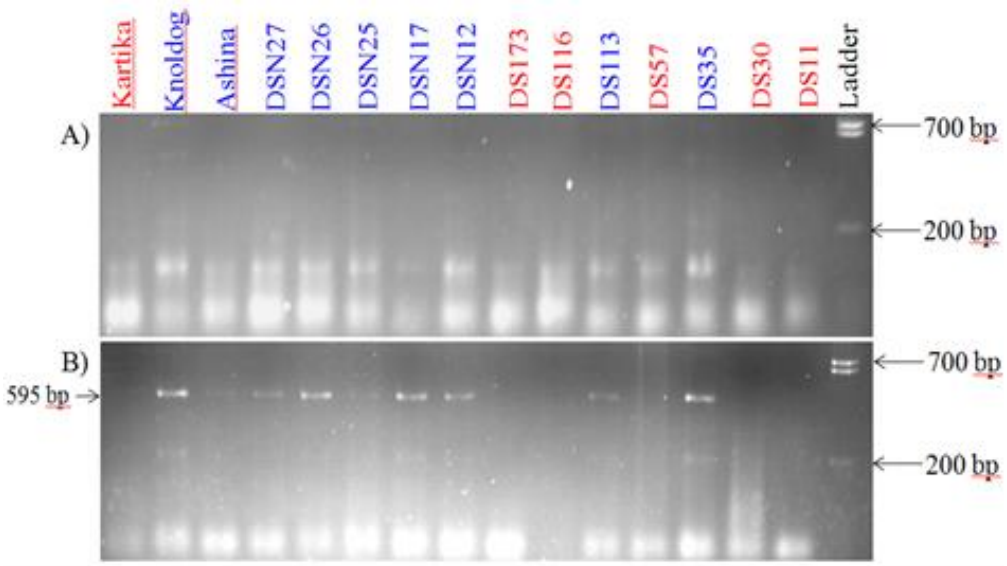

Figure 1. The agarose gel electrophoresis pattern of two SCAR molecular markers (A-ROC11 and B-SG6)for BCMV disease in lablab bean genotypes.

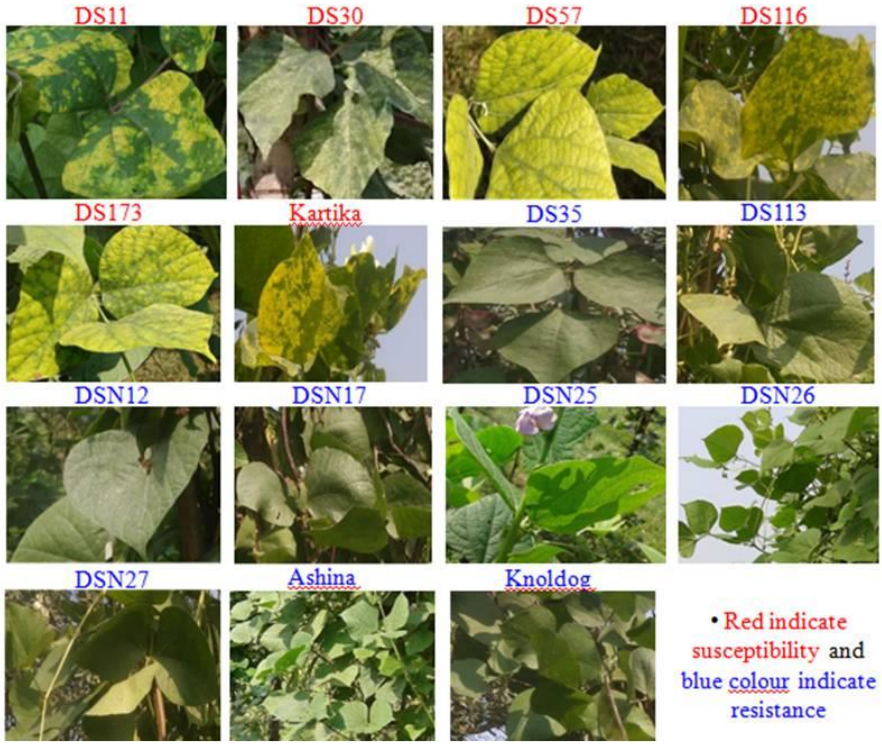

Figure 2. The disease reaction scoring criteria used in this study for BCMV of lablab bean. Scales: 0; no visible symptoms = Resistant (R) and 1; typical mosaic pattern symptoms= Susceptible (S). Red color indicates susceptibility and blue color indicates resistance genotypes.

(Table 2). Similar results were reported by others (Asaduzzaman *et al.,* 2014; Afsan and Roy, 2020). The possible reason might be genotypic inherent properties influenced the morphological traits. Similar results were found by others with different genotypes or varieties (Afsan and Roy, 2020). Coefficient of variation indicated that PCV was higher than the corresponding GCV for the maximum traits indicating they are interacted with the environment to some extent (Asaduzzaman *et al.,* 2015, Ullah *et al*., 2011; Afsan and Roy, 2020). High heritability and low genetic advance which may be attributed to non-additive gene interaction with some extent of influence of environment governing these traits, and these characters can be improved through hybridization and hybrid vigor. Similar result was found by Hossain and Motiur (2013). However, some traits showed high heritability along with high genetic advance which indicated that the predominance of additive gene interaction for the expression of these traits; which is fixable or improvable in subsequent generations. Similar findings were observed by other researchers for the traits (Parmar *et al.,* 2013; Singh *et al.,* 2011). Some associations were recorded as positive but non-significant (Table 5) at phenotypic level. Similar associations were reported by Asaduzzaman *et al.* (2015) and Islam *et al*. (2011). These positive and significant associations these traits suggesting the additive genetic model thereby less affected by the environmental variation. However, each contributing character has two parts of action *viz*., the direct and the indirect effects through yield contributing characters on economic characters which are not revealed from the correlation studies (Parmar *et al*., 2013). In this situation, the indirect causal factors could be considered for simultaneous selection. Similar observations were found by other authors (Parmar *et al*., 2013; Raffi and Nath, 2005).

It is possible that another gene near or very closely linked to *bc-3* gene (Johnson *et al*., 1997). Similar results were observed by some authors (Koenig and Gepts, 1989; Mukeshimana *et al*., 2005). The linked gene might have some important functions for the resistant mechanism against BCMV. Hegay (2013) suggested two Kyrgyz cultivars, Kytayanka-5 and Lopatka-1 as homozygous for recessive *bc-3* using SG6 marker.

CONCLUSION

The traits characterized by high GCV and PCV with high heritability; high GA with positive or negative direct effect on seed yield that should be focused on varietal development and would be more helpful for individual parental selection. Path coefficient analysis revealed the traits whether they had positive or negative direct effect on seed yield. Considering both green seed and pod yield, DS57 and DS116 performed well followed by DSN27 and DS35; also the genotype DSN12 performed well with exception of green and dry yield parameters. As well, molecular analysis revealed the nine genotypes showed resistance namely, DS35, DS113, DSN12, DSN17, DSN25, DSN26, DSN27, Ashina and Knoldog, respectively. Therefore, considering whole analyses, the genotypes like DS35 and DSN27 could be utilized in a breeding program for exploring QTL analysis and development of a variety with higher yield and resistance to BCMV.

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