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MOLECULAR CHARACTERIZATION OF HYACINTH BEAN GERMPLASM COLLECTED FROM BANGLADESH AND EXOTIC SOURCES

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Abstract

Hyacinth bean (*Lablab purpureus* (L.) Sweet) has versatile use as vegetable, pulse, fodder, hay, silage, green manure and cover crop. The experiment was conducted with 65 accessions of hyacinth bean from 17 countries of Asia, Africa and Europe for molecular characterization and phylogenetic relationship. Simple sequence repeat marker (SSR) derived from Medicago and cowpea was used. A total of 47 polymorphic alleles were counted. Two to five alleles per marker were detected with an average of 3.62. PIC values ranged from 0.21 to 0.72 with a mean of 0.53. Average gene diversity was exhibited 0.59 ranging from of 0.24 to 0.76. The maximum genetic distance (0.86) was observed between accessions of Bangladesh and India. Phylogenetic analysis classified the 65 accessions into 8 groups ranging from 1 to 22 accessions per group. Small values of genetic dissimilarities (0 to 0.29) showed without relating geographic distribution. Comparatively greater diversity was exhibited among the accessions in Bangladesh than the remaining 16 countries. The analysis of molecular variance indicated a fixation index of 0.0399 suggesting a low differentiation among the hyacinth bean accessions in Bangladesh and free gene flow among the populations. Genetically diverse accessions were identified that could be potentially source of germplasm for further crop improvement in the country.

Keywords: *Lablab purpureus*, SSR markers, genetic diversity, phylogenetic relationship, Bangladesh.

Introduction

Hyacinth bean is known as country bean or shim, mainly grown for its young pods and immature seeds for vegetable purpose, dry seed is used as pulse and often as soup and in many food preparations in Bangladesh, South and South-East Asia (Yawalkar and Ram, 2004). Lablab has two botanical types, the garden types and the field types. The garden type produces a twining stem and branches and is used as a green vegetable. Field type is erect and bushy, and is used for seed production, forage, green manure, as well as being a cover crop to prevent soil erosion and control weeds. Garden types are cultivated more than the field types all over in

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Bangladesh. Verdcourt (1970) recognized three sub-species: uncinatus, purpureus and bengalensis It was supposed that ssp. *purpureus* and ssp. *bengalensis* were genetically very similar and most of the domesticated hyacinth bean in Bangladesh belongs either to ssp. *purpureus* or ssp. *bengalensis*. BARI have released 10 and Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh released five varieties of hyacinth bean. Hyacinth bean grown on approximately 17,126 ha of land across the country during the winter season, yield an average of 5.50 t of fresh pods per ha for a total yield of about 94,356 t (BBS, 2014).

Hyacinth bean has advantages in terms of environmental tolerances and unique nutritional profiles when compared to the widely cultivated crops. Its unique alleles that could be applied in future agriculture and crop breeding (Varshney *et al.*, 2010). Diversity and relationship among 103 accessions of *Lablab purpureus* from 26 countries of diverse origin were measured through AFLP marker (Maass *et al.* 2005). AFLP and a number of EST-derived markers from a range of legumes were used to test 78 Lablab accessions from India, CPI (Australia) and ILRI (Ethiopia) for genetic diversity (Venkatesha *et al.* 2007). AFLP diversity analysis clearly showed limited genetic variation within the accessions from India compared to a more diverse set of 15 accessions from CPI and ILRI. The biggest challenge for molecular characterization and evaluation of hyacinth bean is the lack of SSR markers. There are many different legume genera and species at PGRC collection and it is expensive and time consuming to develop SSR markers for each species. Many accessions need to be processed with a large set of markers in order to access genetic diversity. It is necessary therefore that DNA marker be abundant in the genome, highly variable among accessions, easy to use and potentially high-throughput. Microsatellites or simple sequence repeats generally meet these requirements for assessing genetic diversity. SSRs markers are generally considered more effective than other techniques because they are co-dominant and highly reproducible (Mondini *et al.,* 2009).

Polymorphic bands in RAPD, AFLP and SAMPL, can also be converted into locus specific SCARs, which is then prove to be as effective as SSR markers. Transferred SSR markers of Medicago, soyabean, cowpea and peanut have been used for characterization and evaluation across the legume family including *Lablab purpureus* (Wang *et al.*, 2004; Wang *et al.*, 2006a, b). Research has previously focused on morphological variation in order to understand the origin and diversity of a crop, however in the past 20 years, studies of molecular markers have taken over as they are not affected by the environment in the same way of morphology which are typically highly variable (Robotham and Chapman, 2017; Rai *et al.*, 2011; Tertivanidis *et al.*, 2008). Bangladesh is rich in hyacinth bean diversity. Systematic research such as collection, conservation, characterization and evaluation and utilization of hyacinth bean has been done at Plant Genetic

Resources Centre (PGRC) of BARI (Islam *et al.*, 2002; Islam 2008; Islam and Haque, 2009). Few studies at molecular level have been already done in different countries but a few or none reports included germplasm from Bangladesh. Therefore, extensive research in this area is needed. PGRC of BARI conserved 751 accessions in the gene bank. The purpose of this study was to characterize and evaluate the accessions of hyacinth bean andto know the genetic diversity of the accessions and to establish a phylogenetic relationship of the accessions from Bangladesh with the other countries.

Materials and methods

Plant materials

The experiment was carried out at the Biotechnology Laboratory of the Bangladesh Institute of Nuclear Agriculture (BINA) Mymensingh, Bangladesh. Sixty five accessions of hyacinth bean of which 38 accessions from 37 districts, 4 varieties of BARI and 23 accessions from 16 countries of Asia, Africa and Europe were used in this study (Table 1). The accessions were selected on the basis of different qualitative and quantitative characters, different geographical locations (district or country) and diversity analysis. The Bangladeshi 38 accessions were collected from 20º35' to 26º75' N latitude and 88º03' to 92º75' E longitude of 37 districts. The varieties, BARI Hyacinth Bean-1, BARI Hyacinth Bean-2 and BARI Hyacinth Bean-4 were grown all over Bangladesh during winter (photo sensitive) but BARI Hyacinth Bean-3 was grown during summer and winter (photo insensitive) (Table 1). The 23 exotic accessions from 16 countries were collected from AVRDC, Taiwan (TOT prefixed before the number). There were eight accessions namely CPI 34777, CPI 35894, CPI 52508, CPI 76996, CPI 81626, CPI 100602, CPI 106548 and ILRI 14437 selected from Core Collection (Pengelly and Maass, 2001 and Maass *et al.,* 2005). The accessions were obtained from the Commonwealth Scientific and Industrial Research Organization (CSIRO), Tropical Agriculture, Brisbane, Australia (CPI prefixed before the number) and the International Livestock Research Institute (ILRI) Addis Ababa, Ethiopia (ILRI-prefixed before the number). The germplasm were accessioned at PGRC BARI by prefixing BD before the number (Table 1). Nine seeds per accession were planted into plastic pots having 15 cm diameter and 20 cm height containing a mixture of loamy soil and decomposed cowdung (5:1). Three seeds were planted in each plastic pot. Watering was done just after seed sowing for proper germination of seeds. Young, actively growing fresh leaves of hyacinth bean were collected from 25-days old seedlings for extracting genomic DNA. Leaves were washed in distilled water and ethanol and were kept in ice and then preserved in -80^{0}C freezer for subsequent use.

Table 1. List of hyacinth bean accessions collected from different countries for SSR analysis

BARI Acc. No.	Collector's number	Name of district	Origin	BARI Acc. number	Collector's number	Name of district	Origin
BD-6	NH-13	Comilla	Bangladesh	BD-8785	MHML-173	Feni	Bangladesh
BD-31	MOI-46	Pabna	Bangladesh	BD-8802	MHQ-98	Jamalpur	Bangladesh
BD-82	MOI-99	Rangpur	Bangladesh	BD-8808	MHQ-164	Sherpur	Bangladesh
BD-86	MOI-104	Gaibandha	Bangladesh	BD-8815	MRA-29	Cox's Bazar	Bangladesh
BD-101	MOI-121	Naogaon	Bangladesh	BD-8835	BARI Hya-1	Variety	Bangladesh
BD-111	SMH-137	Magura	Bangladesh	BD-8867	AEMM-371/1	Chittagong	Bangladesh
BD-114	AM-140	Hobiganj	Bangladesh	BARI Hya-3	-	Variety	Bangladesh
BD-122	AM-148	Hobiganj	Bangladesh	BARI Hya-4	-	Variety	Bangladesh
BD-128	MOI-154	Dinajpur	Bangladesh	**Bangladesh: Total Accession-42, District-37**			
BD-130	MOI-156	Panchagarh	Bangladesh	**BARI Acc. No.**	**AVRDC no.**	**Other no.**	**Origin**
BD-1785	ODMH-19	Netrakona	Bangladesh	BD-8529	36950172	Etho-1	Ethiopia
BD-1799	MMH-162	Barguna	Bangladesh	BD-9441	TOT 7255	CPI 34777*	India
BD-1805	MMH-140	Patuakhali	Bangladesh	BD-9442	TOT 7256A	CPI 81626*	India
BD-1809	MMH-71	Jhalakati	Bangladesh	BD-9444	TOT 7260	CPI 100602*	Kenya
BD-1816	MMH-211	Gopalganj	Bangladesh	BD-9446	TOT 7261B	CPI 106548*	India
BD-1818	MMH-38	Barisal	Bangladesh	BD-9447	TOT 7262A	CPI 35894*	Denmark
BD-1830	MHML-56	Luxmipur	Bangladesh	BD-9449	TOT 7263	CPI 52508*	Mozambique
BD-1839	MHML-111	Noakhali	Bangladesh	BD-9451	TOT 7264B	CPI 76996*	Zambia
BD-2884	AEMM-115	Khagrachhari	Bangladesh	BD-9452	TOT 7396A	CAM 177	Cambodia

BARI Acc. No.	Collector's number	Name of district	Origin	BARI Acc. number	Collector's number	Name of district	Origin
BD-7775	BARI Hya 2	Variety	Bangladesh	BD-9455	TOT 7777	CAM 417	Cambodia
BD-7978	M-226	Sirajganj	Bangladesh	BD-9457	TOT 7265	ILRI 14437*	Zimbabwe
BD-7985	MH-250	Mymensingh	Bangladesh	BD-9459	TOT 6752	PD 13	India
BD-7988	MT-143	Faridpur	Bangladesh	BD-9461	TOT 2029	LV 4018	Indonesia
BD-7993	MT-222	Rajbari	Bangladesh	BD-9462	TOT 4008A	HPL 98-13	Laos
BD-8001	OD-325	Rangamati	Bangladesh	BD-9463	TOT 4008B	HPL 98-13	Laos
BD-8005	OS-32	Jessore	Bangladesh	BD-9469	TOT 7118	LHL 03-0025	Malaysia
BD-8006	OS-164	Narail	Bangladesh	BD-9472	TOT 2713	PHL 2631	Philippines
BD-8018	T-137	Kushtia	Bangladesh	BD-9473	TOT 2716	PHL 2635	Philippines
BD-8027	T-240	Meherpur	Bangladesh	BD-9477	TOT 4881		Taiwan
BD-8039	T-495	Chuadanga	Bangladesh	BD-9478	TOT 0583		Thailand
BD-8737	MA-74	Natore	Bangladesh	BD-9481	TOT 2465		Thailand
BD-8752	MA-225	Nawbabgonj	Bangladesh	BD-9483	TOT7905		Uzbekistan
BD-8757	MA-254	Rajshahi	Bangladesh	BD-9484	TOT3932	TB 90	Viet Nam
BD-8770	MHA-67	Gazipur	Bangladesh	**Exotic: Total accession-23, Country-16**			

Where, '*'Selected the accessions from core collection (Pengelly and Maass 2001 and Maass *et al.* 2005).

DNA preparation

Approximately 0.1 g leaf samples were ground by pestle and mortar using 660 µl extraction buffer and 40 µl 20% SDS. DNA extraction was done by CTAB mini-preparation method (IRRI, 1997). DNA was dissolved and diluted in 0.1 X TE (1mM Tris, 0.1mM EDTA, P^H8.0) to make the concentration of 25ng/µl as PCR templates. DNA preparation was confirmed with 1% agarose gel electrophoresis at 120 V for 1 hour 20 minutes. DNA concentration was measured at A260 nm absorbance through Double bean Spectrophotometer (SQ-4802 USA).

PCR primer selection and amplification

Ten pairs of primers from cowpea (*Vigna unguiculata* (L) Walp genomic SSRs and 15 pairs of primers from Medicago (*Medicago truncatula*) expressed sequence tag (EST)-derived SSRs were selected for polymorphism survey from the hyacinth bean germplasm evaluation by Wang *et al.*, (2007 and 2004). Among them, 13 primers were selected which showed clear polymorphism (Table 3). The PCR was performed in a SIGMA PCR System with a 48-PCR tube format. The PCR tubes (0.2 ml) were set on the wells of the thermocycler plate. Two µl of genomic DNA were used in a 15 µl total volume with a final concentration of 10X PCR buffer, 1.0 µl dNTPs, 1.0 µl each primer, 0.5 µl Tag DNA polymerase and 8.25 µl ddH_2O. The PCR was programmed as initial denaturing at 94P^{oP}C for 4 minutes, denaturing at 94P^{oP}C for 1 minute, annealing at 50P^{oP}C for 30 seconds, polymerizing at 72P^{oP}C for 40 seconds. Step 2 to 4 for 10 cycles. Again denaturing at 94P^{oP}C for 1 minute, annealing at 45P^{oP}C for 30 seconds, polymerizing at 72P^{oP}C for 40 seconds. Step 6 to 8 for 35 cycles. Extension at 72P^{oP}C for 10 minutes. Completion of cycling programme, reactions were held at 4°C (Wang *et al.*, 2007). Each of the amplifications was repeated at least twice for ensuring the results.

PCR fragment separation and microsatellite data analysis

Four µl loading dye was added with the PCR products. Ten µl PCR products were run in 3.5% agarose gel. DNA marker (100 bp) was loaded on either side of the gel. The electrophoresis was run for 2.30 hours at 90 V. The gel was stained for 20 minutes in ethidium bromide. DNA bands were visualized under UV light using a Gel DOC. The bands representing particular alleles at the microsatellite loci were scored by AlphaEase FC 4.0 (Software). Power Marker 3.23 (Liu and Muse, 2005) package software has been used in microsatellite data analysis (Table 3). The population was divided through F-statistics (Wright, 1965). The genetic structure of the accessions was investigated by the analysis of molecular variances (AMOVA). The fixation index was used for analyzing the genetic structure (Kiambi *et al.*, 2005). Genetic distance measures used for frequency data were calculated following Nei (1973). The unweighted pair-group method with arithmetic mean (UPGMA) dendrogram was drawn, and Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4 (Tamura *et al*., 2007).

Results and discussion

Allelic variation

Thirteen SSR markers generated clear polymorphic amplicons (DNA fragments or bands in agarose gels) among the 65 accessions of hyacinth bean (Table 3, Fig.1). A total of 47 bands were scored from 13 SSR markers. The maximum 5 alleles were exhibited in primers VM71, AQ842128 and MtSSRNFBF46 while the minimum, 2 alleles from VM22, AW127626 and MtSSRNFBG31. The primers exhibited an average of 3.62 alleles among the 65 accessions of hyacinth bean (Table 3). On the other hand, 42 accessions from Bangladesh and the 23 accessions from other 16 countries showed an average of 3.23 and 3.00 alleles, respectively (Table 4). The informativeness of SSR markers was measured by PIC values. The highest PIC value 0.72 was found in AQ842128 followed by 0.70 in MtSSRNFBF46 while the lowest 0.21 was found in VM22 with an average of 0.53. More PIC value was found in accessions from Bangladesh (0.50) than other countries (0.41). The maximum heterozygocity was observed in MtSSRNFBF46 (0.86) followed by VM71 (0.72) and AW127626 (0.57) with an average of 0.23 among the 65 accessions. The lowest heterozygosity was exhibited in VM70 (0.03), while 6 markers (VM31, VM22, AQ842128, AW584539, BF647899 and AW688861) did not detect any heterozygosity. Major allele frequency ranged from 0.29 (AW584539) to 0.86 (VM22) with an average of 0.52. However, the lower major allele frequency was obtained from Bangladesh (0.55) than other countries (65). The highest size of allele 213 bp was found in AW584539 and their range was 200 to 218 bp. The second highest size of allele 183 bp was reported in VM31.The lowest size of allele 52 bp was exhibited in VM71 ranging from 52 to 85 bp among the 65 accessions. The banding pattern of AW584539 primer is shown in Fig. 1.

Gene diversity and genetic distance

The maximum gene diversity was exhibited in AQ842128 (0.76) and the minimum was observed in VM22 (0.24). The average gene diversity for all microsatellite loci in 65 accessions was 0.59 (Table 3). Gene diversity of 0.56 and 0.47 was exhibited in accessions from Bangladesh and exotic countries, respectively. It is noted that a marker detecting a lower number of alleles also showed the lower gene diversity, compared to markers detecting a higher number of alleles that showed the higher gene diversity. There were significant deviations from Hardy-Weinberg Equilibrium for all microsatellite loci from 65 accessions (Table 5). The values of pair-wise comparison of Nei’s (1973) genetic distance (D) between

Table 2. List of the selected primer which were used for molecular characterization

Name	Origin	Repeats	Forward primer	Reverse primer	Amplication size
VM14	Cowpea	(AG)24	5'-.AATTCGTGGCATAGTCACAAGAGA	5'-TAAAGGAGGGCATAGGGAGGTAT	144
VM22	Cowpea	(AG)12	5'- GCG GGT AGT GTA TAC AAT TTG	5'- GTACTGTTCCATGGAAGATCT	217
VM31	Cowpea	(CT)16	5'- CGCTCTTCGTTGATGGTTATG	5'-GTGTTCTAGAGGGTGTGATGGTA	200
VM70	Cowpea	(AG)20	5'- AAA ATC GGG GAA GGA AAC C	5'-GAAGGCAAAATACATGGAGTCAC	186
VM71	Cowpea	(AG)12	5'-TCGTGGCAGAGAATCAAAGACAC	5'-TGGGTGGAGGCAAAAACAAAAC	225
AQ842128	*Medicago*	(TA)23	5'-TCAATGCTGATGCCATTTTC	5'-TCGCGTATTATAGCACAACACC	209
AW584539	*Medicago*	(ACA)8	5'-TTGATGGGCAATACATGTCG	5'- GTTGAAGGAAGGTGGTGGTG	204
AW127626	*Medicago*	(GTTT)7	5'- CATTTTGAAGGAAGGAAGAAGG	5'-ATTTGGAAGCGGAATGTGAA	191
AW688861	*Medicago*	(CAACT)7	5'-TTGTTGTGTGGCTTCTTTGG	5'-AAACCAACCACCTGTGTTGAC	195
BF647899	*Medicago*	(AT)34	5'- CTGTCAACAAGGGGTTAGGTG	5'-TGCATCTACACCCAAAACAAA	266
MtSSRNFAW16	*Medicago*	(TTC)17	5'- ATCGTCCCCACTGTGTCTTC	5'- GTGGGGTTGGTGAGAGTGTT	142-175
MtSSRNFBF46	*Medicago*	(GTAT)6	5'- ACCCCTGCTGAAACAGCATA	5'- CTCTCCCCTAGCCTCAAAGC	174-196
MtSSRNFBG31	*Medicago*	(GGTTT)5	5'- GAGCAAAGGGGTTTGTCTCA	5'-GCAACTCCAGCTGCATCTTT	176-200

Table 3. Allelic variation of the microsatellite loci in 65 accessions of hyacinth bean from Bangladesh and Exotic countries

Locus	Repeats	No. of genotype	No. of allele	Amplicon size (bp)	Allele with highest size	Major allele frequency	Availability	Gene diversity	Hetero zygosity	PIC
VM14	(AG)24	5	3	61-110	61	0.55	0.98	0.59	0.22	0.53
VM22	(AG)12	2	2	54-58	58	0.86	1.00	0.24	0.00	0.21
VM31	(CT)16	4	4	177-190	183	0.39	0.88	0.72	0.00	0.67
VM70	(AG)20	4	4	58-90	64	0.60	1.00	0.56	0.03	0.50
VM71	(AG)12	5	5	52-85	52	0.35	1.00	0.73	0.72	0.68
AQ842128	(TA)23	5	5	90-119	98	0.36	0.91	0.76	0.00	0.72
AW584539	(ACA)8	4	4	200-218	206, 213	0.29	0.89	0.74	0.000	0.69
AW127626	(GTTT)7	3	2	56-76	76	0.67	1.00	0.44	0.57	0.34
AW688861	(CAACT)7	3	3	50-62	56	0.58	1.00	0.53	0.00	0.44
BF647899	(AT)34	4	4	100-117	107	0.40	0.97	0.68	0.00	0.62
MtSSRNFAW16	(TTC)17	4	4	51-75	57	0.60	1.00	0.58	0.09	0.53
MtSSRNFBF46	(GTAT)6	6	5	50-86	86	0.38	1.00	0.74	0.86	0.70
MtSSRNFBG31	(GGTTT)5	2	2	150-190	150	0.77	0.88	0.35	0.46	0.29
Average		**3.92**	**3.62**			**0.52**	**0.96**	**0.59**	**0.23**	**0.53**

Where, PIC-Polymorphism Information Content

Table 4. Allelic variation of the microsatellite loci in 42 accessions of hyacinth bean from Bangladesh and 23 accessions from exotic countries separately

Locus	No. of genotype		No. of allele		Amplicon size (bp)	Major allele frequency		Gene diversity		Hetero zygosity		PIC	
	BGD	EXO	BGD	EXO	BD/OBD	BGD	EXO	BGD	EXO	BGD	EXO	BGD	EXO
VM14	5	4	3	3	61-110	0.43	0.76	0.65	0.39	0.17	0.30	0.57	0.35
VM22	2	1	2	1	54-58	0.79	1.00	0.34	0.00	0.00	0.00	0.28	0.00
VM31	4	3	4	3	177-190	0.38	0.61	0.71	0.55	0.00	0.00	0.66	0.49
VM70	3	3	3	3	58-70	0.57	0.65	0.53	0.48	0.00	0.09	0.43	0.40
VM71	4	3	4	3	52-85	0.44	0.67	0.65	0.46	0.76	0.65	0.59	0.39
AQ842128	5	4	5	4	90-126	0.27	0.59	0.78	0.60	0.00	0.00	0.75	0.55
AW584539	4	4	4	4	200-218	0.38	0.39	0.73	0.67	0.00	0.00	0.68	0.61
AW127626	3	3	2	2	56-76	0.75	0.52	0.38	0.50	0.40	0.87	0.30	0.37
AW688861	3	2	3	2	50-62	0.79	0.78	0.36	0.34	0.00	0.00	0.33	0.28
BF647899	4	3	4	3	100-117	0.53	0.61	0.63	0.55	0.00	0.00	0.57	0.49
MtSSRNFAW16	3	4	3	4	51-65	0.55	0.70	0.60	0.48	0.00	0.26	0.53	0.44
MtSSRNFBF46	4	4	3	5	55-86	0.48	0.39	0.59	0.72	0.81	0.96	0.50	0.67
MtSSRNFBG31	2	2	2	2	150-190	0.78	0.75	0.34	0.38	0.44	0.50	0.28	0.30
Average	**3.54**	**3.08**	**3.23**	**3.00**		**0.55**	**0.65**	**0.56**	**0.47**	**0.20**	**0.28**	**0.50**	**0.41**

Where, ‘BGD’ and ‘EXO’, indicate accessions from Bangladesh and Exotic country, respectively.

accessions were computed from combined data for 13 primers and 65 accessions (Table 6). The higher genetic distance (0.86) was observed between accessions of Bangladesh (BD-8006, Narail) and India (BD-9459). However the lowest genetic distance (0.0) was obtained between accessions BD-7978 (Sirajganj) and BD-2884 (Khagrachhari), BD-8770 (Gazipur) and BD-8785 (Feni) from Bangladesh. Considering the 42 accessions from Bangladesh, the higher genetic distance (0.79) was exhibited between accessions BD-82 (Rangpur) and BD-114 (Hobiganj), and between accessions BD-101 (Naogoan) and BD-1799 (Barguna). However, the lowest (0.0) was obtained from the same accessions as earlier from Bangladesh. Considering the 23 accessions from Asia, Africa and Europe (exotic accessions), the higher genetic distance (0.62) was exhibited between accessions BD-9461 (Indonesia) and BD-9462 (Laos), and the lower genetic distance (0.02) was found between accessions BD-9441 (CPI 34777, India) and BD-9457 (ILRI 14437, Zimbabwe) (Table 7).

Germplasm characterization is an important component of breeding programme for an effective and efficient utilization of plant genetic resources. Microsatellite markers have shown high levels of polymorphism in many crops including rice, wheat, barley, maize, sorghum, soyabean, beans, Brassica species, alfalfa, sunflower and tomato. The lowest heterozygosity was exhibited in VM70 (0.03), while 6 markers did not detect any heterozygosity. Lack of heterozygocity could be attributed by the inbreeding nature of hyacinth bean. The proportion of heterozygocity is likely to be low due to self-pollination. Similar findings were observed in cowpea by Padulosi (1993). The number of allele amplified per primer pair was from 3 to 25 for rice (Yang *et al.*, 1994), 11 to 26 for soybean (Rongwen *et al.*,1995), 3 to 16 for wheat (Plaschke *et al.*, 1995), 2 to 23 for maize (Senior *et al.*, 1998) and 2 to 7 for cowpea (Li *et al.*, 2001). In this study, two to five alleles with an average of 3.62 per primer pair were amplified from the 65 accessions of hyacinth bean. This result is agreed with the results of Wang *et al.*, (2007). Wang *et al.* (2007) studied on 47 accessions of hyacinth bean and found a total of 90 bands with a mean of 3.6 alleles per SSR marker and the markers were derived from *Medicago*, cowpea and soybean. Thus the level of microsatellite polymorphism in hyacinth bean, although relatively high, is much lower than other crops. The possible reason for the low level of microsatellite polymorphism is that the cultivated hyacinth bean is relatively narrow genetic base compared with other crops.

Genetic diversity of cultivated hyacinth bean and its wild species have been extensively investigated by means of RAPD (Liu, 1996; Sultana *et al.* 2000; Rai *et al.* 2010), AFLP (Maass *et al.*, 2005) and Transferred SSR (Wang *et al.*, 2007, 2004, 2006a, b). The cultivated hyacinth bean had lower genetic diversity than many other crops including legume crops (Pasquet, 1993; Pasquet, 1999). The low genetic diversity in cultivated hyacinth bean may be a result of narrow genetic base. The low level of genetic diversity at DNA level among hyacinth bean accessions could be increased by using its wild relatives to broaden the genetic base. A high level of genetic variation was observed between the accessions of cultivated and wild form (Liu, 1996); However the lower or moderate level of variations were exhibited from the accessions of cultivated form Sultana *et al.*, 2000; Maass *et al.*, 2005; Wang *et al.*, 2007).

Table 5. Hardy-Weinberg Equilibrium of different accessions of hyacinth bean

Name of marker	Degrees of freedom	χ^2 value	χ^2 P-value
VM14	3	57.70	0.000
VM22	1	65.00	0.000
VM31	6	171.00	0.000
VM70	6	130.80	0.000
VM71	10	114.88	0.000
AQ842128	10	236.00	0.000
AW584539	6	174.00	0.000
AW127626	1	5.31	0.021
AW688861	3	130.00	0.000
BF647899	6	189.00	0.000
MtSSRNFAW16	6	130.38	0.000
MtSSRNFBF46	10	119.69	0.000
MtSSRNFBG31	1	4.98	0.026
Total	**69**		

Table 6. Analysis of molecular variance of hyacinth bean accessions

Sources of variation	Sum of squares	% of variation	F statistics (Fst)
Among populations	100.90	11	0.0399
Within population (BGD)	483.20	51	
Within population (EXO)	182.75	19	
Within accessions (BGD)	108.00	11	
Within accessions (EXO)	80.00	8	
Total	954.85	100	

Where, 'BGD' and 'EXO' indicate accessions from Bangladesh and Exotic countries

Population structure and phylogenetic tree analysis

The analysis of molecular variances (AMOVA) for the total accessions among the populations (Bangladesh and exotic countries) revealed 11% genetic variation while the variation within population from Bangladesh and exotic countries were 51% and 19%, respectively (Table 6). Variation within accessions from

Bangladesh and exotic countries exhibited 11% and 8%, respectively (Table 6). The fixation index (Fst) was low 0.0399, indicating low differentiation among hyacinth bean accessions in Bangladesh. Sixty-five accessions from 17 countries of Asia, Africa and Europe were used to make dendrogram based on Nei's (1973) genetic distance using UPGMA (Fig.2). There were 42 accessions from 37 districts of Bangladesh and 23 accessions from 16 countries. The dissimilarity value ranged from 0.00 to 0.29. The 65 accessions were classified into eight groups by selecting a cut off point at 0.23 on dissimilarity within the dendrogram. Number of accession in each group was ranged from 1 (Group-II) to 22 (cluster-VI). Group-I formed with 3 accessions from Bangladesh. Only one accession, BD-1839 from Noakhali in Bangladesh formed Group II.

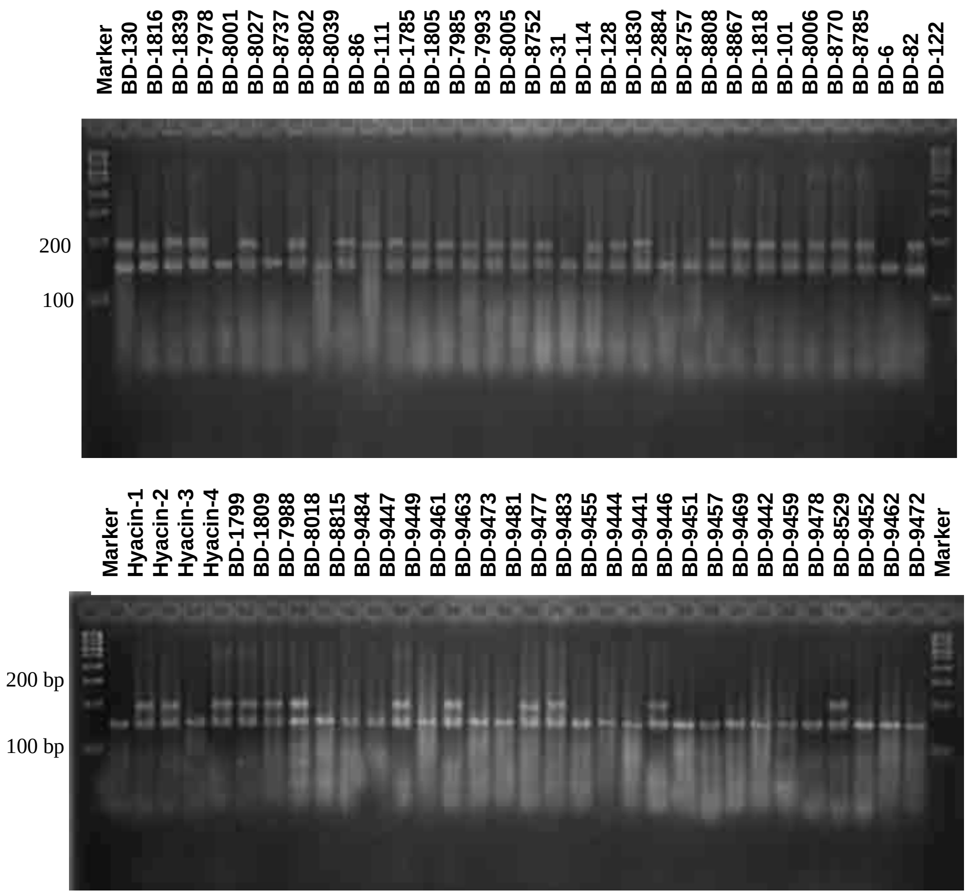

Fig. 1. Amplicons generated by PCR and separated by agarose gel electrophoresis. Each lane contained either 5 µl molecular marker (100 bp ladder) or 15µl PCR products in reaction solution from one of the 65 accessions. Fragments were separated by electrophoresis in a 3.5% agarose gel. PCR products were generated with the primer pair AW 584539.

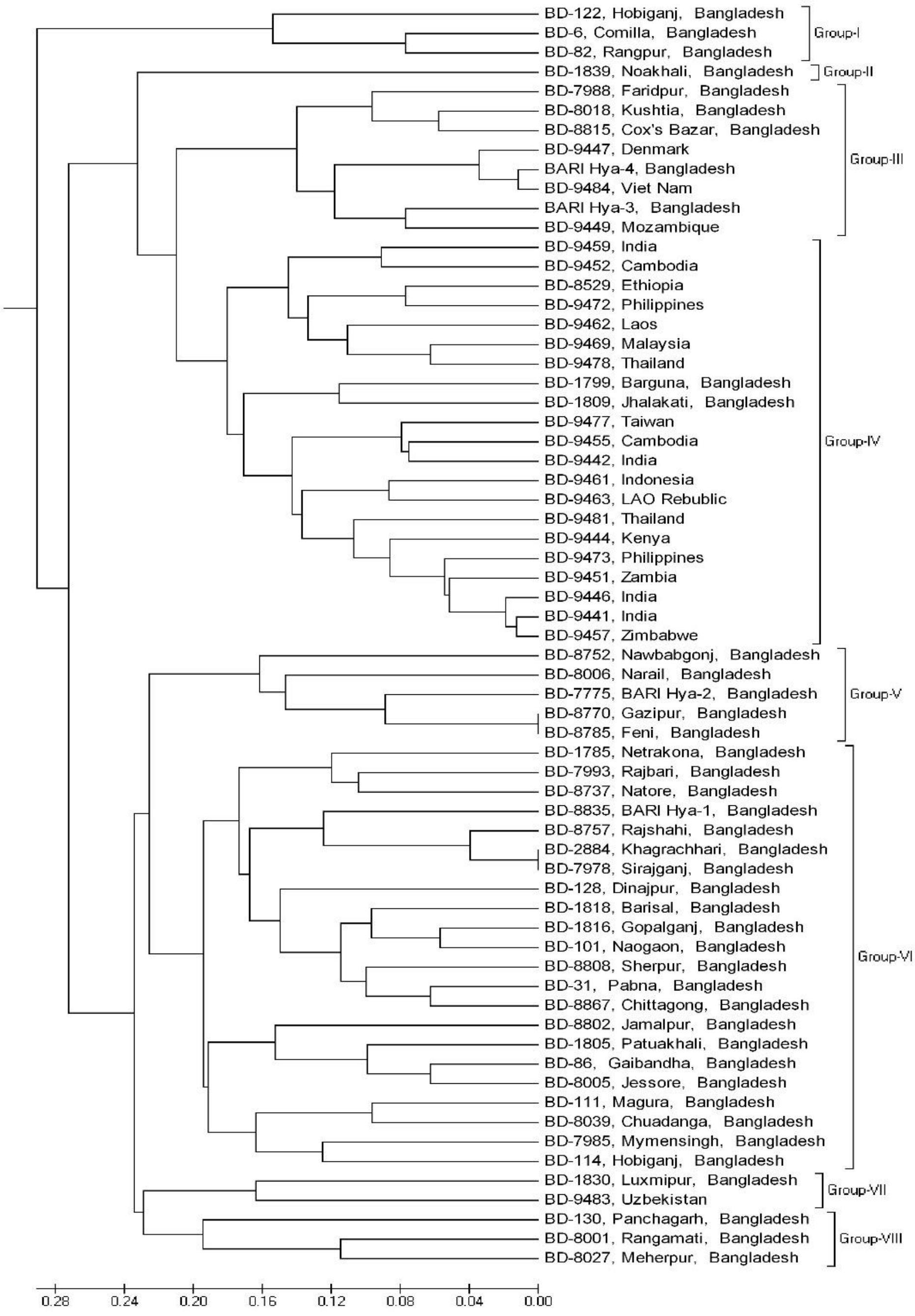

Fig. 2. Dendrogram of genetic relationship (UPGMA) among 65 *Lablab purpureus* accessions from Bangladesh and 16 countries of Asia, Africa and Europe.

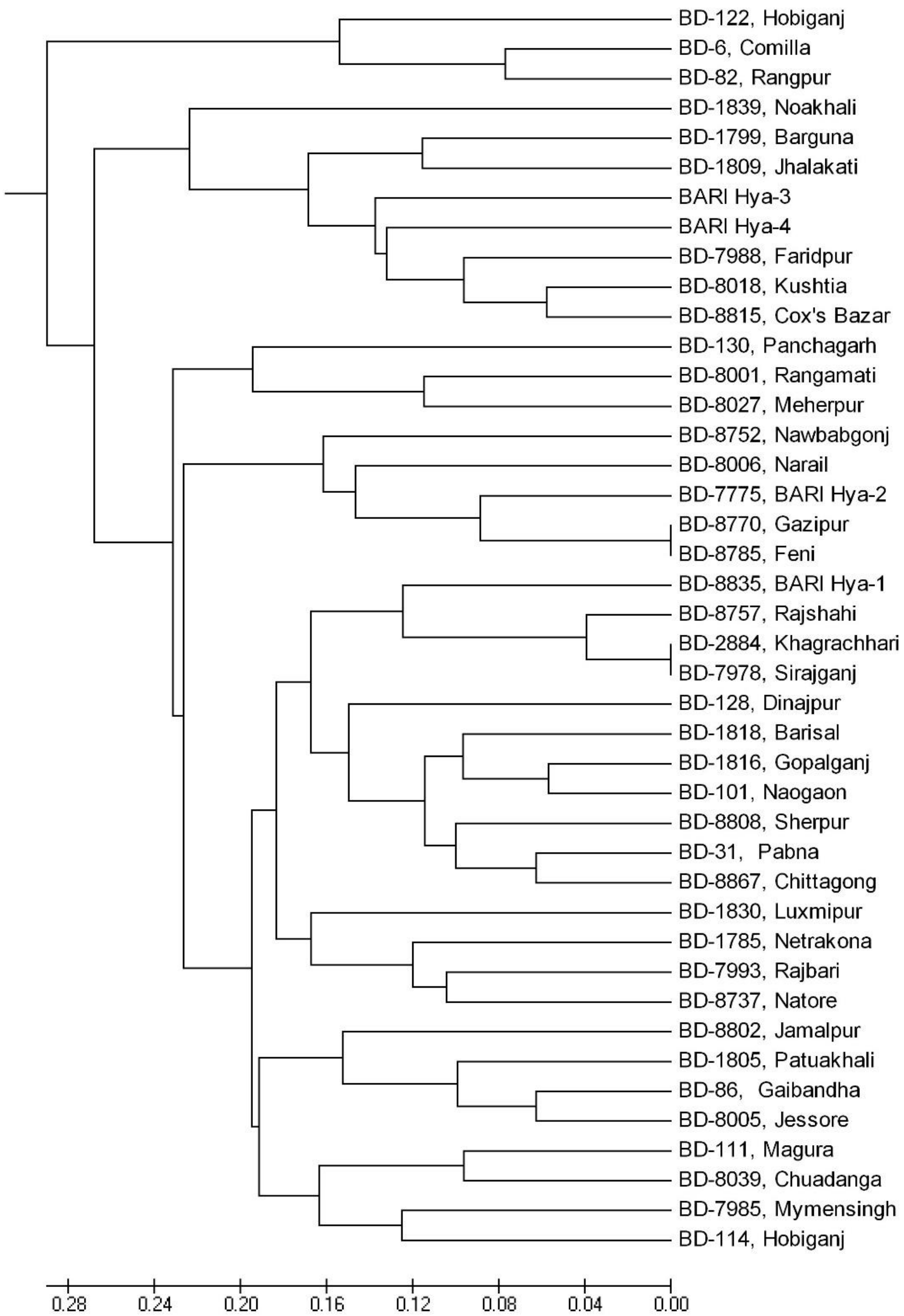

Fig. 3. Dendrogram of genetic relationship (UPGMA) among 42 *Lablab purpureus* accessions from Bangladesh.

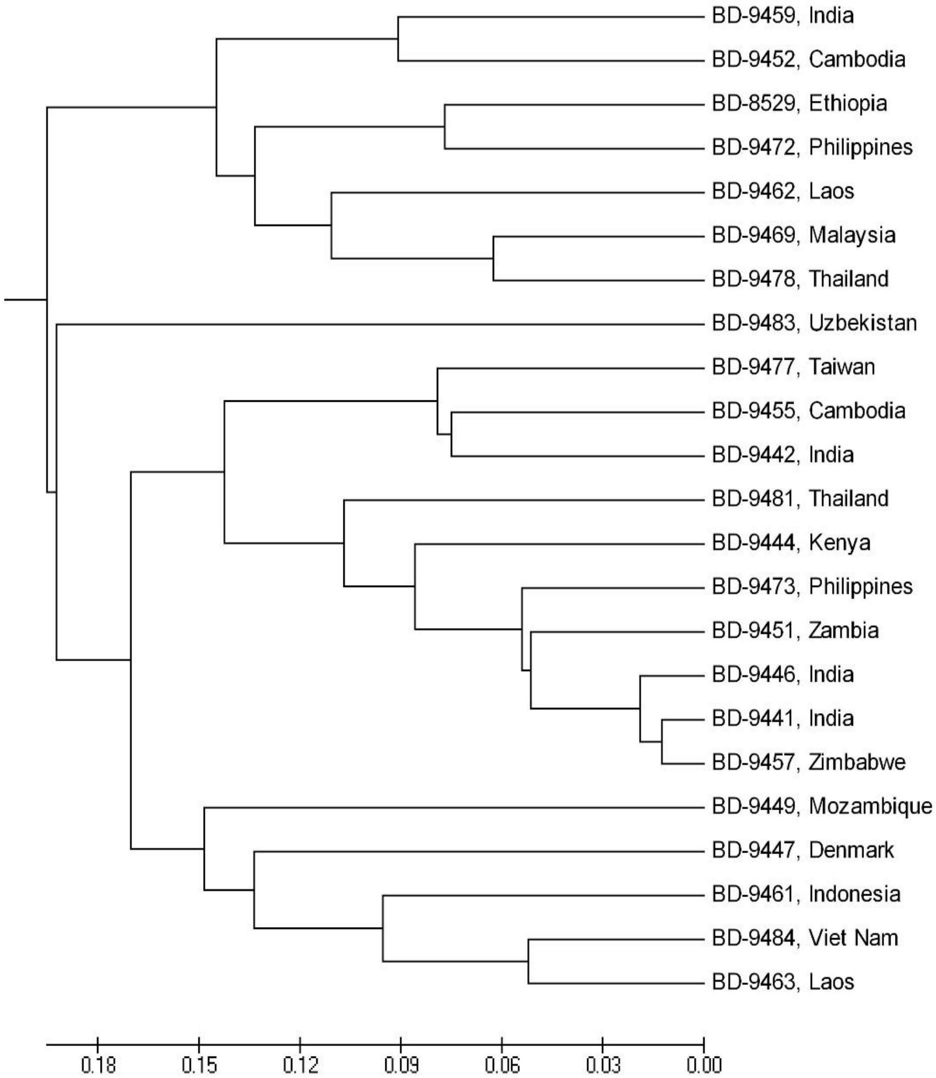

Fig. 4. Dendrogram of genetic relationship (UPGMA) among 23 *Lablab purpureus* accessions from 16 countries of Asia, Africa and Europe (except Bangladesh).

The accession from Denmark (CPI 35894A/BD-9447), Mozambique (CPI 52508/BD-9449), two released variety of BARI (BARI Hyacinth bean-3 and BARI Hyacinth bean-4) and other three accessions from Bangladesh formed Group-III. Group IV composed of 21 accessions from 13 countries of Asia and Africa. Among them, four accessions from India, two accessions each from Bangladesh, Laos, Philippines, Thailand and Cambodia, one accession each from Malaysia, Indonesia, Taiwan, Ethiopia, Zambia, Kenya and Zimbabwe. This is an important group having six accessions, CPI 34777 (India), CPI 106548 (India), CPI 81626 (India), CPI 100602 (Kenya), CPI 76996 (Zambia) and ILRI 14437 (Zimbabwe) from core collection of Pengelly and Maass (2001). Group-V formed with five accessions from Bangladesh. Group VI is the largest group and it contains 22 accessions from 20 districts in Bangladesh indicate the close relationship. BARI hyacinth bean-1 and BARI hyacinth bean-2 were present in groups VI and V, respectively. However, these two varieties were also accommodated into two separate groups in morphological study (Islam, 2012). Similarly, BD-1830 (Bangladesh) and BD-9483 (TOT 7905, Uzbekistan) were aggregated into Group-VII (Fig.2) and they were distributed in separate clusters in morphological study. Three accessions from Bangladesh formed Group-VIII. The 63 accessions were distributed among the 10 clusters with different combinations in D^2 analysis (Islam, 2012). Forty two accessions from Bangladesh and 23 accessions from 16 countries of Asia, Africa and Europe (except Bangladesh) were again used to make dendrogram based on Nei's (1973) genetic distance using UPGMA. The dissimilarity value ranged from 0.00 to 0.285 (Fig.3) and 0.0 to 0.185 (Fig. 4) from the accessions of Bangladesh and exotic countries, respectively. The results showed distinct differentiation in variation among many accessions while less distinct differentiation in others. The accessions from Bangladesh have distinctness and more diverse than the accessions of exotic countries.

Wright (1978) and Kiambi *et al.* (2005) suggested that the fixation index (Fst) in a range of 0- 0.05 indicated little differentiation, 0.05-0.15 moderate differentiation, 0.15–0.25 large differentiation and above 0.25 indicates a very large differentiation. The lack of differentiation among regions and hyacinth bean accessions is an indication of both high level of gene flow between regions as well as lack of sufficient time for significant genetic differentiation among geographic regions. Gene flow can be considered as a function of seed exchange in different ways is influenced by natural and human selection pressures. The low differentiation is in agreement with a random amplified polymorphic DNA (RAPD) study on cowpea germplasm (Tosti and Negri, 2002).

Groupings of the 65 accessions based on microsatellite polymorphisms generally agreed with the morphological studied (Table 7). Several discrepancies were present, such as the CPI 35894 and CPI 52508 in group III, CPI 100602 and CPI 81626 in group IV while they fall in group IX and VIII, respectively in morphological study (Islam, 2012). Such incongruities were also observed in the other studies (Plaschke *et al.* 1995, Senior *et al.* 1998). Comparison of the grouping produced by the present study with that constructed by morphological study showed consistency only in the large group. This lack of consistency between different marker techniques was also observed in soyabean (Powell *et al.* 1996). This may be due to the fact that different markers systems detected different components of DNA variation, subject to different evolutionary mechanisms. In this study, there is not any wild accession to compare easily between morphological and molecular

Table 7. Genetic distance of hyacinth bean accessions based on 13 microsatellite alleles

Accession number	BD 114	BD 2884	BD 101	BD 8006	BD 8770	BD 8785	BD 82	BD 1799	BD 7978	BD-9484	BD-9447	BD-9449	BD-9461	BD-9481	BD-9477	BD-9483	BD-9455	BD-9455	BD-9441	BD-9450	BD-9457	BD-9469	BD-9459	BD 8529	BD-9462	BD-9472
BD 114	**0**																									
BD 2884	0.33	**0**																								
BD 101	0.56	0.38	**0**																							
BD 8006	0.48	0.54	0.38	**0**																						
BD 8770	0.45	0.53	0.43	0.23	**0**																					
BD 8785	0.58	0.56	0.4	0.33	**0**	**0**																				
BD 82	0.79	0.73	0.58	0.5	0.33	0.48	**0**																			
BD 1799	0.65	0.63	0.79	0.56	0.5	0.54	0.48	**0**																		
BD 7978	0.27	**0**	0.33	0.58	0.47	0.52	0.71	0.69	**0**																	
BD-9484	0.46	0.44	0.6	0.52	0.5	0.5	0.69	0.25	0.48	**0**																
BD-9447	0.35	0.46	0.54	0.54	0.47	0.6	0.58	0.44	0.46	0.11	**0**															
BD-9449	0.58	0.48	0.48	0.56	0.5	0.38	0.71	0.38	0.44	0.17	0.27	**0**														
BD-9461	0.48	0.38	0.62	0.62	0.58	0.6	0.75	0.42	0.42	0.21	0.4	0.42	**0**													
BD-9481	0.4	0.52	0.77	0.73	0.56	0.58	0.65	0.48	0.48	0.39	0.39	0.52	0.35	**0**												
BD-9477	0.6	0.5	0.73	0.73	0.73	0.71	0.6	0.42	0.54	0.29	0.48	0.5	0.19	0.31	**0**											
BD-9483	0.48	0.46	0.62	0.46	0.58	0.6	0.6	0.35	0.5	0.46	0.48	0.5	0.38	0.44	0.42	**0**										
BD-9455	0.56	0.58	0.63	0.71	0.56	0.6	0.4	0.5	0.55	0.36	0.34	0.5	0.33	0.27	0.17	0.46	**0**									
BD-9455	0.63	0.46	0.71	0.71	0.69	0.6	0.71	0.29	0.5	0.32	0.5	0.38	0.25	0.31	0.25	0.21	0.43	**0**								
BD-9441	0.53	0.68	0.78	0.73	0.57	0.6	0.63	0.23	0.68	0.19	0.38	0.38	0.23	0.15	0.23	0.33	0.3	0.13	**0**							
BD-9450	0.4	0.56	0.65	0.73	0.67	0.67	0.73	0.4	0.52	0.34	0.43	0.48	0.27	0.21	0.31	0.35	0.32	0.23	0.1	**0**						
BD-9457	0.45	0.6	0.75	0.7	0.57	0.63	0.6	0.25	0.6	0.22	0.35	0.4	0.25	0.18	0.25	0.3	0.3	0.15	0.02	0.13	**0**					
BD-9469	0.6	0.63	0.71	0.63	0.58	0.6	0.56	0.25	0.59	0.36	0.43	0.33	0.33	0.32	0.38	0.38	0.38	0.2	0.15	0.23	0.18	**0**				
BD-9459	0.69	0.77	0.77	0.86	0.67	0.67	0.65	0.4	0.75	0.52	0.61	0.56	0.38	0.44	0.35	0.54	0.34	0.44	0.2	0.4	0.23	0.3	**0**			
BD 8529	0.6	0.54	0.65	0.62	0.65	0.67	0.52	0.35	0.5	0.5	0.52	0.46	0.27	0.35	0.31	0.31	0.33	0.29	0.23	0.31	0.23	0.13	0.29	**0**		
BD-9462	0.67	0.77	0.69	0.62	0.53	0.48	0.56	0.35	0.75	0.54	0.6	0.42	0.62	0.54	0.58	0.56	0.52	0.52	0.4	0.56	0.43	0.25	0.35	0.38	**0**	
BD-9472	0.58	0.52	0.63	0.63	0.73	0.65	0.58	0.48	0.48	0.52	0.54	0.4	0.37	0.4	0.29	0.48	0.29	0.38	0.38	0.4	0.35	0.19	0.38	0.15	0.33	**0**

diversity. Another difficulty is that out of 150 accessions from morphological study, 63 accessions were present in molecular analysis. Two released varieties namely, BARI Hyacinth bean-1 and BARI Hyacinth bean-2; and other 8 accessions from core collection (Pengelly and Maass, 2001) have been compared with respect to morphological and molecular diversity which supports this finding. The transferred SSR classification was consistent with previous reports from SSR, RAPD and AFLP studies. Transferred SSRs markers have been used to assess genetic diversity and to examine phylogenetic relationships among plant germplasm (Wang *et al.* 2004; 2006a, b). Sultana *et al.* (2000) found the dissimilarity value ranged from 0.006 to 0.134 in cultivated accessions and in wild accessions it was from 0.070 to 0.250 through RAPD analysis on 102 accessions. Liu (1996) reported a high level of genetic variation was detected through RAPD analysis between cultivated and wild accessions of hyacinth bean and genetic variation among Asian collections was significantly higher than that among African collections from cultivated form.

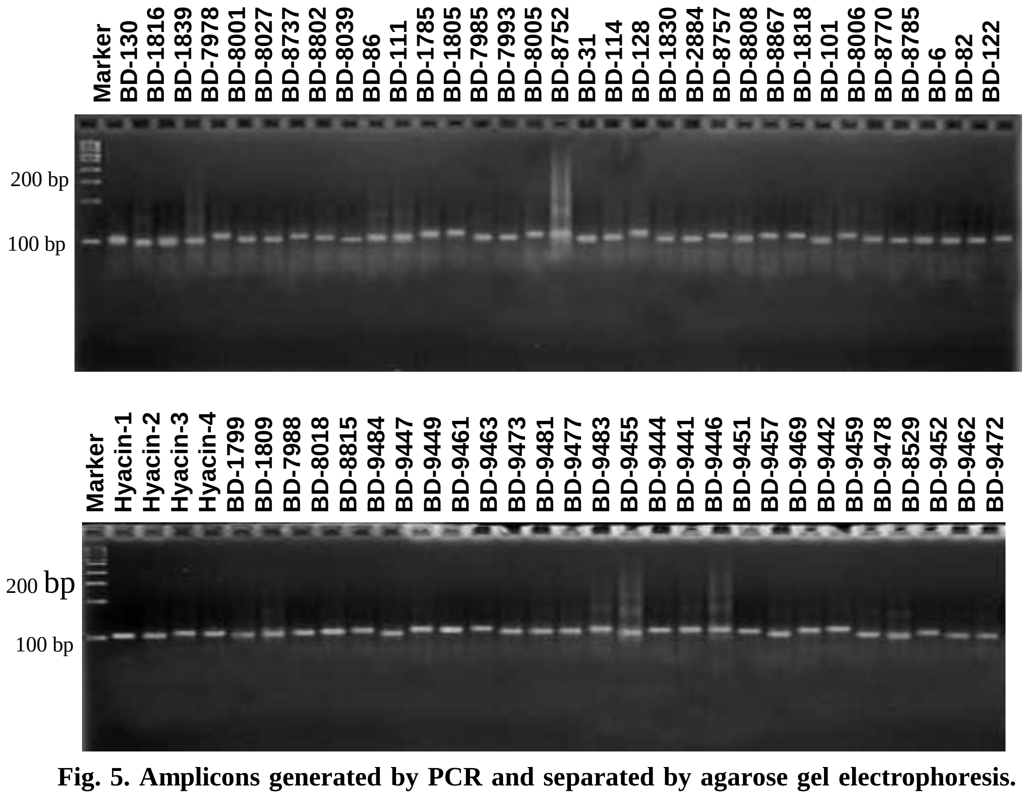

Fig. 5. Amplicons generated by PCR and separated by agarose gel electrophoresis. Each lane contained either 5 µl molecular marker (100 bp ladder) or 15µl PCR products in reaction solution from one of the 65 accessions. Fragments were separated by electrophoresis in a 3.5% agarose gel. PCR products were generated with the primer pair BF647899

Phylogenetic relationships observed in this study were partially agreed with morphological and molecular characters. This relationship observed in molecular markers was not in full agreement with that observed by morphological characters (Sultana. *et al.* 2000; Rai *et al.* 2010; Maass *et al.* 2005). In general, classification based on morphological characters has often showed confusion for taxonomists but the discriminatory power of molecular markers can provide information that will complete taxonomic studies (Carolin, 1992).

The success of cross-species amplification rate could be improved by using SSRs based on express sequence tags rather than using genomic SSR, since express sequence tags come from transcribed regions of the genome, and are likely to be conserved across a broader taxonomic range. In conclusion, microsatellite markers are polymorphic in hyacinth bean. They can be used to distinguish accessions of hyacinth bean. The degree of the polymorphism is relatively low in hyacinth bean compared with the other crops. No significant relation was detected between geographic region and genetic diversity. The polymorphism needs to be expanded. There are 751 accessions of hyacinth bean in the PGRC collection. Only 65 accessions were used in this experiment. Thus the collection should be expended to include more of the genetic diversity available including wild species. This study would aid selection and utilization of germplasm in crop improvement programmes in hyacinth bean.

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INFLUENCE OF THREE HYDROPONIC NUTRIENT SOLUTIONS ON THE YIELD AND QUALITY OF LEAFY VEGETABLES GROWN IN HYDROPONICS CULTURE

M. ASADUZZAMAN[1], M. RAZZAB ALI[2] AND F. ISLAM[3]

Abstract

A study was conducted to evaluate three hydroponic nutrient solutions to grow six types of leafy vegetables in a deep water culture hydroponics system at the glasshouse of Olericulture Division of Horticulture Research Centre (HRC), Bangladesh Agricultural Research Institute (BARI) during the period from 26 November 2020 to 31 January 2021.For this purpose plants of eleven varieties of six types of leafy vegetables (three varieties of lettuce viz. BARI Lettuce-1, Green Wave and New Red Fire; two varieties of spinach viz. BARI Palongshak-1 and BARI Palongshak-2; one variety of Pak Choi named BARI Batishak-1; three varieties of mizuna, namely Late White Stem, Mustard Green and Salad Kyomizuna; one variety (unknown) of Rocket Salad and one variety (unknown) of Chard) were grown in 300-litre culture boxes through deep water culture hydroponics using Modified Cooper's Solution-1 (1.5 dS/m) (MCS-1) (full strength), Modified Cooper's Solution-2 (1.5 dS/m) (MCS-2) (full strength) and Enshi-Shoho nutrient solution (ENS) (1/2 strength) on a completely randomized design (CRD). Results showed that growth of the selected leafy vegetables were varied significantly in three types of hydroponic nutrient solution. In general, it was evident that the overall growth performances of tested vegetables were found greater in MCS-1 than in MCS-2 and ENS. This result was followed by MCS-2 and lower growth of the tested vegetables was obtained from ENS Quality characteristics such as total soluble solids, shoot sap pH and tritratable acidity were found higher in MCS-1 followed by MCS-2. Results also revealed that lower amount of chlorophyll pigments and carotenoid content was recorded in plants grown in MCS-2 than other two nutrient solutions. Therefore, Modified Coopers solution-1 can be used for growing leafy vegetables following deep water culture hydroponics in the winter season of Bangladesh.

Introduction

Hydroponic is a profitable, sustainable, agricultural method and environmentally friendly technology for growing plants without soil. It is the fastest growing agricultural sector, rapidly gaining momentum and popularity, and could dominate food production in future. Hydroponic cultivation is revolutionizing agricultural crop production techniques all over the world owing to its minimal environmental footprint, enhanced pest control, and provide high crop yield (Al-Meselmani, 2022). Vegetables refer to mostly herbaceous annual plants of which some portion

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is eaten, either cooked or raw, during the principal part of the meal to complement starchy food and other food items (Anon., 2019). In other word, a plant raised for some edible part of it, such as the leaves, roots, fruit or flowers, but excluding any plant considered to be a fruit, grain, or spice in the culinary sense. They are classified into root vegetables (potatoes, sweet potatoes, cassava and yams), stem vegetables (stem amaranth, stolons of aroid etc.), leafy vegetables (arugula, pak choi, collards, kale, lettuce, spinach, swiss chard, mustard greens, watercress etc.), flower vegetables (broccoli, cauliflower etc.) and fruity vegetables (tomato, brinjal, bottle gourd, okra etc.) based on the parts of a plant used as vegetables.

Leafy green vegetables are packed with important and powerful nutrients that are critical for good health. Vegetable intake recommendation by FAO/WHO (2003) of ≥400 g/day (5 servings of 80 g) is for fruit and vegetables. As most countries follow the FAO/WHO guidelines and the majority suggest that at least three servings (240 g/day) should come from vegetables. According to Rashid (1996) daily vegetable demand of an adult should be 300 g (tuber and root crops, spices and other vegetables), of which at least 100 g to be leafy vegetables. As most of the leafy vegetables are consumed directly as fresh vegetable (salad), their leaves should be quality produce and free from any contamination (insects and other agrochemicals). Quality leafy vegetables can be produced through hydroponic culture. In Bangladesh, our daily dietary composition include potato based mixed curry with rice. It greatly lacks roots and fresh vegetables in the form of pickles and salad. As a result, our people are suffering from non-communicable disease due to intake of low-quality vegetables with heavy metals, environmental toxicants and pesticidal residues. Inclusion of safe and high-quality leafy vegetables in the dietary composition will provide health benefits and help prevent several food borne diseases.

Nutrient solution and its management are the cornerstone of a successful hydroponic system and are the most important determinant of crop production and quality, which is largely dependent on the extent to which plant nutrients are acquired from the nutrient solution. A hydroponic nutrient solution is basically the liquid that contains essential nutrients to influence this plant's root and stimulate growth. Yield and quality of vegetables greatly depends on the hydroponic nutrient composition, hydroponics production system, cultivation season, and handling procedures after harvest (Ding *et al.,* 2018; Kwack *et al.,* 2015; Kang and Kim, 2007). Thus, recommendation of specific vegetables for specific nutrient solution recipes is necessary. The study was, therefore, conducted to evaluate three hydroponics nutrient solutions for growing six types of leafy vegetables through deep water culture hydroponics.

Materials and Methods

The experiment was conducted in the glasshouse having Hydroponics and Controlled Environment Agriculture research facility at Olericulture Division, Horticulture Research Centre (HRC), Bangladesh Agricultural Research Institution (BARI), Gazipur-1701, Bangladesh during the period from 26 November 2020 to January 2021. The study area was situated in 23.9917° N longitude and 90.4137° E latitude at an altitude of 9 meter above the sea level. Temperature inside the glasshouse was between 19ºC and 26ºC and was greater by 1.5-2.0ºC compared to outside, while the inside average relative humidity was remained around 75%. The experimental treatments consisted of two factors where the first factor was comprised of three hydroponic nutrient solutions, namely Modified Cooper's solution-1 (full strength, 1.5 dS/m) (MCS-1), Modified Cooper's solution-2 (full strength, 1.5 dS/m) (MCS-2) and Enshi-Shoho nutrient solution (1/2 strength) (ENS), and the second factor was comprised of eleven varieties of six leafy vegetables (three varieties of lettuce viz. BARI Lettuce-1, Green Wave and New Red Fire; two varieties of spinach viz. BARI Palongshak-1 and BARI Palongshak-2; one variety of Pak Choi named BARI Batishak-1; three varieties of mizuna, namely Late White Stem, Mustard Green and Salad Kyomizuna; one variety (unknown) of Rocket Salad and one variety (unknown) of Chard. The experiment was conducted in a completely randomized design (CRD) with three replications. The seeds of lettuce var. BARI Lettuce-1, spinach var. BARI Palongshak-1 & 2 and Pak choi var. BARI Batishak- were collected from Olericulture Division, HRC, BARI and the seeds of the remaining varieties of mizuna, lettuce, rocket salad (variety unknown) and chard (variety unknown) were collected from Takii Seeds and Tohuku Seeds, Japan (Table 1).

The seeds of eleven varieties of six leafy vegetables were sown in plastic cell trays (36 cells) filled with cleaned coco-coir on 26 November 2020. Previously coco-coir's were washed thoroughly and treated with potassium permanganate ($KMnO_4$) @ 20 g per 100 L tap water for general disinfection from fungal infection. After 24 hours the treated coco-coir were washed three times with tap water and sun dried before use. Only tap water was used to moisten the coco-coir until seedling emergence. Seedlings were nourished with standard MCS-1 (full strength, 1.5 dS/m EC) (Table 2) until transplanting. Rectangular culture boxes (frames only) (90 cm × 396 cm) were prepared with 15 cm wooden liner placed in the floor of glasshouse. Then two layers of green color plastic polythene (0.10 mm thickness) sheets were placed over the wooden boxes to hold water with nutrient solution. Each culture box can hold 300 L water.

There were three types of hydroponic nutrient solutions used in this study viz., MCS-1 (Asaduzzaman *et al.*, 2021), MCS-2 (composition unpublished) and ENS (Hori, 1966) (Table 2). In each culture box, 300 L working nutrient solution was prepared taking each of three types of hydroponic nutrient solutions.

Table 1. List of varieties of six types of leafy vegetables with specialty characters, English name, botanical name and family grown in deep water culture hydroponics in glasshouse of HRC, BARI, Gazipur

SL #	Variety	Specialty character	English name	Botanical name	Family	Organization/ Company
1.	BARI Lettuce -1	Green leaf lettuce, loosely fit with larger leaf	Lettuce	*Lactca sativa* L.	Asteraceae	HRC, BARI, Bangladesh
2.	Green Wave	Green color lettuce				
3.	New Red Fire	Red color lettuce				
4.	BARI Palongshak-1	Green color leaf having good palatability	Spinach	*Spinacea oleracea* L.	Chenopodiaceae	HRC, BARI, Bangladesh
5.	BARI Palongshak-2	Broad leaved, having heat tolerance				
6.	Unknown	Multiple colour having antioxidants	Chard	*Beta vulgaris* subsp. *vulgaris*	Chenopodiaceae	Bright light^z
7.	BARI Batishak-1	Green fleshly leaf with bioactive compounds	Pak Choi	*Brassica rapa* subsp. *chinensis* L.	Brassicaceae	HRC, BARI, Bangladesh
8.	Late White Stem	Green, huge branching	Mizuna	*Brassica rapa* subsp. *nipposininca*	Brassicaceae	Takii Seeds, Japan
9.	Mustard Green	Purple, single stem habit, rich in anthocyanin				
10.	Salad KyoMizuna	Green, single stem habit				
11.	Unknown	Rich in antioxidants	Salad Rocket	*Eruca vesicaria* subsp. *sativa*	Brassicaceae	Bright light^z

^{z} Country not known.

Table 2. Mineral nutrient elements composition in hydroponic nutrient solutions used in the study

Nutrient element	Cooper's solution (full strength, mg/L) (Shah *et al*., 2011)	Enshi-shoho solution (full strength, mg/L) (Hori, 1966)
Nitrogen (N)	236	245.8
Phosphorous (P)	60	41.8
Potassium (K)	300	312.5
Calcium (Ca)	185	161.3
Magnesium (Mg)	50	49.3
Sulfur	68	65.4
Iron (Fe) (EDTA)	12	3.8
Sodium (Na)	-	1.6
Copper (Cu)	0.1	0.012
Zinc (Zn)	0.1	0.05
Manganese (Mn)	2.0	0.46
Boron (B)	0.3	0.52
Molybdenum (Mo)	0.2	0.008

In each culture box one urethane styrofoam sheet (85 cm × 390 cm) was placed having 66 holes (11 rows, each containing 6 holes) where 11 varieties of six vegetables were transplanted in each row (6 plants of each variety) maintaining 36 cm distance between rows and 15 cm between plants. Silver color plastic mulch films were used to cover the styrofoam sheet which prevents algae from growing in the nutrient solution. Healthy seedlings of each variety having the optimum characters (3-5 leaf stage depending on the test vegetables) were transplanted in the culture boxes with urethane foam blocks for holding the plants tight and upright. Nutrient solutions were aerated frequently by circulating the water around using an automatic timer (10/30 min.; operation/stop). Culture solutions were not changed throughout the growing period for all the vegetables grown. All the leafy vegetables were allowed to grow for 4-6 weeks to grow and harvested at the particular date when they reached marketable stage.

At harvest three sets of plants from each vegetable grown in each type of nutrient solution were sampled for dry weight measurement. Three shoot samples for each vegetable from each treatment were stored in freezer (– 30°C) for subsequent analyses of quality characteristics such as total soluble solids (%), pH, titratable acidity (%), reducing sugar (%), total sugar (%), and also photosynthetic pigments content such as Chlorophyll a (Chl a) (mg g^{-1} FW), Chlorophyll b (Chl b) (mg g^{-1} FW), Total Chlorophyll (Chl t) (mg g^{-1} FW), and Carotenoids content (Car) (mg g^{-1} FW). Data on growth parameters, namely plant height (cm), number of leaves/plant, maximum leaf length (cm), maximum leaf width (cm), longest root length (cm), shoot fresh weight/plant (g), shoot dry matter/plant (g) and root dry weight (g) per plant for all vegetables were also recorded at harvest.

Total soluble solids of leaf extract of 11 leafy vegetable varieties were determined by hand refractometer (RF10, San Diego, USA) and pH was determined by portable pH meter (HANNA, HI98107, Romania). Titratable acidity (%), reducing sugar (%) and total sugar (%) were determined according to method and formula given by AOAC (1994). To determine photosynthetic pigment content, three plant individuals of each variety of six leafy vegetables were randomly selected. Total chlorophyll and carotenoids were extracted by 80% acetone from the leaves of 11 vegetable varieties (fully expanded, exposed) at a similar position for each treatment group. The absorbance of the extracts was measured with a UV-1200 spectrophotometer (SP-75, Shanghai spectrum instruments co., LTD, China) at 663 nm (A663), 645 nm (A645), and 470 nm (A470), respectively Arnon (1949) and Lichtenthaler (1987). Chlorophyll a (Chl a), Chlorophyll b (Chl b), and total carotenoids were calculated using the following equations:

Chlorophyll a (mg/g) = [12.7 (A663) – 2.69 (A645)] × V/ (1000 × W)

Chlorophyll b (mg/g) = [22.9 (A645) – 4.68 (A663) × V]/ (1000 × W)

Total chlorophyll (mg/g) = [20.2 (A645) – 8.02 (A663)] × V/ (1000 × W)

Total carotenoids (mg/g) = (1000 × A470 - 1.82 × Chl a – 85.02 × Chl b)/198

Where, A= Absorbance at specific wavelengths; V= Final volume of chlorophyll extract in 80% acetone;

W= Weight of the fresh tissues extracted

All the data on growth and quality characteristics were analyzed using Statistrix 10.0 software. Means separation was done by Tukey's HSD test at 5% level of probability. The photosynthetic pigment characters were calculated from three samples of each treatment.

Results and discussion

Main effect of hydroponic nutrient solutions and leafy vegetable varieties on growth characters

Plant height, maximum leaf length, longest root length, shoot FW/plant, shoot DW/plant, and root DW/plant except number of leaves/plant and maximum leaf width were significantly influenced by three hydroponic nutrient solutions (Table 3). Plant height (38.8 cm), leaf length (35.2 cm), shoot FW/plant (88.3 g), and shoot and root DW/plant (4.20 g and 1.8 g, respectively) were found highest from MCS-1. No significant differences were found between MCS-2 and ENS in respect of these five growth characters except root DW/plant which was recorded higher from ENS (1.38 g). Maximum longest root length (36.8 cm) was obtained from ENS.

All the growth characters were significantly influenced by different leafy vegetables varieties irrespective of nutrient solution (Table 3). Mizuna var. Late White Stem recorded maximum plant height (48.2 cm), highest maximum leaf length (46.5 cm), maximum longest root length (43.9 cm) and maximum shoot DW (5.56 g/plant), whereas Mizuna var. Salad Kyomizuna produced maximum number of leaves/plant (108.0). No significant differences were found among three varieties of Mizuna in respect of plant height, maximum leaf length, and longest root length, shoot FW/plant and, shoot and root DW/plant. Among the eleven varieties of 6 leafy vegetables, lettuce var. BARI Lettuce -1 and New Red Fire produced highest maximum leaf width (18.7 cm) and shoot FW/plant (131.5 g), whereas lettuce var. Green Wave gave maximum root DW/plant (1.61 g). There were no significant differences found among three varieties of lettuce, namely BARI Lettuce-1, Green Wave and New Red Fire with regards to number of leaves/plant, maximum leaf length, maximum leaf width, longest root length, and shoot and root DW/plant. Two varieties of spinach viz., BARI Palongshak -1 and BARI Palongshak-2 showed identical results in respect of all growth characters. In three cultivars of lettuce. Pak Choi var. BARI Batishak-1 performed better in respect of shoot dry weight (3.78 g/plant) and root dry weight (1.22 g/plant), whereas Chard showed better performance with regard to root dry weight (1.17 g/plant). Moraes *et al*. (2020) found average leaf number (9.27/plant), fresh mass of areal part (31.53 g/plant) and fresh mass of root (6.0 g), leaf length (14.42 cm) and root length (23.14 cm) in Nutrient Film Technique (NFT) hydroponic system harvested at 40 days after planting. Lee *et al.* (2022) obtained plant height (25.70-36.80 cm), number of leaves/plant (24.73-40.58), fresh weight of shoot (76.75 - 284.28 g) from different amount of nutrient solutions and two varieties of lettuce at 34 days after sowing. Sundar and Chen (2020) got leaf number (7.0-10.9), shoot fresh weight (55.3-71.4 g), root fresh weight (4.87-8.24 g), shoot DW (3.40-5.99) and root dry weight (0.47-1.60 g) in aquaponic system through using Floating Raft, Closed Capillary Water Distribution and NFT hydroponics system in Pak Choi vegetables. Wiangsamut and Koolpluksee (2020) reported that they obtained plant height (26.8 cm), number of leaves/plant (16.7), fresh weight of plant (118.8 g) in NFT system at 49 days after sowing (37 days after planting) in Pak Choi. In two cultivars of lettuce, Sapkota *et al*. (2019) obtained average fresh weight/plant (49.53-104.25 g), number of leaves/plant (10.5-20.5) and root length (11.93-26.14 cm) when harvested at 30 days after the initiation of treatment consisting differential nutrient concentration of N, K and Ca.

Interaction effect of hydroponic nutrient solutions and leafy vegetable varieties on growth characters

Plants of Mizuna var. Late White Stem grown in MCS-1 gave maximum plant height (51.3 cm) which was statistically similar to those of Mizuna var. Mustard Green (46.8 cm), Mizuna var. Salad Kyomizuna (49.3 cm), spinach var. BARI Palongshak-1 (41.5 cm), lettuce var. Green Wave (41.0 cm) grown in same type of nutrient solution, all the three varieties of Mizuna grown in MCS-2 and ENS, and plants of spinach var. BARI Palongshak-2 grown only in ENS (Table 4). The leafy vegetable Rocket Salad under MCS-1 showed the poor result in respect of plant height. Plants of Mizuna var. Salad Kyomizuna grown in ENS recorded maximum number of leaves/plant (114.3) which was identical with those of Mizuna of the same variety grown in MCS-1 (109) and MCS-2 (100.7). The remaining treatment combinations produced the similar number of leaves/plants. Maximum leaf length was found highest from the plants of Mizuna var. Late White Stem grown in MCS-1 (50.3 cm) closely followed by those of the same vegetable crop var. Mustard Green (45.2 cm) and Salad Kyomizuna (48.0), and spinach var. BARI Palongshak-1 (39.0 cm) grown in the same nutrient solution, plants of Mizuna of all three varieties and spinach var. BARI Palongshak-2 grown in both MCS-2 and ENS. Plants of Rocket Salad grown in three nutrient solutions gave the poor result in respect of number of leaves/plants. Lettuce var. New Red Fire grown under MCS-1 gave the maximum leaf width (20.7 cm) which was statistically similar to all the remaining combinations except Mizuna var. Salad Kyomizuna, Rocket Salad and Chard each in combination with MCS-1 and MCS-2, and spinach var. BARI Palongshak-1, Mizuna var. Salad Kyomizuna and Rocket salad grown under ENS (Table 4). Plants of Rocket Salad grown in three nutrient solutions gave the inferior result in respect of maximum leaf width. Mizuna var. Late White Stem grown in the ENS produced maximum longest root length (61.3 cm) which was identical with Mizuna var. Mustard Green (58.0 cm) and Salad Kyomizuna (35.0 cm) each in combination with the same nutrient solution, and spinach var. BARI Palongshak-1 (41.0 cm) and Mizuna var. Late White Stem (41.7 cm) each grown under MCS-1 (Table 4). The Rocket Salad coupled with MCS-2 showed the poor result in respect of longest root length. Lettuce var. BARI Lettuce-1 grown under MCS-1 produced maximum shoot FW/plant (160.2 g), which was identical with Pak Choi var. BARI Batishak-1 (119.5 g), Mizuna var. Late White Stem (101.5 g) and Salad Kyomizuna (101.0 g), and lettuce var. Green Wave (107.3 g) and New Red Fire (114.3 g) each grown under MCS-1 and all the varieties of lettuce in combination with MCS-2). Mizuna var. Late White Stem in combination with MCS-1 produced the maximum shoot DW/plant (6.67 g) which was statistically similar to all the remaining combinations except Rocket Salad coupled with the same solution, spinach var. BARI Palongshak-1, Rocket Salad and Chard each coupled with MCS-2, and Pak Choi var. BARI Batishak-1, lettuce var. Green Wave and Rocket Salad each in combination with ENS (Table 4).

All the combinations of leafy vegetables and three nutrients solutions except lettuce var. BARI Lettuce -1, Mizuna var. Mustard Green, Rocket Salad each coupled with MCS-2 and Rocket Salad grown under ENS gave identical results in respect of root DW/plant. Lettuce var. BARI Lettuce -1, Mizuna var. Mustard Green, Rocket Salad each grown under MCS-2 and Rocket Salad grown in ENS recorded the poorest result in respect of root DW/plant (Table 4). In spinach, Agarwal *et al*. (2021) obtained maximum leaf length (44.64 cm), leaf width (8.19

cm), number of leaves/plant (16.57), fresh weight of leaves/plant (77.07 g), dry weight of leaves/plant (6.54 g), fresh weight of roots/plant (11.09 g) and dry weight of roots/plant (1.38 g), root length (15.40 cm) from hydroponics using nutrient solution (all in ppm: N 150, P 40, K 200, Fe 5, Ca 50, Mg 60, Zn 3, Mn 3, Cu (<1), B (<4), Mo (<1), Na (<1) and S 50). Oztekin *et al.* (2018) obtained maximum plant height (13.8 cm), shoot fresh weight (4.96 g), shoot dry weight (0.52 g), root fresh weight (0.60 g), root dry weight (0.046 g) from hydroponic solution (ppm: N 150, P 50, K 150, Fe 5, Ca 150, Mg 50, Zn 0.05, Mn 0.05, Cu 0.03, B 0.50, Mo 0.02) in spinach in Turkey. Shah and Shah (2009) reported in lettuce that number of leaves/plant (13.67), leaf length (17.53 cm), root length (227.3 cm) and leaf yields/plant (53.89 g) were recorded from full strength Cooper's Solution.

Main effect of hydroponic nutrient solutions and leafy vegetable varieties on quality characters

Total soluble solid (%), pH, titratable acidity (%), reducing sugar (%) and total sugar (%) were significantly influenced by different nutrient solutions (Table 5). MCS-1 gave maximum pH (6.65) and titratable acidity (1.36%) while MCS-2 recorded maximum total Soluble Solid (5.9%) which was statistically similar to that of MCS-1 (5.8%). Reducing sugar (18.0%) and total sugar (13.2%) were recorded maximum from ENS. Total soluble solid was influenced little by 11 leafy vegetable varieties (Table 5). Maximum total soluble solid was obtained from Mizuna var. Mustard Green (6.3%) which was closely followed by Mizuna var. Late White Stem (6.2%), spinach var. BARI Palongshak-2 (6.0) and BARI Palongshak-1 (5.7%), Pak Choi var. BARI Batishak-1, Mizuna var. Salad Kyomizuna (5.6%), Rocket Salad (5.6%) and lettuce var. BARI Lettuce-1 (5.2%) and lettuce var. New Red Fire gave the lowest total soluble solid (4.6%). Lettuce var. Green Wave gave maximum pH (6.73) followed by spinach var. BARI Palongshak-2 (6.63) and then followed by Pak Choi var. BARI Batishak-1 (6.59) and spinach var. BARI Palongshak-1 (6.57) and its lowest value was obtained from lettuce var. New Red Fire (6.07). Maximum titratable acidity was recorded from Mizuna var. Mustard Green (1.89%) followed by Pak Choi var. BARI Bartishak-1 (1.72%) and minimum titratable acidity was obtained from lettuce var. BARI Letuuce-1 (0.74). Lettuce var. New Red Fire gave maximum reducing sugar (17.5%) which was significantly higher than other vegetable varieties. The second maximum reducing sugar was recorded from lettuce var. Green Wave (14.1%) and its minimum value was obtained from spinach var. BARI Palongshak-2 (6.8%). Maximum total sugar was obtained from lettuce var. BARI Lettuce-1 (15.6%) which was followed by spinach var. BARI Palongshak-1 (15.4%) and then followed by Rocket Salad (12.8%). The lowest total sugar was recorded from Mizuna var. Mustard Green (11.3%). Research results indicated that crop species responded differently in different hydroponics nutrient formulations, as it was found that "Korea Wonshi" nutrient solution can enhance growth and quality of romaine lettuce while "Yamazaki" nutrient solution enhances the growth of lettuce, red color of beet and red radish (Kwack *et al.*, 2015).

Table 3. Main effect of hydroponic nutrient solution and leafy vegetables varieties on the growth parameters of leafy vegetables grown in deep water culture hydroponics

Experimental treatments	Plant height (cm)	Number of leaves/ plant	Maximum leaf length (cm)	Maximum leaf width (cm)	Longest root length (cm)	Shoot FW/plant (g)	Shoot DW/plant (g)	Root DW/plant (g)
Nutrient solution								
Modified Cooper's solution-1	38.8 a[z]	23.0 a	35.2 a	13.4 a	31.3 b	88.3 a	4.20 a	1.80 a
Modified Cooper's solution-2	36.1 b	21.7 a	32.4 b	12.5 a	27.9 b	68.2 b	3.32 b	0.74 c
Enshi-shoo solution	35.5 b	21.3 a	33.6 ab	12.1 a	36.8 a	61.2 b	3.24 b	1.38 b
Level of Significance	**	ns	*	ns	**	**	**	**
Vegetables with variety								
Lettuce var. BARI Lettuce-1	33.5 cd	14.9 bc	27.8 ef	18.7 a	24.6 c	131.5 a	4.94 ab	1.50 ab
Lettuce var. Green Wave Lettuce	35.9 b-d	11.4 bc	25.7 f	16.4 a-c	31.2 a-c	90.3 bc	3.00 b-d	1.61 a
Lettuce. Var. New Red Fire Lettuce	27.3 e	15.4 bc	25.7 f	18.7 ab	30.4 bc	96.1 b	3.17 bc	1.39 ab
Spinach var. BARI Palongshak-1	38.3 bc	9.9 bc	36.0 cd	12.5 c-f	31.0 a-c	54.7 de	3.39 bc	1.17 ab
Spinach var. BARI Palogshak-2	38.9 b	11.1 bc	38.7 bc	11.2 def	31.7 a-c	60.6 c-e	4.33 a-c	1.39 ab
Pak Choi var. BARI Batishak-1	32.8 d	12.0 bc	28.6 ef	11.0 def	29.0 bc	82.9 b-d	3.78 a-c	1.22 ab
Mizuna var. Late White Stem	48.2 a	23.6 b	46.5 a	13.6 b-e	43.9 a	82.2 b-d	5.56 a	1.33 ab
Mizuna var. Mustard Green	45.1 a	16.9 bc	43.2 ab	14.8 a-d	41.0 ab	61.8 b-e	3.78 a-c	1.33 ab
Mizuna var. Salad Kyomizuna	44.9 a	108.0 a	43.8 ab	7.9 fg	34.1 a-c	83.7 b-d	3.89 a-c	1.39 ab
Rocket Salad var. unknown	25.6 e	11.3 bc	23.7 f	5.7 g	25.6 c	14.2 f	1.11 d	0.89 b
Chard var. unknown	33.9 b-d	7.6 c	31.7 de	8.9 e-g	29.9 bc	40.6 ef	2.50 cd	1.17 ab
Level of Significance	**	**	**	**	**	**	**	**

[z]values within a column followed by different letters are significantly different according to the Tukey's HSD test at $P \leq 0.05$.

ns, *, **indicates not significant or significant at $p \leq 0.05$, $p \leq 0.01$, respectively.

Table 4. Interaction effect of hydroponic nutrient solution and leafy vegetables varieties on the growth parameters of leafy vegetables grown in deep water culture hydroponics

Nutrient solution × Leafy vegetables with variety		Plant height (cm)	Number of leaves /plant	Maximum leaf length (cm)	Maximum leaf width (cm)	Longest root length (cm)	Shoot FW /plant(g)	Shoot DW /plant (g)	Root DW /plant (g)
Modified Cooper's solution-1	Lettuce var. BARI Lettuce-1	35.2 e-l[z]	16.7 b	27.5 g-k	20.0 ab	26.0 cd	160.2 a	6.00 ab	2.17 a
	Lettuce var. Green Wave	41.0 a-g	12.0 b	27.3 g-k	18.5 a-c	31.2 b-d	107.3 a-e	3.50 a-d	2.00 ab
	Lettuce var. New Red Fire	29.5 i-m	17.7 b	27.7 f-k	20.7 a	34.0 b-d	114.3 a-d	3.17 a-d	1.67 a-c
	Spinach var. BARI Palongshak-1	41.5 a-g	10.7 b	39.0 a-g	11.3 a-g	41.0 a-d	70.5 c-h	5.00 a-d	1.50 a-c
	Spinach var. BARI Palongshak-2	38.0 c-j	10.3 b	36.5 b-i	10.8 a-g	24.3 cd	68.8 c-h	4.67 a-d	1.33 a-c
	Pak Choi var. BARI Batishak-1	33.7 f-l	11.3 b	28.5 e-k	12.3 a-g	31.7 b-d	119.5 a-c	4.67 a-d	1.67 a-c
	Mizuna var. Late White Stem	51.3 a	30.0 b	50.3 a	15.3 a-f	41.7 a-d	101.5 a-e	6.67 a	2.17 a
	Mizuna var. Mustard Green	46.8 a-d	17.7 b	45.2 a-c	15.3 a-f	31.3 b-d	66.0 c-g	4.17 a-d	1.83 a-c
	Mizuna var. Salad Kyomizuna	49.3 ab	109. 0 a	48.0 ab	9.2 c-g	34.3 b-d	101.0 a-e	4.33 a-d	2.00 ab
	Rocket Salad vr. unknown	26.8 k-m	10.3 b	25.6 i-k	5.7 fg	25.5 cd	15.5 gh	1.17 d	1.67 a-c
	Chard var. unknown	33.2 f-m	7.7 b	31.5 d-j	8.5 c-g	23.7 cd	46.5 d-g	2.83 a-d	1.83 a-c
Modified Cooper's solution-2	Lettuce var. BARI Lettuce-1	38.3 c-j	15.3 b	31.3 d-j	20.0 ab	19.7 cd	145.5 ab	5.00 a-d	0.50 c
	Lettuce var. Green Wave	37.7 c-k	12.7 b	24.7 i-k	15.5 a-f	23.0 cd	109.8 a-e	3.67 a-d	1.00 a-c
	Lettuce var. New Red Fire	27.7 j-m	15.3 b	25.7 i-k	17.0 a-e	26.0 cd	98.0 a-e	2.83 a-d	0.67 bc
	Spinach var. BARI Palongshak-1	34.2 e-l	10.7 b	31.3 d-j	17.0 a-e	29.0 cd	41.5 e-h	2.00 b-d	0.83 a-c
	Spinach var. BARI Palongshak-2	37.3 d-k	11.0 b	40.0 a-e	9.5 b-g	43.3 a-c	41.8 e-h	2.67 a-d	0.83 a-c
	Pak Choi var. BARI Batishak-1	31.3 g-m	13.3 b	25.5 i-k	10.2 a-g	27.7 cd	65.8 c-h	4.33 a-d	1.17 a-c

Nutrient solution	Leafy vegetables with variety	Plant height (cm)	Number of leaves /plant	Maximum leaf length (cm)	Maximum leaf width (cm)	Longest root length (cm)	Shoot FW /plant(g)	Shoot DW /plant (g)	Root DW /plant (g)
	Mizuna var. Late White Stem	48.2 a-c	25.7 b	44.2 a-c	13.0 a-g	28.8 cd	80.5 b-g	5.67 a-c	0.83 a-c
	Mizuna var. Mustard Green	45.0 a-e	17.7 b	42.7 a-d	14.3 a-g	33.7 b-d	67.0 c-h	4.50 a-d	0.50 c
	Mizuna var. Salad Kyomizuna	45.0 a-e	100.7 a	43.7 a-c	8.2 c-g	33.0 b-d	71.5 c-h	3.00 a-d	0.67 bc
	Rocket Salad vr. unknown	22.4 m	10.0 b	19.2 k	4.6 g	15.0 d	9.0 h	1.17 d	0.50 c
	Chard var. unknown	29.8 h-m	6.0 b	28.5 e-k	7.9 d-g	27.7 cd	20.2 f-h	1.67 cd	0.67 bc
Enshi-shoho solution	Lettuce var. BARI Lettuce-1	27.0 k-m	12.7 b	24.6 i-k	16.2 a-f	28.0 cd	88.8 b-f	3.83 a-d	1.83 a-c
	Lettuce var. Green Wave	29.2 i-m	9.7 b	25.0 i-k	15.2 a-g	39.3 a-d	53.8 c-g	1.83 cd	1.83 a-c
	Lettuce var. New Red Fire	24.6 lm	13.3 b	23.8 jk	18.3 a-d	31.2 b-d	76.0 b-h	3.50 a-d	1.83 a-c
	Spinach var. BARI Palongshak-1	39.3 b-i	8.3 b	37.7 b-h	9.2 c-g	23.0 cd	52.2 c-h	3.17 a-d	1.17 a-c
	Spinach var. BARI Palongshak-2	41.5 a-g	12.0 b	39.5 a-f	13.2 a-g	27.3 cd	71.0 c-h	5.67 a-c	2.00 ab
	Pak Choi var. BARI Batishak-1	33.3 f-l	11.3 b	31.7 d-j	10.5 a-g	27.7 cd	63.3 c-h	2.33 b-d	0.83 a-c
	Mizuna var. Late White Stem	45.0 a-e	15.0 b	45.0 a-c	12.3 a-g	61.3 a	64.5 c-h	4.33 a-d	1.00 a-c
	Mizuna var. Mustard Green	43.4 a-f	15.3 b	41.6 a-d	14.6 a-g	58.0 ab	52.3 c-h	2.67 a-d	1.67 a-c
	Mizuna var. Salad Kyomizuna	40.5 a-h	114.3 a	39.7 a-e	6.5 e-g	35.0 a-d	78.5 b-h	4.33 a-d	1.50 a-c
	Rocket Salad var. unknown	27.6 j-m	13.7 b	26.3 h-k	6.8 e-g	36.2 a-d	18.0 f-h	1.00 d	0.50 c
	Chard var. unknown	38.7 b-i	9.0 b	35.0 c-j	10.4 a-g	38.3 a-d	55.0 c-h	3.00 a-d	1.00 a-c
Level of Significance		**	**	*	*	**	*	*	**

[z]values within a column followed by different letters are significantly different according to the Tukeys HSD test at $P \leq 0.05$.

*, **indicates significant at $p \leq 0.05$, $p \leq 0.01$ respectively.

FW = Fresh weight, DW = Dry weight

During cultivation of micro-greens (1 true leaf stage), Bulgaria *et al*. (2017) obtained, 0.74 mg/g FW reducing sugar and 2.21 mg/g FW total sugar in Swiss Chard, and 1.42 mg/g FW reducing sugar and 4.22 mg/g FW total sugar content in Rocket. Miceli *et al.* (2019) reported that they obtained TSS (2.8 ^{o}Brix), titratable acidity (28.5 mg/100 g FW) in lettuce while TSS (5.5 ^{o}Brix) and titratable acidity (45.3 mg/100 g) in Rocket. Ramos-Sotelo *et al.* (2021) obtained pH (6.10), titratable acidity (0.12% on FW), TSS (2.54%), moisture (90.4%) and carbohydrate (5.10%) content at 50 days after sowing in lettuce. Rossi *et al*. (2020) reported that they obtained pH (5.90), TSS (2.20%) and titratable acidity (1.90 g/100 ml leaf extracts from green Romanela lettuce in NFT hydroponics system. Ahmed *et al*. (2000) obtained reducing sugar (4.63-6.22 mg/g FW) and total sugar (6.53 -8.64 mg/g FW) in Rocket. Quality parameters in lettuce can also be influenced by the growing season or environmental factors, and nutrient compositions (Sublett *et al*., 2018).

Table 5. Main effect of hydroponic nutrient solutions and leafy vegetables varieties on the quality parameters of leafy vegetables grown in deep water culture hydroponics

Experimental treatments	Total soluble solids (%)	pH	Titratable acidity (%)	Reducing sugar (%)	Total sugar (%)
Nutrient solution					
Modified Cooper's solution-1	5.8 a^z	6.65 a	1.36 a	5.8 c	13.0 b
Modified Cooper's solution-2	5.9 a	6.58 b	1.15 b	6.6 b	12.2 c
Enshi-Shoho solution	5.2 b	6.06 c	0.52 c	18.0 a	13.2 a
Level of Significance	**	**	**	**	**
Cultivars					
Lettuce var/ BARI Lettuce-1	5.2 ab	6.47 d	0.74 f	7.3 h	15.6 a
Lettuce var. Green Wave	5.5 ab	6.73 a	0.41 g	14.1 b	11.4h
Lettuce var. New Red Fire	4.6 b	6.07 g	0.38 g	17.5 a	12.3 f
Spinach var. BARI Palongshak-1	5.7 ab	6.57 c	0.79 f	9.4 f	15.4 b
Spinach var. BARI Palogshak-2	6.0 a	6.63 b	0.88 e	6.8 i	12.1 g
Pak Choi var. BARI Batishak-1	5.7 ab	6.59 c	1.72 b	8.6 g	12.5 e
Mizuna var. Late White Stem	6.2 a	6.19 f	1.24 c	9.6 e	12.0 g
Mizuna var. Mustard Green	6.3 a	6.47 d	1.89 a	10.4 d	11.3 i
Mizuna var. Salad Kyomizuna	5.6 ab	6.17 f	1.18 d	10.5 c	12.6 d
Rocket Salad	5.6 ab	6.40 e	0.84 e	7.3 h	12.8 c
Chard	-	-	-	-	-
Level of Significance	**	**	**	**	**

^{z}values within a column followed by different letters are significantly different according to the Tukeys HSD test at $P \leq 0.05$.

**indicates significant at $p \leq 0.01$.

Interaction effect of hydroponic nutrient solutions and leafy vegetable varieties on quality characters

The quality characters, pH, titratable acidity, reducing sugar and total sugar were significantly affected by the interaction of nutrient solutions and leafy vegetables varieties but total soluble solid remained non-significant (Table 6). Plants of spinach var. BARI Palongshak-1 grown under MCS-1 gave maximum pH (7.17), which was followed by the spinach var. BARI Palongshak-2 grown in same nutrient solution (7.00) and Rocket salad grown under MCS-2 (6.97). The higher pH in BARI palongshak-1, BARI Palongshak-2 and Rocket salad under MCS-1 and MCS-2 indicates bitterness in taste. The vegetable lettuce var. New red Fire gave lower pH value under MCS-1 (5.60) and MCS-2 (5.90), and spinach var. BARI Palongshak-1 (5.80), Mizuna var. Late White Stem (5.50) and Rocket Salad (5.50) recorded lower pH values under ENS. Overall lower pH values in the tested 11 varieties of vegetables were observed in ENS compared to MCS-1 and MCS-2. MCS-1 in combination with Mizuna var. Mustard Green gave maximum titratable acidity (2.83%) followed by MCS-1 coupled with Pak Choi var. BARI Batishak-1 (2.43%), then followed by two varieties of Mizuna viz., Late White Stem and Mustard Green (2.30%) except Salad Kyomizuna (1.80%) coupled with MCS-1, and spinach var. BARI Batishak-1 in combination with MCS-2 (2.20%) and Mizuna var. Salad Kyomizuna under MCS-1 (1.80%) also gave fair amount of titratable acidity. Overall lower titratable acidy content in the tested leafy vegetables varieties were observed under ENS. Mizuna var. Late White Stem grown under ENS produced maximum reducing sugar (25.8%) which was followed by Mizuna var. Mustard green grown under the same nutrient solution (24.8%), then followed by Mizuna var. Late white stem' grown in ENS (22.8%). Spinach var. BARI Palongshak-1 grown in ENS (21.8%) and lettuce var. Green Wave and New Red Fire grown under MCS-2 (20.8%) recorded fair amount of reducing sugar. Lettuce var. BARI Lettuce-1 grown under MCS-1 and spinach var. BARI Palongshak-1 grown under ENS gave maximum total sugar content (17.5%) which was identical with Rocket Salad grown in ENS (15.5%). Spinach var. BARI Palongshak-1 produced moderate amount of total sugar (14.7%) when grown in MCS-1. Lettuce var. BARI Lettuce-1 grown under MCS-2 and ENS also gave moderate amount of total sugar (14.7). Agarwal *et al*. (2021) also got maximum total sugar content (0.42 g/100 g) from hydroponics using nutrient solution (all in ppm: N 150, P 40, K 200, Fe 5, Ca 50, Mg 60, Zn 3, Mn 3, Cu (<1), B (<4), Mo (<1), Na (<1) and S 50). It is also reported that quality of some leafy vegetables like vitamin-C content showed negative correlations with NO_3^- content in Pak choi, romaine lettuce and chungchima lettuce (Kang and Kim, 2007).

Table 6. Interaction effect of hydroponic nutrient solution and leafy vegetables varieties on the quality parameters of leafy vegetables grown in deep water culture hydroponics

Nutrient solution × Leafy vegetables with variety		Total soluble solids (%)	pH	Titratable acidity (%)	Reducing sugar (%)	Total sugar (%)
Modified Cooper's solution-1	Lettuce var. BARI Lettuce-1	5.1 a^{z}	6.87 de	0.80 i	3.3 l	17.5 a
	Lettuce var. Green Wave	5.3 a	6.70 g	0.40 l	18.3 g	11.9 i
	Lettuce var. New Red Fire	4.6 a	5.60 q	0.30 m	14.8 j	13.0 f
	Spinach var. BARI Palongshak-1	6.4 a	7.17 a	1.20 f	2.8 n	14.7 c
	Spinach var. BARI Palogshak-2	6.7 a	7.00 b	1.03 g	2.8 n	12.2 h
	Pak Choi var. BARI Batishak-1	5.7 a	6.90 cd	2.43 b	3.6 k	13.3 e
	Mizuna var. Late White Stem	6.9 a	6.50 j	2.30 c	3.1 m	11.7 j
	Mizuna var. Mustard Green	6.1 a	6.50 j	2.30 c	2.8 n	13.3 e
	Mizuna var. Salad Kyomizuna	5.2 a	6.50 j	1.80 e	3.3 l	11.2 k
	Rocket Salad	6.2 a	6.73 fg	1.03 g	3.3 l	11.7 j
	Chard	-	-	-	-	-
Modified Cooper's solution-2	Lettuce var. BARI Lettuce-1	5.5 a	6.53 ij	0.90 h	2.9 n	14.7 c
	Lettuce var. Green Wave	5.6 a	6.80 ef	0.30 m	20.8 e	11.3 k
	Lettuce var. New Red Fire	4.7 a	5.90 o	0.30 m	20.8 e	10.8 l
	Spinach var. BARI Palongshak-1	5.1 a	6.73 fg	0.53 k	3.6 k	14.0 d
	Spinach var. BARI Palogshak-2	6.1 a	6.90 cd	1.20 f	2.8 n	10.8 l
	Pak Choi var. BARI Batishak-1	6.6 a	6.67 gh	2.20 d	3.3 l	12.2 h
	Mizuna var. Late White Stem	6.5 a	6.57 ij	0.80 i	2.9 n	11.2 k
	Mizuna var. Mustard Green	6.8 a	6.30 l	2.83 a	2.6 o	12.7 g
	Mizuna var. Salad Kyomizuna	6.0 a	6.40 k	1.20 f	3.3 l	13.3 e
	Rocket Salad	6.1 a	6.97 bc	1.20 f	2.8 n	11.2 k
	Chard	-	-	-	-	-

Enshi-shoo solution	Lettuce var. BARI Lettuce-1	5.0 a	6.00 n	0.53 k	15.8 i	14.7 c
	Lettuce var. Green Wave	5.6 a	6.70 g	0.53 k	3.1 m	11.2 k
	Lettuce var. New Red Fire	4.5 a	6.70 g	0.53 k	16.8 h	13.3 e
	Spinach var. BARI Palongshak-1	5.7 a	5.80 p	0.63 j	21.8 d	17.5 a
	Spinach var. BARI Palogshak-2	5.2 a	6.00 n	0.40 l	14.8 j	13.3 e
	Pak Choi var. BARI Batishak-1	4.7 a	6.20 m	0.53 k	18.8 f	12.2 h
	Mizuna var. Late White Stem	5.4 a	5.50 r	0.63 j	22.8 c	13.3 e
	Mizuna var. Mustard Green	6.1 a	6.60 hi	0.53 k	25.8 a	7.9 m
	Mizuna var. Salad Kyomizuna	5.7 a	5.60 q	0.53 k	24.8 b	13.3 e
	Rocket Salad	4.4 a	5.50 r	0.30 m	15.8 i	15.5 a
	Chard	-	-	-	-	-
Level of Significance		ns	**	**	**	**

[z]values within a column followed by different letters are significantly different according to the Tukeys HSD test at $P \leq 0.05$.
ns, **indicates not significant or significant at $p \leq 0.01$, respectively.

Effect of hydroponic nutrient solutions and leafy vegetables varieties on the photosynthetic pigments of leafy vegetables

Photosynthetic pigment content in ten leafy vegetables (except Chard) grown in three nutrient solutions varied significantly when grown in deep water culture hydroponics (Table 7). Results revealed that comparatively lower chlorophyll pigments and carotenoid content obtained in plants grown in MCS-2 than other two nutrient solutions. In MCS-1, Mizuna var. Late White Stem and Mizuna var. Mustard Green showed significantly lower chlorophyll a and it was also evident in MCS-2 however, Mizuna var. Salad Kyomizuna had lower chlorophyll a in ENS. ENS has lower EC value compared to MCS-1 and -2, thus showed lower photosynthetic pigments which is consistent with the findings of Roosta and Hamidpour (2011), Ding *et. al*., (2018), and Wortman, (2015). In general, chlorophyll a, b and t were comparatively higher in BARI Lettuce-1, BARI Palongshak-1 and -2 in all the nutrient solution used. Carotenoid content also followed similar trend. Lee *et al.* (2022) obtained chlorophyll content (0.87-2.03 mg/g FW) from different amount of nutrient solutions and two varieties of lettuce at 34 days after sowing. In case of spinach, it was reported variation in Chl a (1.37 mg/g FW), Chl b (0.68 mg/g FW) and Chl t (2.05 mg/g FW) content when grown in hydroponics using nutrient solution (all in ppm: N 150, P 40, K 200, Fe 5, Ca 50, Mg 60, Zn 3, Mn 3, Cu (<1), B (<4), Mo (<1), Na (<1) and S 50) (Agarwal *et al.*, 2021). While in Turkey, it was reported Chl t content (1.67 mg/g FW) from hydroponic solution (ppm: N 150, P 50, K 150, Fe 5, Ca 150, Mg 50, Zn 0.05, Mn 0.05, Cu 0.03, B 0.50, Mo 0.02) in spinach (Oztekin et al., 2018). Bulgaria *et al.* (2017) obtained 0.510 mg/g FW Chl a, 0.191 mg/g FW Chl b and 0.0.701 mg/g FW Chl t and 0.122 mg/g FW carotenoid in Swiss Chard, and 0.740 mg/g FW Chl a, 0.260 mg/g FW Chl b, 1.007 mg/g FW Chl t, and 0.171 mg/g FW Car in Rocket.

Table 7. Effect of hydroponic nutrient solutions on the photosynthetic pigments of leafy vegetables grown in deep water culture hydroponics.

Nutrient solution	Leafy vegetables	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)	Chl t (mg g^{-1} FW)	Car (mg g^{-1} FW)
Modified Cooper's solution-1	Lettuce var. BARI Lettuce-1	39.7[z]	38.6	34.8	0.095
	Lettuce var. Green Wave				
	Lettuce var. New Red Fire				
	Spinach var. BARI Palongshak-1	32.7	24.1	21.9	0.045
	Spinach var. BARI Palogshak-2	38.5	29.6	26.8	0.068
	Pak Choi var. BARI Batishak-1	39.7	38.3	34.6	0.088
	Mizuna var. Late White Stem	4.0	2.9	2.7	0.020
	Mizuna var. Mustard Green	9.4	6.9	6.3	0.030
	Mizuna var. Salad Kyomizuna	36.2	27.4	24.9	0.070
	Rocket Salad	37.7	29.0	26.3	0.016
	Chard	-	-	-	-

Nutrient solution	Leafy vegetables	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)	Chl t (mg g^{-1} FW)	Car (mg g^{-1} FW)
Modified Cooper's solution-2	Lettuce var. BARI Lettuce-1	17.5	13.1	11.9	0.061
	Lettuce var. Green Wave	15.7	11.8	10.7	0.064
	Lettuce var. New Red Fire	27.0	19.3	17.6	0.050
	Spinach var. BARI Palongshak-1	21.8	16.8	15.2	0.043
	Spinach var. BARI Palogshak-2	11.8	9.3	8.4	0.035
	Pak Choi var. BARI Batishak-1	13.9	10.2	9.2	0.037
	Mizuna var. Late White Stem	9.5	7.4	6.7	0.006
	Mizuna var. Mustard Green	12.0	8.8	8.0	0.015
	Mizuna var. Salad Kyomizuna	16.5	12.1	11.0	0.032
	Rocket Salad	37.5	29.8	27.0	0.107
	Chard	-	-	-	-
Enshi-shoho solution	Lettuce var. BARI Lettuce-1	32.1	23.5	21.3	0.071
	Lettuce var. Green Wave	36.6	28.7	26.0	0.075
	Lettuce var. New Red Fire	30.3	20.6	18.7	0.059
	Spinach var. BARI Palongshak-1	40.3	46.1	41.5	0.066
	Spinach var. BARI Palogshak-2	37.8	28.5	25.9	0.053
	Pak Choi var. BARI Batishak-1	11.3	8.2	7.5	0.044
	Mizuna var. Late White Stem	40.1	39.8	35.8	0.092
	Mizuna var. Mustard Green	39.6	36.4	32.9	0.094
	Mizuna var. Salad Kyomizuna	5.8	4.6	4.2	0.020
	Rocket Salad	22.5	16.3	14.8	0.018
	Chard	-	-	-	-

zValues were calculated from the Spectrophotometric absorbance for each sample.

Chl a = Chorophyll a, Chl b = Chlorophyll b, Chl t = Total Chlorophyll, Car = Carotenoid, FW = Fresh weight

Conclusions

Based on the above results and discussion it is observed that growth of eleven varieties of six leafy vegetables was varied significantly in three types of hydroponic nutrient solutions in deep water culture system. The growth performances of tested vegetables were found better in Modified Cooper's solution-1 (MCS-1) which was followed by Modified Cooper's solution-2 (MCS-2) and the lower performance was obtained from Enshi-shoho nutrient solution (ENS). In case of quality characteristics such as total soluble solids, pH, and titratable acidity were found higher in MCS-1 followed by MCS-2. Lower chlorophyll pigments (Chl a, Chl b and Chl t) and carotenoid content were recorded from plants grown in MCS-2 compared to other two hydroponic nutrient solutions. All three varieties of lettuce, two varieties of spinach, variety of Pak Choi and three varieties of Mizuna performed better when their plants were grown in MCS-1. Therefore, MCS-1 can be recommended for growing these leafy vegetables in hydroponics following deep water culture technique. In further studies, hydroponic systems, and growing season will be considered for evaluating performances of these selected leafy vegetables.

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ASSESSMENT OF ADVANCED FIELD CORN HYBRIDS AT DIFFERENT LOCATIONS OF BANGLADESH

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Abstract

Fourteen promising crosses of maize and four check varieties (BHM-9, 981, Elite and NK-40) were assessed for genotype environment interaction (GEI) and stability in three agro-ecological zones of Bangladesh. The experiment was laid out in RCB design with three replications during rabi 2018-19. The AMMI (additive main effects and multiplicative interaction) model was used to select desired hybrid having higher yield and other potential attributes. Significant variation among the genotypes was noticed in all the characters except plant height and ear height whereas environment (E) was found significant for all the characters studied. The environment of Gazipur was poor and Dinajpur and Burirhar were suitable for the tested maize hybrids. Considering the mean, bi and S2di value all the genotypes showed differential response to adaptability under different environmental conditions. Hybrids E5 (BIL28×BIL79), E7 (BIL153×BIL95) and E9 (CML161×CML172) exhibited the higher yield as well as stable across location based on response (bi) and stability (S2di) parameter.

Keyword: Maize, single cross, stability, across location.

Introduction

Maize is one of the most important food grains in the world as well as in developing countries like Bangladesh. It is the highest yielding grain crop having various uses. A great combination of high market demand with relatively low production cost, ready market and high yield has generated great interest among the farmers in maize cultivation in Bangladesh. It is gaining popularity in the country due to vast demand, particularly for poultry industry. In 2020-21, maize was cultivated in 4.80 lakh hectare of land and produced 4.11 million tons in Bangladesh (BBS, 2022). Multi-environment yield trials are used commonly to release superior genotypes for target sites in plant breeding programs. GEI is universal phenomenon when different genotypes are tested in a number of environments. The large GEI variation usually impairs the accuracy of yield estimation and reduces the relationship between genotypic and phenotypic values (Nachit *et al.* 1992). Numerous methods for multi-environment trials data have been developed to expose patterns of G×E interaction (Yamada, 1962), joint regression (Finlay and

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Wilkinson, 1963; Eberhart and Russel, 1966), AMMI (Gauch, 1992) and GGE biplot (genotype main effect plus genotype by environment interaction). AMMI model combines the analysis of variance of genotypes and the environment main effects with principal component analysis of the GEI into a unified approach (Gauch and Zobel, 1996). Crossa et al. (1990) indicated that the AMMI model can be used to analyze the GEI and to identify the superior hybrid maize genotypes and the best environment . More precise GEI estimates can be obtained with the AMMI model which makes to easier to interpret the results (Durate and Vencovsky, 1999). The objective of this study was to use the AMMI analysis model to assess the stability of some single cross maize hybrids and verify the influence of a sample of environments at different locations of Bangladesh (Gazipur, Dinajpur and Burirhat) in the productive performance of these hybrids.

Materials and Methods

The experiment was conducted at three locations namely Gazipur, Dinajpur and Burirhat during rabi 2018-19. Fourteen single cross maize hybrids namely, E1 (BIL28 × BIL95), E2 (BIL50(1) × BIL28), E3 (BIL51(1) × BIL28), E4 (Lmly08 × BILl79), E5 (BIL28 × BIL79), E6 BIL121 × BIL95), E7 (BIL153 × BIL95), E8 (BIL26 × BIL79), E9 (CML161 × CML172), E10 (CML172 × CML191), E11 (CML193 × CML161), E12 (CML193 × CML162), E13 (CML491 × CML502), E14 (CLQRCWQ10 × CLQRCWQ26) and four check varieties (BHM-9, 981, Elite and NK-40) were evaluated in this study. The experiment was laid out in RCB design with 3 replications. Seeds of each entry were sown in two rows, 4m long plots with 60 cm and 25 cm spacing between rows and hills, respectively. Seeds were sown at Gazipur on 25 November, Dinajpur on 29 November and Burirhat on 19 November, 2018. One healthy seedling per hill was kept after thinning. Fertilizers were applied @ 250, 55, 110, 40, 5 and 1.5 kg/ha of N, P, K, S, Zn and B respectively. Standard agronomic practices and plant protection measures were taken as required. Two border rows at both end of each replication were used for minimize the border effect. Data on days to pollen shedding, days to silking was recorded on whole plot basis. Ten randomly selected plants were used for recording observations on plant and ear height. All the plants in two rows were considered for plot yield and converted to t/ha. The analysis of variance (ANOVA) was used and the GE interaction was estimated by the AMMI model (Zobel et al. 1988). In this procedure, the contribution of each genotype and each environment to the GE interaction is assessed by use of the biplot graph display in which yield means are plotted against the scores of the first principal component of the interaction (IPCA1). The computational program for AMMI analyses is supplied by Duarte and Vencovsky (1999). The stability parameters, regression coefficient (bi) and deviation from regression (S2di) were estimated according to Eberhart and Russel (1966). Significance of differences among bi value and unity was tested by t-test while between S2di and zero by F-test. All the data were processed and analyzed using PB Tools program.

Results and Discussion

Results of combined analysis of variance for five characters of eighteen hybrids at three environments are presented in Table 1. The mean sums of squares for the genotypes were significant for days to pollen shedding, days to silking and yield which revealed the presence of genetic variability in the material under studied. Environments mean sum of squares were highly significant for all of the characters. The highly significant effects of environment indicate high differential genotypic response across the different environments. The variation in soil structure and moisture across the different environments were considered as a major underlying causal factor for the G×E interaction. Environment relative magnitude was much higher than genotypic effect all trails suggesting that performance of each genotype is influenced more by environmental factors. Stability analysis for days to pollen shedding, days to siliking, plant height, ear height and yield were presented in Table 2-6. Results of stability and response of the genotypes under different environments according to Eberhart and Russell (1966) are discussed character-wise as follows; Stability parameter i.e. regression coefficient (bi) and deviation from regression (S2di) for days to 50% pollen shedding (DPS), days to 50% silking (DS), plant height (PH), ear height (EH), and grain yield (GY) were presented in Table 2, 3, 4, 5 and 6.

Table 1. Full joint analysis of variance including the partitioning of the G×E interaction of maize hybrids over 3 locations during 2018-19

Source of variation	Df	Mean sum of squares				
		Days to pollen shedding	Days to silking	Plant height (cm)	Ear height (cm)	Grain yield (t/ha)
Genotypes (G)	17	9.28*	9.87*	359.05	269.28	2.70*
Environment (E)	2	606.85**	512.35**	19079.4**	7695.62**	23.51**
Interaction G×E	34	9.72*	4.58	232.96	138.98	2.67*
AMMI Component 1	18	5.91	6.09	210.25	218.16	1.03
AMMI Component 2	16	1.26	2.88	46.01	49.90	0.26
G×E (Linear)	17	2.50	3.72	88.36	89.36	0.90
Pool deviation	17	4.94	5.44	177.57	188.36	0.44
Pooled error	106	6.61	7.30	284.65	222.30	1.28

*P<0.05, **P<0.01

In Eberhart and Russel (1966) model, regression coefficient (bi) was considered as an indication of the response of the genotype to varying environments while deviation from regression (S2di) was used as the criterion of stability. In the present study these two criteria were considered simultaneously to identify stable hybrids. A genotype with unit regression coefficient (bi=1) is said to be average responsive to environment and suitable for all environment therefore, more adaptive. If bi>1 is said to be highly responsive and suitable for favorable environment. If bi<1 is said to be less responsive and the genotype is suitable for unfavorable environment (Nadarajan and Gunasekaran, 2005).

Days to 50% pollen shedding along with the value of phenotypic indices (Pi), environmental index (Ij), regression coefficient (bi) and deviation from regression (S2di) were presented in Table 2. The genotypic mean value over the location ranges from 95 (E18) to 101 (E3 & E14) days. The genotypes which showed positive pi index took longer period and genotypes showing negative pi index took shorter period for day to 50% pollen shedding. The range bi and S2di values for days to 50 % pollen shedding were 0.09 (E2) to 1.38 (E3) and 0 (E12) to 15.57 (E17) respectively.

Table 2. Stability analysis for days to 50% pollen shedding of maize hybrids over three environments

Genotype	Locations			Overall mean	Pi	bi	S2di
	Gazipur	Dinajpur	Burirhat				
E1	95	103	90	96	-2.19	1.08	7.21
E2	94	104	96	98	-0.41	0.09	4.62
E3	100	110	94	101	2.80*	1.38	8.50
E4	96	106	97	100	1.02	0.95	1.53
E5	97	102	92	97	-1.42	0.81	5.89
E6	94	102	97	98	-0.97	0.63	8.27
E7	97	108	95	100	1.58	1.13	0.26
E8	97	107	94	99	0.91	1.19	1.37
E9	96	106	95	99	0.24	1.03	0.01
E10	97	106	93	99	0.02	1.18	3.57
E11	96	106	95	99	0.46	1.05	0.11
E12	97	108	96	100	1.91	1.13*	0.00
E13	98	104	97	100	1.02	0.71*	0.02
E14	97	108	97	101	2.02	1.10	0.43
E15 (BHM-9)	95	106	97	99	0.80**	0.97	3.00
E16 (981)	95	103	89	96	-2.86**	1.09	13.13
E17 (Elite)	92	103	97	97	-1.42	0.78	15.57
E18 (NK-40)	90	101	94	95	-3.53**	0.81	10.60
Mean	96	105	95	99	-	-	-
E. Index (Ij)	-2.77	6.67	-3.90	-	-	-	-
LSD (0.05)	1.92	3.99	3.30	-	-	-	-

Pi= Phenotypic Indices, bi= regression co-efficient and S2di=deviation from regression.

Days to 50% silking along with the value of phenotypic indices (Pi), regression coefficient (bi) and deviation from regression (S2di) are presented in Table 3. The genotypic mean ranged from 99 (E1, E16, E17 & E18) to 104 (E3, E7 & E14). Nine hybrids showed positive Pi index while the rest nine showed negative Pi index for days 50% silking. Genotypes showed positive Pi index took longer period and negative Pi index took shorter period for day to 50% silking. The bi and S2di values for days to 50% silking ranged from 0.64 (E6) to 1.49 (E14) and 0.19 (E12) to 18.15 (E17), respectively.

Table 3. Stability analysis for days to 50% silking of maize hybrids over three environments

Genotype	Locations			Overall mean	Pi	bi	S2di
	Gazipur	Dinajpur	Burirhat				
E1	98	107	94	99	-1.89	1.17	6.56
E2	96	105	99	100	-1.22	0.79	5.65
E3	102	113	98	104	2.99*	1.40	10.65
E4	98	107	100	102	0.43	0.79	2.06
E5	99	105	96	100	-1.34	0.81	4.41
E6	96	104	100	100	-1.34	0.64	11.00
E7	103	110	99	104	2.88*	0.94	7.86
E8	100	108	98	102	0.54	0.99	1.33
E9	99	109	99	102	0.88	1.04	0.20
E10	99	109	96	101	-0.22	1.22	3.44
E11	99	105	98	100	-0.89	0.68	0.21
E12	99	111	99	103	1.54	1.26	0.19
E13	100	107	100	102	0.99	0.75	0.20
E14	99	113	100	104	2.77*	1.49	0.56
E15 (BHM-9)	97	109	100	102	0.77	1.06	4.62
E16 (981)	97	105	94	99	-2.34	1.02	4.39
E17 (Elite)	94	104	100	99	-1.89	0.68	18.15
E18 (NK-40)	93	106	97	99	-2.67**	1.19	11.10
Mean	98	108	98	101	-	-	-
E. Index (Ij)	-3.06	6.16	-3.09	-	-	-	-
LSD (0.05)	1.69	4.23	3.03	-	-	-	-

Plant heights along with the value of phenotypic indices (Pi), regression coefficient (bi) and deviation from regression (S2di) are presented in Table 4. The genotypic overall mean for plant height ranged from 212 cm (E18) to 252 cm (E3 and E16). Nine genotypes showed positive Pi index while the rest nine showed negative Pi index for plant height. The genotypes showed positive Pi index represent taller and negative Pi index represent shorter plant height. The bi and S2di values range for plant height were 0.54 (E1) to 1.35 (E18) and 0.05 (E12) to 769.20 (E18), respectively.

Table 4. Stability analysis for plant height of maize hybrids over three environments

Genotype	Locations			Overall mean	Pi	bi	S2di
	Gazipur	Dinajpur	Burirhat				
E1	202	247	220	223	-15.35*	0.54	410.54
E2	184	257	246	229	-9.46	1.19	127.18
E3	225	268	263	252	13.54	0.71	34.00
E4	197	252	247	232	-6.35	0.93	37.71
E5	191	248	240	226	-12.02	0.93	64.38
E6	199	243	284	242	3.64	1.16	692.47
E7	214	271	267	251	12.20	0.96	25.46
E8	213	262	261	245	7.09	0.85	11.12
E9	203	275	257	245	6.75	1.11	242.18
E10	204	253	279	245	6.98	1.12	257.57
E11	208	253	252	238	-0.68	0.78	10.06
E12	189	255	260	235	-3.46	1.21*	0.05
E13	199	258	274	244	5.31	1.20	76.82
E14	194	245	261	233	-5.13	1.05	70.97
E15 (BHM-9)	207	256	251	238	-0.46	0.80	31.18
E16 (981)	214	265	278	252	14.09	1.02	50.23
E17 (Elite)	209	273	262	248	9.42	1.02	107.61
E18 (NK-40)	163	215	259	212	-26.13**	1.35	769.20
Mean	201	255	259	238	-	-	-
E. Index (Ij)	-37.54	16.98	20.56	-	-	-	-
LSD (0.05)	28.27	21.64	20.14	-	-	-	-

Ear height along with the value of phenotypic indices (Pi), regression coefficient (bi) and deviation from regression (S2di) are presented in Table 5. The genotypic mean for ear height 112 cm (E1) to 142 cm (E2). Eight genotypes showed positive pi index while rest ten showed negative Pi index for ear height. The genotypes showed positive Pi index represent taller and negative pi index represent shorter ear height. The bi and S2di values for ear height ranges from 0.43 (E11) to 10.50 (E6) and 1.58 (E5) to 624.89 (E9), respectively.

Yield along with the value of phenotypic indices (Pi), regression coefficient (bi) and deviation from regression (S2di) are presented in Table 6. The genotypic mean for yield ranged from 10.67 (t/ha) (E2) to 12.71 (t/ha) (E10). Ten genotypes had positive Pi index while the rest eight exhibited negative pi index for grain yield. The genotypes showed positive Pi index represented higher and negative Pi index represented lower yield. The bi and S2di values for yield ranged from -0.12 (E4) to 2.14 (E2) and 0.04 (E15) to 1.61 (E6), respectively.

Table 5. Stability analysis for ear height of maize hybrids over three environments

Genotype	Locations			Overall mean	Pi	bi	S2di
	Gazipur	Dinajpur	Burirhat				
E1	97	128	110	112	-15.51*	0.56	223.76
E2	122	152	152	142	-13.29	0.92	188.49
E3	102	135	120	119	14.82*	0.82	8.33
E4	90	131	135	119	-8.06	0.66	173.87
E5	114	127	167	136	-8.51	1.21	1.58
E6	115	157	148	140	8.93	10.50	614.15
E7	115	136	129	127	12.93	1.02	110.65
E8	112	169	139	140	-0.51	0.44	44.48
E9	105	138	175	139	12.82	1.08	624.89
E10	116	139	127	127	12.15	1.55	421.37
E11	93	137	141	123	0.26	0.43	103.90
E12	103	140	156	133	-3.62	1.29	5.09
E13	107	133	145	128	5.93	1.28	40.11
E14	104	132	142	126	1.26	0.92	25.70
E15 (BHM-9)	91	123	157	124	-1.06	0.95	10.73
E16 (981)	97	126	143	122	-3.40	1.47	339.80
E17 (Elite)	90	117	144	117	-4.84	1.08	60.59
E18 (NK-40)	90	133	118	114	-10.29**	1.20	208.75
Mean	103	136	142	127	-	-	-
E. Index (Ij)	-23.68	9.19	17.51	-	-	-	-
LSD (0.05)	24.68	5.04	12.51	-	-	-	-

The AMMI biplot provide a visual expression of the relationship between the first interaction principal component axis (AMMI component 1) and mean of genotypes and environment (Fig. 1) as well as relationship of IPCA1 and IPCA 2. The fig 1 depicted that hybrids E5, E7 and E9 were high yielder as well as stable across locations because they had low interaction effects and suitable for all environments. The entries E10, E16, E17 and E18 had high mean value and negative interactions which were suitable for unfavorable environment and the rest of the genotypes were low yielding.

Table 6. Stability analysis for grain yield of maize hybrids over three environments

Genotype	Locations			Overall mean	Pi	bi	S2di
	Gazipur	Dinajpur	Burirhat				
E1	10.13	13.16	12.2	11.83	-0.12	1.33	0.11
E2	7.78	12.00	12.24	10.67	-1.23**	2.14	0.55
E3	10.03	12.79	11.73	11.52	-0.43	1.19	0.20
E4	11.58	11.43	11.14	11.38	-0.56	-0.12	0.06
E5	10.53	12.9	12.91	12.11	0.16	1.18	0.10
E6	10.03	12.15	13.45	11.88	-0.07	1.29	1.61
E7	9.81	13.23	12.90	11.98	0.02	1.14	0.04
E8	11.12	12.53	11.93	11.86	-0.9	0.59	0.08
E9	10.89	13.44	12.28	12.21	0.25	1.06	0.11
E10	12.07	13.00	13.04	12.71	0.75	0.87	0.22
E11	11.16	12.85	11.89	11.97	0.01	0.67	0.26
E12	10.35	12.97	12.93	12.08	0.12	1.30	0.10
E13	8.98	12.74	13.20	11.64	-0.31	1.95	0.73
E14	11.11	12.11	13.19	12.13	0.18	0.69	0.91
E15 (BHM-9)	10.24	12.70	12.54	11.83	-0.12	1.19	0.04
E16 (981)	12.07	13.72	12.15	12.65	0.25	0.54	0.96
E17 (Elite)	12.38	13.53	12.09	12.67	0.71	0.31	0.90
E18 (NK-40)	11.46	12.95	11.74	12.05	0.09	0.53	0.53
Mean	10.65	12.79	12.42	11.95	-	-	-
E. Index (Ij)	-1.30	0.83	0.46	-	-	-	-
LSD (0.05)	2.14	1.08	1.31	-	-	-	-

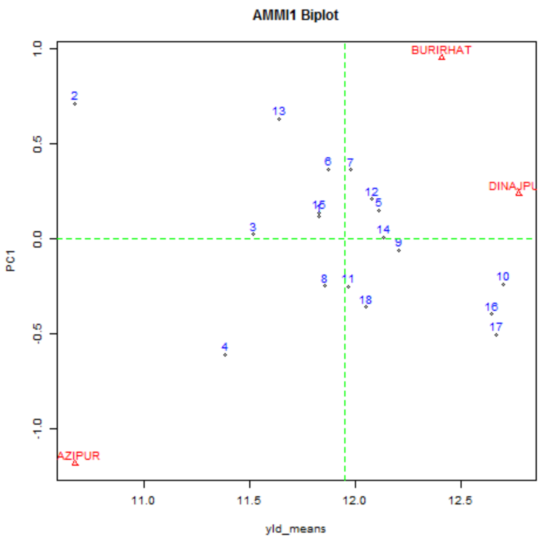

Fig.1. Biplot of the first AMMI interaction (IPCA1) score (Y –axis) plotted against mean yield (X- Axis) of eighteen maize hybrids and three environments

IPCA2 scores also play a significant role in explaining the GEI, the IPCA1 scores were plotted against the

IPCA2 score for further explanation of adaptation (Fig 2). The hybrid E15 was stable over locations for

yield as located within and near the circle. The performances of other hybrids were unstable due to their

dispersed position.

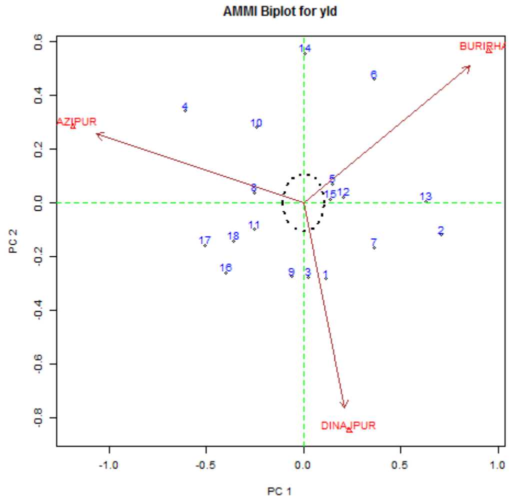

Fig. 2. Biplot of the first AMMI interaction (IPCA2) score (Y –axis) plotted against AMMI interaction (IPCA1) (X-Axis) of eighteen maize hybrids and three environments.

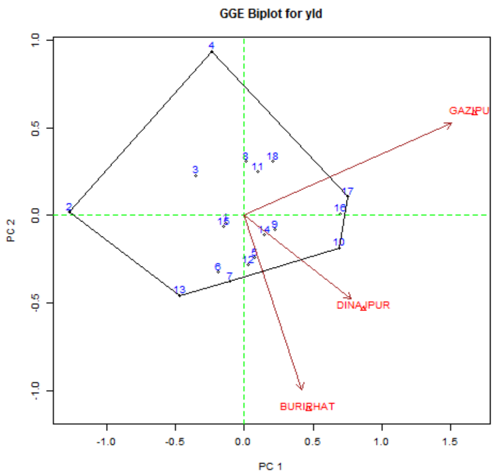

Fig.3. GGE biplot showing "which won where" for yield of eighteen maize hybrids and three environments

The graph "which-won-where" (Fig. 3) enables to identify potential mega-environments (Yan, et al. 2000 and Yan and Hunt, 2001). The genotypes that are utmost from the origin are connected with a straight line forming a polygon. The lines starting from the origin divide the polygon into several sectors. The genotype at the vertex of the polygon performs the best in the environment falling within the sectors (Yan 2002; Yan and Tinker, 2006). The locations within one sector are the ones where the certain genotype had the best yield and can be considered as mega-environments only for that genotype. The hybrid E17 was high yielder and suitable only at Gazipur location. On the other hand the genotype E10 was found high yielder and stable at Dinajpur and Burirhat locations.

Conclusion

Considering the yield potentially and stability parameters, three tested hybrids viz. E5 (BIL28 × BIL79), E7 (BIL153×BIL95) and E9 (CML161×CML172) showed the higher yield as well as stable for overall environments and need to be evaluated further in large plots before release as commercial hybrids across different ecological zones in Bangladesh.

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PRODUCTIVITY AND PROFITABILITY OF INTERCROPPING GARLIC WITH GROUNDNUT IN THE CHAR LAND OF TANGAIL

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Abstract

The experiment was carried out at the farmers' field of On-Farm Research Division, Bangladesh Agricultural Research Institute (BARI), Tangail during two consecutive years 2019-20 and 2020-21 to find out the suitable intercropping system for increasing crop productivity and profitability of groundnut + garlic intercropping system. The treatments were viz. T_1=groundnut (100%) + one row garlic (33%) in between two rows of groundnut, T_2=groundnut (100%) + two rows of garlic (67%) in between two rows of groundnut, T_3= groundnut (100%) + three rows of garlic (100%) in between two rows of groundnut, T_4= Sole groundnut (100%) and T_5= Sole garlic (100%). Groundnut var. BARI Chinabadam-9 and garlic var. BARI Rashun-2 were used in this trial. The experiment was laid out in a randomized complete block design with six dispersed replications. Among three intercropped treatments, groundnut (100%) + two rows of garlic (67%) within two rows of groundnut showed higher groundnut equivalent yield (5.40 t ha^{-1}) and garlic equivalent yield (6.48 t ha^{-1}) which provided the yield advantages of 106 and 26 % over the respective sole crops. The highest land equivalent ratio (1.43), gross return (Tk 3,23,800 ha^{-1}) and benefit cost ratio (2.93) were achieved in groundnut (100%) + two rows of garlic (67%) within two rows of groundnut compared to other intercropping and sole cropping systems. The higher values of all competition functions were also exhibited in groundnut (100 %) + two rows of garlic (67 %) in between two rows of groundnut (T_2). Thus, it is stated that two rows of garlic in between two rows of groundnut could be the most productive and profitable combination for the farmers of Tangail regions.

Keywords: Intercropping systems, economic, equivalent yield, groundnut and garlic.

Introduction

Bangladesh is an agriculture-based country and about 11.52 % gross domestic product (GDP) comes from agriculture in Bangladesh (AIS, 2023). The main challenge of the new millennium is to increase yield of per unit area by at least 50 % through manipulating the limited land resource.

Groundnut (*Arachis hypogaea* L.) is the third most important legume crop in Bangladesh which grown on 34,956 ha of land and produces 66,745 metric tons of nut with an average yield of 1.91 t ha^{-1} (BBS, 2021). It is used as edible oil, make cake, biscuit and bakery in the food industries. Recently the area of groundnut is being decreased due to the competition with *rabi* crops like wheat, potato, *boro rice* and mustard (Alom *et. al*., 2009). Moreover, most of the char land of

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Bangladesh become inundated in *kharif* season which also one of the causes the decline of groundnut production area.

On the other hand, garlic (*Allium sativum* L.) is one of the important spices crops in Bangladesh. In Bangladesh, it ranks third next to onion and chilli. It is grown all over the country in the *rabi* season and consumed by most of the people in Bangladesh. It is cultivated in 72,889 ha land and produces 5,01,612 metric ton with an average yield of 6.08 t ha^{-1} (BBS 2021). Now a days, intercropping plays an important role in increasing the productivity and stability of yield in order to improve resource utilization and environmental factors (Alizadeh *et al*., 2010). Consequently, it is a time demanding technology for cultivation of crops. So, intercropping of garlic with groundnut will help to retain spices crops and ensure highest productivity per unit area as well as supply of oil in our food menu. Groundnut var. BARI Chinabadam-9 is short in stature and it does not occupy more space in the field. Groundnut and garlic are row seeded crops while they are grown in the same season. The growth of groundnut is very slow in the winter season. So, during this time for utilization the space between two rows of groundnut, garlic can be cultivated as intercrop. Groundnut and garlic in intercropping systems may increase their production and fulfill the demand of groundnut and garlic. With this view, the present study was undertaken to find out the suitable intercrop combination of garlic with groundnut for higher productivity and profitability.

Materials and Methods

The study was carried out at the farmers' field under the supervision of On-Farm Research Division, Bangladesh Agricultural Research Institute (BARI), Tangail during two consecutive years 2019-20 and 2020-21 to find out the suitable intercropping system for increasing crop productivity and profitability of groundnut and garlic intercropping system. The experimental site belongs to Active Brahmaputra-Jamuna Floodplain Agro-ecological Zone (AEZ-7) of Tangail. The land was medium high and the soil of the study area was sandy loam in texture with well drainage system with acidic to slightly alkaline in reaction having pH range of 4.6 to 7.9. Organic matter content is low to medium. General fertility level including N, P, K, S, Zn and B was low to medium. Maximum rainfall was received during the months of April to September. The crop received (140.5 mm) rain from October to March. Monthly mean maximum and minimum air temperature (31.9 and 19.3^{o}C), total rainfall (2018 mm) and relative humidity (82.7 %) were prevailing during the study period.

The treatments were viz. T_1 = groundnut (100%) + one row garlic (33%) in between two rows of groundnut, T_2 = groundnut (100%) + two rows of garlic (67%) in between two rows of groundnut, T_3 = groundnut (100%) + three rows of garlic (100%) in between two rows of groundnut, T_4 = Sole groundnut (100%) and T_5 = Sole garlic (100%). Groundnut var. BARI Chinabadam-9 and garlic var.

BARI Rashun-2 were used in this trial. The experiment was laid out in a randomized complete block design with six dispersed replications. The unit plot size was 8.0 m × 5.0 m. Groundnut was the main crop and garlic as the intercrop in the study. Garlic was intercropped in between two rows groundnut @ 33, 67 and 100% population. The groundnut and garlic were planted on 14 November, 2019 and 17 November 2020 in line with seed rate of 100 kg ha^{-1} and 400 kg ha^{-1}, respectively. The plant spacing was 40 cm × 15 cm for groundnut and 10 cm × 10 cm for garlic. The sole crop of groundnut and intercrops were fertilized @ 47-38-25-14-1.0 kg ha^{-1} of NPKSB with 5 t ha^{-1} cowdung where half of nitrogen and other fertilizers were used as basal. Remaining N was top dressed at flowering stage and covered with soil for proper establishment of crop. Other intercultural operation such as weeding, fertilizer application, earthing up and crop protection measure were done properly. The garlic was harvested on 23 March, 2020 and 25 March, 2021 and groundnut harvested on 03 May, 2020 and 10 May, 2021 at physiological maturity. The crops were threshed and dried properly to take the clove and nut yield, respectively. The yield contributing characters of garlic and groundnut were recorded from 10 randomly selected plants in both the years. The following parameters were recorded for intercrop aspects:

Harvest index (HI) was calculated as per following equation (Rahman *et al*., 1989).

$$\text{HI (\%)} = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

Yield of individual crop was converted into equivalent yield on the basis of the prevailing market price of individual crop (Prasad and Srivastava, 1991).

$$\text{Groundnut equivalent yield (GEY)} = \text{Yield of intercrop groundnut} + \frac{\text{Yiga} \times \text{Pga}}{\text{Pg}}$$

and

$$\text{Garlic equivalent yield (GaEY)} = \text{Yield of intercrop garlic} + \frac{\text{Yig} \times \text{Pg}}{\text{Pga}}$$

Where, Yiga= Yield of intercrop garlic, Pga= Price of garlic, Pg= Price of groundnut and Yig= Yield of intercrop groundnut

In intercropping systems, relative yield was quantified by (Jokinen, 1991).

Relative Yield of Groundnut (RYg) = $\frac{\text{Yig}}{\text{Ysg}}$, Relative Yield of Garlic (RYga) = $\frac{\text{Yiga}}{\text{Ysga}}$ and Relative yield total (RYT) = RYg + RYga

Where Yig= Yield of intercrop groundnut, Ysg= Sole yield of groundnut, Yiga= Yield of intercrop garlic and Ysga= Sole yield of garlic.

Various competitive function like land equivalent ratio (LER), area time equivalent ratio (ATER), system productivity index (SPI), replacement value of intercropping (RVI), monetary advantage index (MAI), aggressivity index (A), competitive ratio (CR) and relative crowding coefficient (RCC) were worked out by using standard procedures to find out the benefit of intercropping and the effect of competition between the treatments. The competition functions were calculated by using the following formula:

(a) LER values were calculated by adding the partial LER for each crop and it was calculated by the following formula (Mead and Willey, 1980)

$$\text{LER}=\frac{\text{Yig}}{\text{Ysg}}+\frac{\text{Yiga}}{\text{Ysga}}$$

Where, Yig=Yield of intercrop groundnut, Ysg=Yield of sole groundnut, Y_{iga}=Yield of intercrop garlic and Y_{sga}=Yield of sole garlic

(b) Adetiloye *et. al*. (1983) uses the concept of land equivalent coefficient (LEC) defined as the product of land equivalent ratio in the intercrop. It was developed to assess the interaction and productive potential in crop mixtures.

(c) $\text{LEC}=\frac{\text{Yig}}{\text{Ysg}}+\frac{\text{Yiga}}{\text{Ysga}}$

where, Ysg= Sole yield of groundnut, Ysga= Sole yield of garlic, Yig= Intercrop yield of groundnut and Yiga= Intercrop yield of garlic

(d) The area time equivalent ratio (ATER) was calculated by the formula proposed by Hiebsch (1978) as follows:

$\text{ATER}=\left[\frac{\text{Yig}}{\text{Ysg}}\times Tg+\frac{\text{Yi}ga}{\text{Ysga}}\times Tga\right]\div T$, Where Y_{ig}= Yield of groundnut in intercropping, Y_{sg}=yield of groundnut in sole cropping, Y_{iga}= yield of garlic in intercropping, Y_{sga}= yield of garlic in sole cropping, T_g= Duration of groundnut, T_{ga}= Duration of garlic and T= Total duration of intercropping system.

(e) The replacement value of intercropping (RVI) was calculated according to Moseley (1994) as follows: $\text{RVI}=\frac{Y\text{ig}\times Pg+Y\text{iga}\times Pga}{Y\text{sg}\times Pg-C\text{sg}}$ Where Y_{ig} & Y_{iga} are the yield of groundnut and garlic intercrops, P_g & P_{ga} are the respective market price of these crops, Y_{sg} & C_{sg} are the yield and input cost of the groundnut in sole stand.

(f) The monetary advantage index (MAI) which gives an indication of the economic advantage of the intercropping system was calculated according to Ghosh (2004) as follows:

$$\text{MAI}=\frac{\text{Monetary value of combined intercrops yield}\times(\text{LER}-1)}{\text{LER}}$$

(g) Relative crowing coefficient (K), which is a measure of the relative dominance of one species over the other in a mixture (Willey and Rao, 1980) was calculated as follows:

$K_{groundnut} \times$, $K_{garlic,}$ where $K_{groundnut} = \frac{Yig \times Zgap}{(Ysg - Yig) \times Zgp}$, and

$K_{garlic} = \frac{Yiga \times Zgp}{(Ysga - Yiga) \times Zmp}$ where Z_{gp} and Z_{gap} were the proportion of groundnut and garlic in the mixture, respectively.

(h) Aggressivity (A) index is a measure of competitive relationships between two crops in inter cropping system. This was expressed according to Dhima *et al*. (2007) as follows:

$A_{groundnut} = \frac{Yig}{Ysg \times Zgp} - \frac{Yiga}{Ysga \times Zgap}$ and $A_{garlic} = \frac{Yiga}{Ysga \times Zgap} - \frac{Yig}{Ysg \times Zgp}$. Aggressivity value would be zero when the component species were equally competitive and in all the other cases value of one species would be positive (dominant) and the other would be negative (undominated).

(i) Competitive Ratio (CR): The competitive ratio for groundnut and garlic in mixture was calculated by the formula proposed by (Willey and Rao, 1980).

$CR_{groundnut} = \frac{LERgroundnut}{LER\ garlic} \times \frac{Zgap}{Zgp}$ and $CR_{garlic} = \frac{LERgarlic}{LER\ groundnut} \times \frac{Zgp}{Zgap}$

(j) The system productivity index (SPI) was calculated as described by Odo (1991)

$SPI = (\frac{Ysg}{Ysga} \times Yiga) + Yig$, Where Ysg and Ysga= Mean yield of groundnut and garlic in sole cropping, Yig and Yiga= Mean yield of groundnut and garlic in intercropping.

Pooled analysis was done as because there was no significant difference yield and yield contributing characters between two years. The collected data on different parameters were statistically analyzed with the help of computer package MSTAT-C and mean comparison among the treatments was made by LSD test at 5% level of significance (Gomez and Gomez, 1984).

Economic analysis was done on the basis of prevailing market price of the commodities. The inputs used included seed, fertilizer, labour and insecticides. The two years average results were analyzed for economic benefits using the methodology prescribed by CIMMYT (1988).

$$\text{Benefit Cost Ratio (BCR)} = \frac{\text{Gross return}}{\text{Total cost}}$$

Results and Discussion

Yield and yield attributes of groundnut

The yield and yield contributing characters of groundnut was significantly different due to intercropping system (Table 1). The considerable variation was found in branches plant^{-1}, kernel plant^{-1} and nut yield per hectare while plant height, shelling percent and 100-kernel weight were found insignificant (Table 1). The highest nut yield was recorded in monoculture (T_4) compared to intercropping systems might be due to no intercrop competition for light, nutrients, moisture and space. These nut yield differences were mainly due to higher kernel number plant^{-1}, and numerically higher 100-kernel weight and shelling percent of groundnut. The nut yield of groundnut was reduced 18 to 42 % in intercropping system than sole crop of groundnut though the plant population of groundnut was constant regardless of treatment. The nut yield was reduced probably due to intercrop competition between groundnut and garlic. This result corroborates with the findings of Khan *et al*., (2017) who reported that less groundnut yield was obtained from intercropping system. In Groundnut (100%) + two rows of garlic (67%) in between two rows of groundnut had higher nut yield due to rows planting system fovoured the growth of intercropped groundnut and judicious use of growth resources compared to others. These results are in conformity with the findings of Islam *et al*., (2016). Higher harvest index (HI) of groundnut was observed in T_4 (Sole groundnut) with intercropping system. It was possibly owing to use of more assimilates to the reproductive organs and had higher values in T_4> T_2 T_1>T_3 (Table 1).

Table 1. Nut yield, yield attributes and harvest index of groundnut intercropping with garlic during 2019-20 and 2020-21 (Pooled)

Treatments	Plant height (cm)	Branch plant^{-1} (no.)	Kernel plant^{-1} (no.)	100 kernels wt. (g)	Shelling (%)	Nut yield (t ha^{-1})	% yield decreased over sole	Harvest index (%)
T_1	36.6	7.3	15.3	49.2	66.4	2.15	18	58
T_2	36.0	7.1	15.2	48.8	66.7	1.98	24	59
T_3	35.4	6.8	13.1	48.4	64.4	1.52	42	55
T_4	38.2	7.9	17.4	49.6	66.8	2.62	-	60
LSD ($_{0.05}$)	ns	1.01	2.14	ns	ns	0.32	-	-
CV (%)	6.77	5.84	6.90	5.32	8.33	7.62	-	-

Note: - T_1= Groundnut (100%) + One row of garlic (33%) in between two rows of groundnut, T_2= Groundnut (100%) + two rows of garlic (67%) in between two rows of groundnut, T_3= Groundnut (100%) + three rows of garlic (100%) in between two rows of groundnut and T_4=Sole groundnut

Yield and yield attributes of garlic

The bulb yields of garlic were significantly influenced by groundnut + garlic intercropping systems (Table 2). Higher bulb yield (5.14 t ha^{-1}) was recorded from T_5 (sole garlic) which was statistically identical to T_2 treatment. Lower bulb yield (2.24 t ha^{-1}) was obtained from T_3 which was at par to T_2. The lower bulb yield might be due to lower yield attributes. Higher bulb yield of garlic was observed in monoculture compared to intercropping systems might be due to no intercrop competition for light, nutrients, moisture and space. This result corroborates with the findings of Khan *et al*., (2017). Garlic gave 20 to 56 % lower bulb yield in intercropping treatments as compared to their corresponding monoculture. The bulb yield of garlic was reduced probably due to intercrop competition between garlic and groundnut. However, additional yield from garlic not only compensated the deficit but also gave extra income. This finding is in conformity with Islam *et al*., (2016). The harvest index (HI) of garlic differs by the intercropping systems; it had higher values in T_5> T_2> T_1 >T_3 (Table 2).

Table 2. Bulb yield, yield attributes and harvest index of garlic intercropping with groundnut in Tangail during 2019-20 and 2020-21 (Pooled)

Treatments	Plant height (cm)	leaf plant^{-1} (no.)	Cloves bulb^{-1} (no.)	Bulb wt. (g)	Yield of garlic (t ha^{-1})	% yield decrease d over sole	Harvest index (%)
T_1	68.25	9.10	19.66	11.15	2.43	53	78
T_2	66.34	8.76	18.70	10.93	4.10	20	82
T_3	61.15	7.88	15.28	9.72	2.24	56	75
T_5	71.39	9.35	20.45	11.56	5.14	-	84
LSD ($_{0.05}$)	ns	ns	2.83	ns	1.97	-	-
CV (%)	11.5	6.73	7.32	7.44	6.42	-	-

Note: - T_1= Groundnut (100%) + One row of garlic (33%) in between two rows of groundnut, T_2= Groundnut (100%) + Two rows of garlic (67%) in between two rows of groundnut, T_3= Groundnut (100%) + Three rows of garlic (100%) in between two rows of groundnut and T_5=Sole garlic.

Equivalent yield and partial relative yield

All the intercropping systems gave much higher groundnut equivalent yield than that of sole crop. The treatment T_2 gave the highest groundnut equivalent yield among the combinations, similar trend was followed in same treatment for garlic equivalent yield over sole (Table 3). It's indicated that higher bulb yield and consequently more efficient use of land and available resources under intercropping than sole cropping. The highest groundnut equivalent yield (5.40 t ha^{-1}) as well as garlic equivalent yield (6.48 t ha^{-1}) were recorded from T_2 (Groundnut 100% + two rows of garlic (67%) in between two rows of groundnut which covered the yield advantages of 24 and 20 % over their respective sole crops. Such yield advantage might be due to combined yield of both the crops. The results are in agreement with the finding of Islam et. *al.*, (2016). The partial relative yields of intercropped groundnut varied from 0.58 to 0.82 and intercropped garlic ranged from 0.44 to 0.80 (Table 3). Groundnut yield was reduced (18 to 42 %) among the intercropping system but garlic yield was drastically reduced (20 to 56 %). The yield was reduced due to lower plant population. The result showed that treatment T_2 well accommodative in competitiveness in groundnut + garlic intercropping system (Table 3).

Table 3. Equivalent yields and relative yields of groundnut intercropping with garlic during 2019-20 and 2020-21(average)

Treatment	Yield (tha^{-1})		Equivalent Yield (t ha^{-1})		Partial relative yield (t ha^{-1})	
	Groundnut	Garlic	Groundnut	Garlic	Groundnut	Garlic
T_1	2.15	2.43	4.18	5.01	0.82 (18)	0.47 (53)
T_2	1.98	4.10	5.40	6.48	0.76 (24)	0.80 (20)
T_3	1.52	2.24	3.39	4.06	0.58 (42)	0.44 (56)
T_4	2.62	-	2.62	-	1.00	-
T_5	-	5.14	-	5.14	-	1.00

Note: - T_1= Groundnut (100%) + One row of garlic (33%) in between two paired rows of groundnut, T_2= Groundnut (100%) + Two rows of garlic (67%) in between two rows of groundnut, T_3= Groundnut (100%) + Three rows of garlic (100%) in between two rows of groundnut, T_4=Sole groundnut and T_5=Sole garlic.

Economic return of intercropping garlic with groundnut

On the basis of two years average result, the highest gross margin (Tk. 213202 ha^{-1}) was obtained in groundnut (100%) + two rows of garlic (67 %) in between two rows of groundnut (T_2) which gave an additional income of Tk. 151502 and Tk. 54452 ha^{-1} over sole groundnut and garlic, respectively (Table 4). Total cultivation cost was lower in sole crop and higher in intercropping treatments might be due to inclusion of component crops. Intercropping of garlic brought about an increase in return per taka investment. It was evident that intercropping was beneficial and recorded higher benefit cost ratio (BCR) with respect to monoculture of groundnut and garlic. Among the intercropping systems groundnut (100%) + two rows of garlic (67 %) in between two rows of groundnut (T_2) was obtained the highest benefit cost ratio of 2.93 which further indicated the superiority to T_2 over other treatments. These results are in agreement with the findings of Islam *et al*. (2016). Only one treatment T_2 showed higher BCR but other treatments failed to showed higher profitability.

Table 4. Yield of component crops and economics of groundnut and garlic intercropping system during 2019-20 and 2020-21 (average of two years)

Treatment	Yield ($t ha^{-1}$)		Gross return ($Tk. ha^{-1}$)	Total cost ($Tk. ha^{-1}$)	Gross margin ($Tk. ha^{-1}$)	BCR
	Groundnut	Garlic				
T_1	2.15	2.43	250500	99490	151010	2.52
T_2	1.98	4.10	323800	110598	213202	2.93
T_3	1.52	2.24	203200	115580	87620	1. 76
T_4	2.62	-	157200	95500	61700	1.65
T_5	-	5.14	257000	98250	158750	2.62

Note: - T_1= Groundnut (100%) + One row of garlic (33%) in between two rows of groundnut, T_2= Groundnut (100%) + Two rows of garlic (67%) in between two rows of groundnut, T_3= Groundnut (100%) + Three rows of garlic (100%) in between two rows of groundnut, T_4=Sole groundnut and T_5=Sole garlic.

Price: - Groundnut=Tk. 60.00 kg^{-1} and Garlic= Tk. 50.00 kg^{-1}

Effect of intercropping on different competitive functions

Land equivalent ratio (LER)

The data on LER of different intercropping systems were found greater than unity indicating higher land use efficiency of intercropping systems over the respective monoculture. The range of yield advantage over sole cropping of groundnut or garlic was between 0.02 to 56%. However, the total LER value (1.56) was highest in groundnut (100%) + two rows of garlic (67%) in between two rows of groundnut (T_2) where groundnut and garlic achieved 76 and 80% of their sole yields, respectively indicating higher biological and economic efficiency over monoculture (Table 5). It also expressed that by intercropping garlic with groundnut, a farmer can produce 4.10 tons garlic and 1.98 tons groundnut in one hectare of land instead of growing those separately as sole crops.

Land Equivalent Coefficient (LEC)

Land equivalent coefficient (LEC) measure mixture productivity which also measures intercrop interaction proved to be a superior index for the evaluation of mixture performance. Increasing garlic plant density per unit area 33 to 100 % had the lowest LEC. LEC was found 0.61 (by growing 100 % Groundnut + 67% garlic intercropping system), 0.39 (by growing 100 % Groundnut + 33 % garlic intercropping system)) and 0.28 by growing 100 % Groundnut + 100% garlic intercropping system (Table 5). These results probably due to yielding ability of 100 % Groundnut + 67% garlic (T_2) was more constant through the coordination of the interaction for above and/or below ground competition than other intercropping treatments by increasing adverse effect of increasing garlic population. These results show that competitive pressure between 100 %

Groundnut + 67% garlic (T_2) was lower than other treatments when grown together in the same field indicating a substantial land use advantage of intercropping. These results are in the same context of those obtained by Metwally *et al.* (2018).

Area Time Equivalent Ratio (ATER)

The area time equivalent ratio (ATER) included the duration of the intercrops in intercropping systems in the field and also evaluated the crop yield per day basis. ATER values were found greater than unity in T_1 and T_2 treatments in intercropping systems (Table 5). Groundnut (100%) + garlic (67%) in between two lines of groundnut intercropping system (T_2) showed higher ATER value (1.44) which was about 17 and 45% higher than that of ATER values obtained from T_1 and T_3 which indicating higher yield per day (Table 5). So, the intercropping system was found to be advantageous in comparison to sole crop. This was achieved due to the development of temporal as well as spatial complementary.

Replacement Value of Intercropping (RVI)

Replacement value of intercropping (RVI) is one of the better measures of economic advantage of intercropping systems. Maximum value (5.25) of RVI was obtained in groundnut (100 %) + garlic (67 %) in between groundnut lines (T_2) intercropping system (Table 5). This implies that, farmers who practice intercropping of two rows of garlic in between groundnut lines (T_2) could make 425 % more profit than the farmers who are involved in groundnut or garlic monoculture.

System Productivity Index (SPI)

The system productivity index (SPI) which standardized the yield of the secondary crop (garlic) in terms of the primary crop (groundnut) and also identified the combinations that utilized the growth resources most effectively and maintained a stable yield performance (Tajudeen, 2010). SPI values ranged from 2.78 to 4.10. The results showed that groundnut 100% + garlic (67 %) in intercropping system (T_2) gave the higher SPI value (4.10) than other intercropping systems. Decreasing garlic plant density per unit area from 100 to 67 % of sole garlic planting had higher SPI than intercropping system groundnut (100%) + garlic (33%) or groundnut (100%) + garlic (100%). (Table 5). The results could be attributed to decrease in number of garlic plants from 6,66,667 to 4,46,667 per hectare had positive effect on groundnut productivity per unit area through reducing competitive pressure between the intercrops for basic growth resources.

Monetary Advantage Index (MAI)

The monetary advantage index (MAI) values were positive in all intercropping systems (Table 5). The highest MAI (Tk. 97.37 ha^{-1}) was obtained in groundnut

(100 %) + garlic (67%) in between groundnut lines (T_2), which implied that the planting pattern was highly economical and advantageous for the mixtures. The results are in agreement with the finding of Islam *et al*. (2016) who reported that higher MAI values found in turmeric-sesame intercropping systems compared to sole cropping system.

Table 5. Land equivalent ratio (LER), land equivalent coefficient (LEC), area time equivalent ratio (ATER), replacement value of intercropping (RVI), system productivity index (SPI) and monetary advantage index (MAI) of groundnut and garlic intercropping system during 2019-20 and 2020-21(average)

Treatment	LER	LEC	ATER	RVI	SPI	MAI (Tk ha^{-1})
T_1	1.29	0.39	1.23	4.06	3.39	45.17
T_2	1.56	0.61	1.44	5.25	4.10	97.37
T_3	1.02	0.28	0.99	3.29	2.78	10.94
T_4	1.00	-	-	-	-	-
T_5	1.00	-	-	-	-	-

Note: - T_1= Groundnut (100%) + One row of garlic (33%) in between two rows of groundnut, T_2= Groundnut (100%) + Two rows of garlic (67%) in between two rows of groundnut, T_3= Groundnut (100%) + Three rows of garlic (100%) in between two rows of groundnut, T_4=Sole groundnut and T_5=Sole garlic.

Aggressivity (A)

The value of aggressivity determined the competitive ability of the component crops in an intercropping system. Regardless of the intercropping system, there was a positive sign for garlic and a negative sign for groundnut indicating that garlic was dominant while groundnut appeared as dominated crop (Table 6). Results showed positive aggressivity for garlic at groundnut (100%) + garlic (33%) and groundnut (100%) + garlic (67%) in between groundnut lines planting pattern while it proved less competitive and was dominated by garlic at groundnut (100 %) + garlic (100 %) in between groundnut lines intercropping system. Intercropping of 3 lines garlic (100 %) in between two groundnut lines showed minimum competitive ability between the component crops.

Table 6. Aggressivity index (A), relative crowding coefficient (K) and competitive ratio (CR) of groundnut and garlic intercropping system during 2019-20 and 2020-21(average)

Treatment	Aggressivity (A)		Relative Crowding Coefficient (K)		Total Relative Crowding Coefficient (K)	Competitive ratio (CR)		
	Groundnut	Garlic	Groundnut (Kg)	Garlic (Kga)		Groundnut	Garlic	Difference
T_1	-1.61	0.61	1.51	2.72	4.11	0.68	1.48	0.80
T_2	-0.44	0.44	4.11	5.89	12.19	0.76	1.31	0.55
T_3	0.10	-0.10	1.38	0.93	1.28	1.21	0.83	0.38
T_4	-	-	-	-	-	-	-	-
T_5	-	-	-	-	-	-	-	-

Note: - T_1= Groundnut (100%) + One row of garlic (33%) in between two rows of groundnut, T_2= Groundnut (100%) + Two rows of garlic (67%) in between two rows of groundnut, T_3= Groundnut (100%) + Three rows of garlic (100%) in between two rows of groundnut, T_4=Sole groundnut and T_5=Sole garlic.

Relative Crowding Coefficient (K)

Total Relative crowding coefficient (K) of groundnut and garlic was more than unity indicating greater non-competitive interference than the competitive one. The intercropped garlic had higher relative crowding coefficient values than the intercropped groundnut. Positive relative crowding coefficient values were obtained in all intercropping systems (Table 6). Intercropping of groundnut (100 %) + two rows of garlic (67%) in between two groundnut lines had the higher relative crowing coefficient (K) value (12.19) compared to other intercropping system.

Competitive Ratio (CR)

The competitive ratio values showed variation among the intercropping indicating differential competitive ability of component crops as influenced by intercrops of garlic at different populations (Table 6). Garlic showed higher values of CR (0.83-1.48) than groundnut (0.68-1.21) in all intercropping combinations indicating garlic as the best competitor than groundnut. Garlic exhibited better competitive ability in groundnut (100%) + two rows of garlic (67%) in between two groundnut lines (T_2) intercropping system probably garlic plant was highly responsive to intercrops of groundnut. Lower values of CR indicated similarities of competitiveness but higher value of difference in CR indicated dissimilarities of competitiveness between the crops grown in the mixture. Consequently, groundnut (100%) + one row garlic (33 %) in between two groundnut lines intercropping system with higher difference of CR (0.80) exhibited dissimilarities in competitiveness between the component crops (Table 6). However, groundnut

(100%) + three rows of garlic (100%) in between groundnut lines (T_3) intercropping system with lower difference of CR (0.38) showed merely similar competitiveness between the component crops. These results are in agreement with the findings of Islam et al. (2016).

Conclusion

From two years average result indicating that intercropping groundnut with garlic gave maximum productivity as well as economic return than monoculture of component crops. The equivalent yields, relative yields, land equivalent ratio (LER) values and economic return were also found highest in treatment T_2 (two rows of garlic in between two lines of groundnut) in groundnut + garlic intercropping system. Thus, it could be concluded that a planting pattern comprising two rows of garlic in between two lines of groundnut intercropping system, i.e., groundnut (100%) + two rows of of garlic (67%) in between two rows of groundnut could be adopted for better productivity and to get maximum profit. So, the farmers of Tangail regions could be suggested to cultivate groundnut with garlic as intercropped instead of sole crops.

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COMBINING ABILITY ASSESSMENT OF POPCORN CONSIDERING YIELD AND KERNEL POPPING QUALITY

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Abstract

A line × tester experiment was conducted at early generation of popcorn involving eight superior female lines and three testers for grain yield, its components as well as popping quality to determine the effect of combining ability both General combining ability (GCA) and specific combining ability (SCA). Highly significant genotypic differences were observed indicated wide range of variability present among the genotypes. The inbred parents Thai Pop / S_{3}-28 and Thai Pop / S_{3}-20 were identified as the best general combiners due to their good combining ability effect for grain yield and popping quality and also their ability to transmit characters to their progenies for most of the characters. The crosses with high SCA effect for grain yield evolved from high × low general combiner parents were reveled additive × dominance type of gene action. The good combination crosses were Thai Pop /S_3-1 × PCB /S_6-13, Thai Pop /S_3-17 × PCB /S_6-39 and Thai Pop /S_3-20 × PCB /S_6-39 could be used in future breeding program to develop high yielding popcorn hybrids with desirable poped kernel qualities. The information on the nature of gene action with respective variety and characters might be used depending on the breeding objectives.

Keywords: Popcorn, maize, gca, sca, line × tester, popping quality.

Introduction

Popcorn broadly belongs to maize, *Zea mays* L. or corn which is the most plentifully grown cereal in the world. Corn can be categorized into major types namely, Dent, Flint, Pod, flour and sweet corn. Popcorn is a special kind of flint corn that was particularly nominated by Indians in early Western civilizations time.

The difference of popcorn from other flint and dent corns represents firstly by the hard starch type and secondly by very hard pericarp and outer layers of endosperm, which permit the internal pressure and temperature to rise high enough to pop (Cretors, 2001).

Repeated buying of a particular popcorn brand depends greatly much on its taste and quality. Expansion volume and the number of unpopped corn grains are the most critical factors determining the popcorn quality (Song *et al.*, 1991). The expansion volume is a very important quality criterion commercially, because

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commercial popcorn buyers buy by weight and sell the popped corn by volume (Ceylan and Karababa, 2002).

Results from classical quantitative genetics and traditional statistical analysis have shown that popping characteristics are quantitatively inherited, controlled by multiple genes (Clary 1954; Ziegler 2001), influenced by environmental effects (Li *et al.* 2003) and popping methods (Dofing *et al*. 1990).

Hence, hybrid development program was undertaken to exploit maximum heterotic effects for yield and quality of popcorn, where parental selection plays the key role in hybrid development. In this context, Line × Tester analysis by Kempthome (1957) has widely been used for evaluation of inbred lines by crossing them with testers. The value of any inbred line in hybrid breeding ultimately depends on its ability to combine very well with other lines to produce superior hybrids. The present study involving a Line × Tester analysis aimed at to determine both the general combining ability (GCA) and specific combining ability (SCA) of crosses of the popcorn inbred lines for grain yield, yield contributing characters and kernel popping quality and to explore heterotic hybrid combinations. Additionally, this experimemntal approach evaluated the cross performance for yield and popping related traits.

Materials and Methods

The experimental materials comprised of eight selected S_3 generation lines of Thai pop corn (as female parents) and three testers (PCB/S6-11, PCB/S6-13, PCB/S6-39) of popcorn. Total 24 crosses combinations were made through Line × Tester mating design during 2013-14. The 24 F1's along with two checks (NI5820 and Khoibhutta) were sown following Alpha lattice design with two replications at the research field of Bangladesh Agricultural Research Institute, Joydebpur during rabi, 2014-15. Spacing was maintained at 60 cm × 20 cm. Two border rows were used at each end of the replication to minimize the border effect. Fertilizers was applied @ 250, 55, 110, 40, 5 and 1.5 Kg/ha of N, P, K, S, Zn and B, respectively. All the recommended packages of practice were followed and the observations were recorded on ten randomly selected plants for quantitative characters viz. days to 50% tasselling, days to 50% pollen shading, days to 50% silking, plant and ear height, ear length, ear diameter, number of grains per ear, 1000-grain weight, and grain yield. Yield maturity traits were recorded from plot basis and yield was converted to ton/ha. After harvesting and proper drying, the popcorns were tested for quality by popping. Qualitative characters like popping % and popping expansion was recorded. After popping the popped kernels were tasted by 17 evaluators (Scientists and staffs) for scoring the taste of quality following 1-5 scale. During scoring the best one was scored 1 and the worst one 5. All these observations were considered in combining ability analysis (excluding parents) following Sharma (1988) using statistical analysis package 'R 4.2'.

Results and Discussion

The analysis of variance revealed significant differences among the parents, crosses and parent vs. crosses for popping quality and most of the the traits studied, indicating sufficient genetic variability present among them. The analysis of variance also revealed significant differences in the variance due to testers, and Line x Testers in some of the yield and quality traits (Table 1). Similar difference for grain yield and other characters were reported by Sofi and Rathor (2006). No significant differences were observed among the lines but significant difference was observed for the testers regarding tasseling, pollen shedding, silking, plant height and ear height. Significant differences were also existed in the interactions of Line × Testers for yield, yield related traits and popping quality traits, indicating that there were wide range of variability among lines and testers for the traits.

The higher estimate of dominance variance as compared to additive variance for all of the characters was probably due to predominance of non-additive gene action, which suggest the scope of improvement of these characters through heterosis breeding. Similar non-additive gene action was also found by Singh and Singh (1998) and they reported that non-additive gene action for plant height, ear length, kernel rows, 1000- grain weight. Mahto and Ganguly (2001) observed non additive gene action for grain yield. Both additive and dominant genetic effects play very important roles in the inheritance of popping characteristics as reported by Dofing *et al.,* (1991). The contribution of lines, testers and their interactions to total variances are presented in Table 2. The proportional contribution of lines and interactions to total variances was much higher than testers in all the traits except days to tassel, days to pollen shading, days to silking, plant height and ear height. However, the contribution of lines was higher than the interactions to total variances only for test quality and was nearly equal in case of popping expansion. In this study interaction contributed maximum to total variance for quality traits and grain yield which was followed by female lines. Testers contributed lowest to total variance for yield and quality, which is in conformity with Amiruzzaman (2010) and Li *et.al.* (2003).

General combining ability (GCA) effects

The gca effects showed that line Thai Pop/S_{3}-1exhibited significant negative gca effects for pollen shedding and could be utilized for evolving earliness (Table 3). Hussain *et al*. (2003) also observed similar phenomenon in their study. For plant height, line Thai Pop /S_{3}-28 exhibited significant negative effects which could be used for developing shorter plant. Ahmed *et al.* 2017 and Ahmed *et al.* 2017 also

Table 1. Analysis of variance for grain yield, its components and popping quality traits in line x tester analysis of pop corn

Source of variation	df	DT	DPS	DS	PH	EH	EL	ED	NK/R	TSW	Yi (t/ ha)	POP%	PE	TQ
Genotypes	34	30.99**	29.23**	31.82**	1545.2**	492.6**	12.09*	2.21**	99.49**	1280.74**	54.36**	479.83**	85.35**	0.29**
Parents	10	33.136**	28.40**	24.83**	692.43**	185.63*	25.11**	3.58**	56.88**	1673.6**	130.30**	893.04**	97.36**	0.368**
Parents vs. Crosses	1	452.58**	432.46**	476.36**	36321.6**	9323.15**	70.08**	2.79	2322.9**	5234.1**	155.92**	1201.4**	151.55**	0.697*
Crosses	23	11.74**	12.07**	15.54**	403.96**	242.13**	3.91	1.59*	21.35*	938.02**	16.94	340.66**	79.34**	0.257*
Lines	7	1.76	2.93	3.43	145.10	96.61	2.46	1.74	22.95	840.40	20.86	257.08	112.29	0.443
Testers	2	108.52**	96.58**	132.94**	2751.44**	1527.65**	0.90	1.90	13.56	19.27	17.45	211.57	110.30	0.061
Lines ×Testers	14	2.89	4.56	4.82	198.03*	131.24*	5.06	1.47	21.66*	1118.08**	14.91	400.90**	58.44**	0.192
Error	34	3.43	3.05	2.98	77.54	60.03	6.35	0.85	10.54	282.63	15.75	0.97	0.34	0.106
Estimates of components of variance														
σ^2gca		5.283	5.915	5.975	17.784	3.457	0.215	0.097	0.869	27.360	0.039	5.512	0.350	0.005
σ^2sca		-410.98	-460.23	-465.06	-1283.78	-238.82	-16.04	-7.47	-61.33	-2085.75	-1.01	-389.11	-22.39	-0.37
σ^2gca /σ^2sca		-0.013	-0.013	-0.013	-0.014	-0.014	-0.013	-0.013	-0.014	-0.013	-0.039	-0.014	-0.016	-0.013

*, ** indicate significant at 5% and 1% respectively;

DT= Days to 50% tasseling, DPS= Days to 50% pollen shedding, DS= Days to 50% silking, PH= Plant height, EH= Ear height, EL= Ear length, ED= Ear diameter, NK/R=Kernel number per row, TSW= Thousand grain wt., Yi= Yield (t/ha), Pop%= Popping percentage, PE= Popping expansion; TQ=Taste of Quality scale (1= very good, 5 = very poor).

Table 2. Proportional contribution of lines, testers and their interactions to total variance of pop corn

Source	DT	DPS	DS	PH	EH	EL	ED	NK/R	TSW	Yi (t/ ha)	POP%	PE	TQ
Due to line	4.56	7.38	6.71	10.93	12.14	19.184	33.30	32.72	27.27	37.48	22.97	43.08	52.51
Due to tester	80.41	69.61	74.41	59.23	54.86	1.993	10.39	5.52	0.18	8.96	5.40	12.09	2.06
Due to L×T	15.01	23.00	18.88	29.84	32.99	78.823	56.31	61.75	72.55	53.57	71.63	44.83	45.43

Table 3. GCA effects of parents for different yield contributing traits of pop corn

Tester/Tester	DT	DPS	DS	PH	EH	EL	ED	NK/R	TSW	Yi (t/ ha)	POP%	PE	TQ
Testers													
1. PCB/S_6-11	-3.00**	-2.83**	-3.31**	14.56**	11.02**	-0.167	0.36	1.00	-0.104	-0.88	3.86**	1.66**	0.022
2. PCB/S_6-13	1.69**	1.54**	1.94**	-10.88**	-7.60**	-0.104	-0.02	-0.188	1.146	1.16	-3.35**	-3.02**	0.048
3. PCB/S_6-39	1.31**	1.29**	1.38**	-3.69	-3.417	0.271	-0.33	-0.812	-1.042	-0.28	-0.51*	1.36**	-0.07
SE (gi)	0.463	0.437	0.431	2.201	1.937	0.63	0.231	0.812	4.203	0.992	0.24	0.14	0.081
Lines													
1. Thai Pop/S_3-1	-1.25	-1.479*	-1.229	1.75	4.292	-0.458	-0.69	1.0	-16.35*	-1.31	-3.13**	4.92**	0.014
2. Thai Pop/S_3-8	0.083	0.521	0.438	6.417	4.792	0.375	0.81*	1.5	11.98	-0.896	-3.30**	2.97**	-0.011
3. Thai Pop/S_3-16	0.417	-0.146	-0.562	0.083	3.958	0.208	0.81*	-0.5	-9.69	-0.70	0.36	2.13**	0.38**
4. Thai Pop/S_3-17	-0.083	0.188	-0.396	2.417	-1.042	0.042	-0.19	0.167	-6.35	-0.35	-5.30**	0.58*	0.07
5. Thai Pop/S_3-20	-0.083	-0.479	-0.396	4.25	0.125	0.042	-0.19	-1.167	2.81	-0.36	7.69**	3.00**	-0.22
6. Thai Pop/S_3-26	0.250	0.521	0.438	-0.75	-2.375	0.875	-0.19	2.333	8.65	-0.12	7.78**	-2.83**	-0.43**
7. Thai Pop/S_3-28	0.250	0.521	0.938	-7.417*	-5.708	0.208	-0.02	0.667	-8.02	4.52**	5.78**	-2.41**	-0.08
8. Thai Pop/S_3-29	0.417	0.354	0.771	-6.75	-4.042	-1.29	-0.35	-4.0**	16.98*	-0.79	-9.88**	-8.37**	0.35**
SE (gj)	0.756	0.713	0.705	3.595	3.163	1.029	0.38	1.325	6.86	1.62	0.402	0.238	0.132

*, ** indicate significant at 5% and 1% respectively;

DT= Days to 50% tasseling, DPS= Days to 50% pollen shedding, DS= Days to 50% silking, PH=Plant height, EH= Ear height, EL= Ear length, ED= Ear diameter, NK/R=Kernel number per row, TSW= Thousand grain wt., Yi= Yield (t/ha), Pop%= Popping percentage, PE= Popping expansion; TQ =Taste of Quality scale (1 = very good, 5 = very poor).

found similar result in their study. popping expansion. Better taste was scored for the lines Thai Pop/S_3-16 and Thai Pop/S_3-29. The tester PCB/S_6-11showed significant positive GCA effects for quality parameter but negative gca for yield. As gca is generally associated with additive gene action in inheritance of characters, the lines and testers with high gca may be utilized in hybridization programme to improve a particular trait through transgressive segregation. According to Jenkins (1935) and Sprague (1946a), the early generation determination of lines combining ability does not change significantly with continued inbreeding at later generations.

Only one line Thai Pop/S_3-29 had significant positive gca effect for 1000 - grain weight that could be utilized for evolving bold grained corn. The line Thai Pop /S_3-28 exhibited highly significant positive gca effects for yield which also had negative significant effect for plant height. Since, it could be used for developing high yielding dwarf hybrid. Significant gca effect for yield in maize was also reported by Paul and Duara (1991). In case of qualitative characters, the line Thai Pop /S_3-16 only showed positive effect for all the three quality parameters where, popping expansion and taste quality were highly significant. On the other hand, the lines Thai Pop/S_3-20, Thai Pop/S_3-26 and Thai Pop/S_3-28 had positive significant GCA effect for popping % and Thai Pop/S_3- 1, Thai Pop/S_3-8, Thai Pop/S_3-17 and Thai Pop/S_3-20 posses significant effect for

Specific combining ability (SCA) effects

Developing high-yielding hybrids depend on careful choice of parents. Information regarding general and SCA is very important to select desirable parents and crosses.

The Specific combining ability effects and mean of the crosses for grain yield, its components and other qualitative characters are presented in Table 4. Positive sca effect is expected for yield components. Significant positive sca was observed for yield in Thai Pop/S_3-28 × PCB/S_6-13 with higher mean yield, which also exhibited positive significant sca effect for popping% having higher mean value and positive but non-significant sca regarding popping expansion and 1000 grain weight. The genotype Thai Pop/S_3-29 × PCB/S_6-11 possess non-significant but positive sca yield and all its component traits along with all popping parameters except test quality. Considering popping quality, the hybrids; Thai Pop/S_3-8 × PCB/S_6-11, Thai Pop/S_3-17 × PCB/S_6-11, Thai Pop/S_3-1 × PCB/S_6-13, and Thai Pop/S_3-29 × PCB/S_6-13 were credited for having significant positive sca for all the popping traits except test quality where, it showed only non-significant but positive sca against effect. Only the genotype Thai Pop/S_3-26 × PCB/S_6-11 exhibited significant positive sca for test quality but its other traits were not favorable. Considering all the characters under study, the genotype Thai Pop/S_3-17 × PCB/S_6-39 exhibited favorable performances like, negative significant sca for days to

Table 4. SCA effects and mean performance of the F_1 hybrids for grain yield, yield contributing traits and different popping quality of popcorn

Crosses	DT		DPS		DS		PH		EH		EL		ED	
	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean
1. Thai Pop/S_3-1 × PCB/S_6-11	-0.5	77.5	-1.333	81.5	-1.521	81.5	-5.062	171.5	-5.854	79.5	-2.17	14.5	-0.19	11
2. Thai Pop/S_3-8 × PCB/S_6-11	0.667	82	0.667	86.5	0.812	87.5	-3.229	161	-7.854	71	0.5	17.7	1.312*	10.7
3. Thai Pop/S_3-16 × PCB/S_6-11	-0.167	83.5	-0.667	89	-0.688	90	2.104	153.5	6.479	72.5	-1.33	18.5	-0.69	11
4. Thai Pop/S_3-17 × PCB/S_6-11	0.333	80	1	85.5	1.146	85.5	10.77	178	7.979	78	-0.67	18	-0.69	14
5. Thai Pop/S_3-20 × PCB/S_6-11	-1.167	83	-1.333	88.5	-1.354	89.5	-7.062	164	-6.688	72.5	0.333	16.8	-0.19	12.2
6. Thai Pop/S_3-26 × PCB/S_6-11	0.5	84	0.167	89	0.312	89	-3.562	158	-6.688	74	0	18	-0.69	11
7. Thai Pop/S_3-28 × PCB/S_6-11	0.5	79.5	1.167	83.5	1.312	83	1.604	177	7.646	91.5	1.167	16.2	0.646	12
8. Thai Pop/S_3-29 × PCB/S_6-11	-0.167	86	0.333	90	-0.021	90.5	4.438	147	4.979	65	2.17	17.5	0.479	13
9. Thai Pop/S_3-1 × PCB/S_6-13	-0.688	82.5	-0.708	87.5	-0.771	87.5	9.875	157	5.104	65.5	0.77	19.2	-0.31	11.9
10. Thai Pop/S_3-8 × PCB/S_6-13	-1.021	79.5	-0.708	85.5	-0.438	85	8.208	188	0.938	88	-0.56	16.5	-0.31	11
11. Thai Pop/S_3-16 × PCB/S_6-13	1.646	85	1.458	90.5	1.562	90.5	-2.458	147.5	-1.062	66.5	0.104	18	0.688	11.5
12. Thai Pop/S_3-17 × PCB/S_6-13	1.146	82	1.625	86	1.396	86	-4.292	152.5	·11.73*	52.5	0.77	17.5	0.188	11.3
13. Thai Pop/S_3-20 × PCB/S_6-13	0.146	78	-0.208	82.5	-0.104	82.5	-1.125	172	-1.396	74.5	-0.73	17.5	0.188	11.5

Crosses	DT		DPS		DS		PH		EH		EL		ED	
	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean
14. Thai Pop/S_3-26 × PCB/S_6-13	-0.188	84	0.292	88	-0.438	89	3.375	152.5	5.104	63.5	0.44	16.5	0.688	11.7
15. Thai Pop/S_3-28 × PCB/S_6-13	0.312	84.5	-0.208	89.5	0.062	90	-18.95**	169	0.938	72.5	-1.89	18.2	-1.48*	10.8
16. Thai Pop/S_3-29 × PCB/S_6-13	-1.354	80	-1.542	85	-1.271	85	5.375	170.5	-1.062	72	1.104	18	0.354	11
17. Thai Pop/S_3-1 × PCB/S_6-39	1.188	84	2.042	89.5	2.292	89.5	-4.812	152	1.583	59	1.396	18.5	0.5	12
18. Thai Pop/S_3-8 × PCB/S_6-39	0.354	83.5	0.042	88.5	-0.375	89.5	-4.979	156	2.583	72	0.062	18	-1	11
19. Thai Pop/S_3-16 × PCB/S_6-39	-1.479	80	-0.792	86	-0.875	86.5	0.354	169	-5.083	83	1.229	18.5	0	12.5
20. Thai Pop/S_3-17 × PCB/S_6-39	-1.479	84.5	-2.63*	89	-2.542*	90.5	-6.479	123	-13.08*	45	-0.10	15.5	0.5	10
21. Thai Pop/S_3-20 × PCB/S_6-39	1.021	83	1.542	88	1.458	88.5	8.188	166.5	5.75	65	0.396	18.5	0	12.15
22. Thai Pop/S_3-26 × PCB/S_6-39	-0.312	79.5	-0.458	85	0.125	85	0.188	172.5	7.75	82	-0.44	18.1	0	11.8
23. Thai Pop/S_3-28 × PCB/S_6-39	-0.812	83	-0.958	87.5	-1.375	89	17.35**	148	4.083	57	0.729	17	0.833	11.5
24. Thai Pop/S_3-29 × PCB/S_6-39	1.521	85.5	1.208	90	1.292	91	-9.812	140	-3.583	59	-3.27	13.1	-0.83	10.2
SE(sij) / SE	1.309	1.31	1.235	1.24	1.222	1.22	6.227	6.23	5.479	5.48	1.782	1.78	0.651	0.67

Table 4. Cont'd

Crosses	NK/R		TSW		Yi (t/ha)		POP%		PE		TQ	
	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean
1. Thai Pop/S_3-1 × PCB/S_6-11	2	39.5	-14.90	165	-0.23	3.30	-15.37**	74	-3.53**	24.53	-0.087	2.51
2. Thai Pop/S_3-8 × PCB/S_6-11	-1.5	35.8	1.77	175	-0.25	3.83	9.80**	96	2.28**	28.40	0.248	2.74
3. Thai Pop/S_3-16 × PCB/S_6-11	-2	34	-6.56	200	0.08	6.08	-3.87**	86.5	1.49**	26.28	-0.08	2.49
4. Thai Pop/S_3-17 × PCB/S_6-11	-3.167	36.5	-9.90	210	0.28	3.69	7.30**	99	4.34**	28.40	0.015	2.82
5. Thai Pop/S_3-20 × PCB/S_6-11	0.167	37	10.94	190	0.77	5.60	-6.95**	70.5	-3.26**	19.14	-0.18	2.50
6. Thai Pop/S_3-26 × PCB/S_6-11	-1.333	37.5	10.10	225	0.85	5.15	-6.78**	86.5	-7.18**	25.81	0.66**	2.34
7. Thai Pop/S_3-28 × PCB/S_6-11	0.833	34	-13.23	180	-2.98	4.21	0.97	89	3.52**	26.78	-0.19	2.89
8. Thai Pop/S_3-29 × PCB/S_6-11	5.0*	35.5	21.77	220	1.48	5.23	14.89**	82	2.33**	13.26	-0.39	3.22
9. Thai Pop/S_3-1 × PCB/S_6-13	-0.312	35.5	-6.15	160	-1.73	5.59	13.85**	96	5.02**	30.81	0.107	2.73
10. Thai Pop/S_3-8 × PCB/S_6-13	0.188	33.5	-19.48	180	-0.37	4.76	-11.48**	94.5	-2.28**	28.08	-0.103	2.61
11. Thai Pop/S_3-16 × PCB/S_6-13	0.688	36.5	32.19**	170	-0.93	5.98	-3.65**	56	-7.33**	12.56	0.229	2.70
12. Thai Pop/S_3-17 × PCB/S_6-13	1.021	37	-21.15	220	-0.54	5.35	-23.98**	99.5	-6.48**	25.56	0.079	2.41
13. Thai Pop/S_3-20 × PCB/S_6-13	-3.146	35.5	4.69	210	-1.45	5.24	5.52**	93.25	0.66	22.88	-0.141	2.18

Crosses	NK/R		TSW		Yi (t/ha)		POP%		PE		TQ	
	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean
14. Thai Pop/S_3-26 × PCB/S_6-13	0.854	31	18.85	205	-0.98	5.06	5.19**	98.5	5.16**	22.12	-0.335	2.25
15. Thai Pop/S_3-28 × PCB/S_6-13	-1.979	36.5	15.52	182.5	7.50**	5.74	4.44**	97.25	0.78	28.44	-0.045	2.59
16. Thai Pop/S_3-29 × PCB/S_6-13	2.688	37.5	-24.48*	215	-1.50	5.57	10.10**	93.5	4.45**	13.12	0.209	2.81
17. Thai Pop/S_3-1 × PCB/S_6-39	-1.688	38.5	21.042	225	1.96	5.77	1.51*	98.25	-1.48**	20.78	-0.02	1.84
18. Thai Pop/S_3-8 × PCB/S_6-39	1.312	37.5	17.708	175	0.62	5.43	1.68*	97.5	-0.001	22.02	-0.145	1.73
19. Thai Pop/S_3-16 × PCB/S_6-39	1.312	38	-25.63*	175	0.86	6.38	7.51**	99.25	5.83**	24.25	-0.149	2.31
20. Thai Pop/S_3-17 × PCB/S_6-39	2.146	34	31.04**	205	0.27	6.89	16.68**	95.5	2.13**	16.82	-0.094	2.48
21. Thai Pop/S_3-20 × PCB/S_6-39	2.979	36.5	-15.625	185	0.68	5.43	1.43*	88.5	2.59**	16.11	0.321	2.64
22. Thai Pop/S_3-26 × PCB/S_6-39	0.479	37.5	-28.96*	235	0.13	5.52	1.59*	97.5	2.01**	17.11	-0.327	2.55
23. Thai Pop/S_3-28 × PCB/S_6-39	1.146	34	-2.292	190	-4.52*	4.57	-5.41**	85.5	-4.31**	14.53	0.233	3.17
24. Thai Pop/S_3-29 × PCB/S_6-39	-7.69**	23	2.708	215	0.02	4.65	-24.99**	53.25	-6.78**	7.68	0.181	3.03
SE(sij) /SE	2.295	2.30	11.89	11.89	1.96	2.01	0.696	1.02	0.412	0.44	0.230	0.22

*, ** indicate significant at 5% and 1% respectively;

DT= Days to 50% tasseling, DPS= Days to 50% pollen shedding, DS= Days to 50% silking, PH=Plant height, EH= Ear height, EL= Ear length, ED= Ear diameter, NK/R=Kernel number per row, TSW= Thousand grain wt., Yi= Yield (t/ha), Pop%= Popping percentage, PE= Popping expansion; TQ=Taste of Quality scale (1=very good, 5=very poor).

pollen shading, silking, plant height and ear height together with significant positive sca against 1000 grain weight, popping percentage and popping expansion where it also possesses positive sca for kernel number per row and yield. In general, the crosses involved both good general combiners and or at least one good combiner showed high SCA effects are due to additive × additive and additive × dominant gene action. These results agreed with the earlier findings of Hussain *et al*. (2003) in maize.

Conclusion

The parental line Thai Pop /S_3-20 has been identified as the best general combiner due to its good combining ability effects and also its ability to transmit characters to their progenies for most of the characters. The crosses Thai Pop/S_3-17 × PCB/S_6-39 and Thai Pop/S_3-20 × PCB/S_6-39 were marked out as good combinations for yield and quality trait along with high mean performances, could be used in future breeding program to develop high yielding popcorn hybrids with desirable qualities.

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ADOPTION STATUS AND PROFITABILITY ANALYSIS OF MANGO VARIETIES BARI AAM-3 AND BARI AAM-4 IN RAJSHAHI REGION

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Abstract

The study was conducted in three mango growing districts namely Naogaon, Natore, and Chapainawabganj during 2023 to assess the adoption status of mango var. BARI Aam-3 and BARIAam-4 varieties, thier farm level profitability, and constraints to cultivation at the farm level. A total of 60 farmers taking 20 from each district were randomly selected for interviews. The study revealed that BARI Aam-3 is highly adopted in Naogaon district which covered 61% of the total mango production in that area. Besides, 32% of the mango orchards are occupied by BARI Aam-3 variety in Natore district. Whereas, BARI Aam-4 is highly adopted in Naogaon district which shared 7.5% of the total mango production in that area. The highest fruit yield of BARI Aam-3 was found at 12.56 ton ha^{-1} from a more than 8 years old orchard. Gross return and net return was Tk. 7,26,474 ha^{-1} and Tk. 5,58,474 ha^{-1}, respectively from 8 years above old mango orchard. Regular bearings, higher yield, higher profit, sweetness, and less insect-pest infestation were the prime reasons for choosing BARI Aam-3 and BARI Aam-4 at the farmers' level. Farmers observed very low bearing after 15 years, scarcity of quality saplings, unattractive color, and short shelf life were the major constraints to BARI Aam-3 cultivation. Market controlled by a business syndicate, lack of credit facilities, lack of knowledge about modern cultivation, and lack of storage facilities were common problems opined by the sample farmers.

Keyword: Mango, Cultivation, Adoption, Production, Market, Yield, Profitability.

Introduction

Bangladesh is a predominantly agro-based developing country in the world and its fertility status of soil and weather are very expedient for harvesting different varieties of agricultural products, especially mango. In addition, mango is the most important source of nutrients and energy (Mukherjee et al., 2009) because it is rich in amino acids, carbohydrates, fatty acids, minerals, organic acids, proteins, and

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vitamins, mainly vitamins A and C (Moore, 2004). In Bangladesh, mango is called the king of all fruits but it is native to the Indian sub- continent. The largest amounts of mangos are produced in India, China, Thailand, Indonesia, Bangladesh, Pakistan, Mexico, and Brazil. Nowadays, its worldwide trading is increasing, especially in the USA and European markets for better quality and higher standards.

In Bangladesh, a variety of mangoes including Fajli, Langra, Gopal-bhogh, Misribhogh, Arshini, Laksmanbhogh, Mohonbhogh, Raj-bhogh, Himsagar, Chokanan, Khirshapat, Shurjapuri, Chosha, Hari Bhanga, Shatapora, Kachamitha, and Mollika are produced (Azad et al., 2017). These varieties are mostly grown in Rajshahi, Dinajpur, and Nawabganj where the Rajshahi region alone produces over 270 varieties of mangoes. This area's mango has huge demand in the market both domestically and commercially but there is no large industry in the northwestern region. Most of the people are employed in different jobs like nursing, giving areas of production in Rajshahi, Chapainawabganj, Dinajpur, Rangpur, Kushtia, and Natore is ranged from 35,000 ha to 50,000 ha per year and 50,000 people involved in mango production (BBS 2018). Now a day, Khagrachori and Bandarban are also producing huge quantities of mangos. Bangladesh Agricultural Research Institute (BARI) developed 18 mango varieties. For domestic consumption and international export, BARI developed BARI Aam-3 and BARI Aam-4 mango varieties. gainning popularity . However, the adoption status of those varieties throughout the country is still unknown. Therefore, it is important to know the adoption status of different BARI-released varieties, and cultivation practices, and identify region-based problems. The specific objectives of this study were as follows.

- To know the adoption status of BARI Aam-3 and BARI Aam-4 at the farmers' level;
- To estimate the profitability of BARI Aam-3 and BARI Aam-4;
- To identify social, economic, and biological constraints to BARI Aam-3 and BARI Aam-4 cultivation; and
- To recommend short-term and long-term plans of action for policymakers, researchers, and extension personnel to enhance BARI Aam-3 and BARI Aam-4 production.

Materials and Methods

Sampling Technique and Sample Size

Multi-stage and purposive random sampling techniques were used to select the study areas and sample respondents. In the first stage of sampling, three mango growing districts namely Naogaon, Natore, and Chapainawabganj were selected purposively based on the area coverage and production of mango. A total of 60 samples taking 20 farmers from each district were randomly selected for this study. Data were collected by experienced field investigators under direct supervision of the researchers using a pre-tested interview schedule.

Analytical Techniques

Data were categorized according to the age of mango orchard like 1st year, 2nd year, 3rd year, 4-8th year and above 8 years. Tabular methods of analysis using descriptive statistics were used in presenting the results of the study. The following equations were used to calculate the profitability of mango cultivation (Gittinger et al., 1984).

Gross return = GRij = YijPij

Net return = GRij – TCij

Gross margin = GRij – TVCij

Where,

GRij = Gross return of jth orchard forith farmer (Tk./ha)

Pij = Price (Tk./ha) of jth crop received by ith farmer

Yij = Yield of mango of jth orchard for ith farmer(kg/ha)

TC_{ij}= Total cost of jthorchard for ith farmer (Tk/ha)

TVC_{ij}= Total variable cost of jthorchard for ith farmer (Tk/ha)

Analysis of returns to investment

The following techniques were used to estimate the returns to investment of establishing a mango orchard at the farmers' level.

Benefit cost ratio (BCR): BCR analysis is a technique for evaluating a project or investment by comparing the economic benefits with the economic costs of the activity. BCR can be used to evaluate the economic merit of a project.

$$Benefit\ cost\ ratio\ (BCR) = \frac{\sum_{t=1}^{t=n} \frac{B_t}{(1+t)^t}}{\sum_{t=1}^{t=n} \frac{C_t}{(1+i)^t}}$$

Net present value (**NPV):** NPV is the current value of all project net benefits. Net benefits are simply the sum of benefits minus costs. The sum is discounted at the discount rate. The following formula is used to calculate NPV:

$$Net\ present\ value\ (NPV) = \sum_{t=1}^{t=n} \frac{B_t - C_t}{(1+i)^t}$$

Internal rate of return (IRR): IRR is the discount rate for which the present of total benefits equal to the present value of total cost. Generally, the IRR should be greater than the discount rate for a project to be accepted.

$$Internal\ rate\ of\ return\ (IRR) = \sum_{t=1}^{t=n} \frac{B_t - C}{(1+i)^t}$$

$$IRR = Lower\ discount\ rate + Difference\ between\ t(x+a)^n = \sum_{k=0}^{n} \binom{n}{k} x^k a^{n-k}$$

Where,

B_t= Total benefit (Tk./ha) in thet^{th} year

C_t = Total cost (Tk./ha) in thet^{th} year

t = Number of year

i = Interest (discount) rate

Results and Discussion

Adoption of BARI Aam-3 and BARI Aam-4 Mango Variety

Table 1 enumerates the extent of adoption of BARI Aam-3 and BARI Aam-4 in the study areas. Among the three survey areas, BARI Aam-3 is highly adopted in Naogaon district which covered 61% of the total mango production in that area. Besides, 23.32% of the mango orchards are occupied by this variety in Natore district. The lowest adopted area was Rajshahi which covered only 6.49% of the total mango production. The adoption of BARI Aam-4 is highly adopted in Naogaon district which occupied 7.5% of the total mango production in that area. Besides, 2% of the mango orchards are occupied by this variety in Chapainawabganj. The lowest adopted area was Rajshahi and Natore which covered only 1% of the total mango production.

Table 1. Percentage of area coverage at farm level in different district

Variety	Naogaon	Natore	Chapainawabganj	Rajshahi
BARI Aam-3	61.0	23.32	7.0	6.49
BARI Aam-4	7.5	1.0	2.0	1.0

Table 2. Varity-wise area coverage (ha) in different Upazila at Chapainawabganj

Sl No.	Name of the Varieties	Study areas						% of total
		Sadar	Shibgonj	Gomustapur	Nachol	Vulahat	Total	
1.	Fazli	750	4600	985	737	923	7995	21
2.	Arshini	585	5790	1230	833	889	9327	25
3.	Langra	320	1850	395	319	229	3113	8
4.	Gopalbhog	250	540	220	97	84	1191	3
5.	Khirshapat	1088	2250	255	310	305	4205	11
6.	Bombe	585	525	15	28	10	1163	3
7.	Laksmanbhog	140	940	195	20	607	1902	5
8.	BARI Aam-3	450	515	297	1307	51	2620	7
9.	Mollika	35	2	5	0	05	47	0
10.	Mohonbhog	5	0	5	0	0	10	0
11.	Kishanbhog	2	0	0	0	0	2	0
12.	Nagfazli	0	0	0	0	1	1	0
13.	BARI Aam-11	55	2	85	105	5	252	1
14.	BARI Aam-4	270	20	140	350	6	786	2
15.	Ranipochondo	2	1	0	0	15	18	0
16.	Kumrajali	1	2	0	0	0	3	0
17.	Haribhog	7	2	5	1	0	15	0
18.	Kachamitha	1	0	1	0	0	2	0
19.	Dudhsor	10	0	2	0	0	12	1
20.	Gourmoti	65	10	50	0	3	128	0
21.	Katimon	105	80	90	110	12	397	5
22.	Mohanonda	30	0	2	0	0	32	1
23.	Boishak	1	0	0	0	0	1	
24.	Localguti	36	2283	13	30	185	2547	2
25.	Othersguti	375	848	240	24	332	1819	3
	Total	5165	20260	4230	4271	3662	37588	100

Table 3.Varity-wise area (ha)and production (MT) in Naogaon district

SL No.	Variety Name	Area (ha)	Production (MT)	Yield (MT/ha)	% of area
1	Nag fazli	940.75	13170	14	3.14
2	Langra	1559	20270	13	5.20
3	Fazli	1420	21300	15	4.73
4	BARI Aam-3	18313.50	255660	13.9	61.05
5	Gupalbhog	646	8400	13	2.15
6	Khirshapat	1388	17350	12.5	4.63
7	BARI Aam-4	2262.50	27150	12	7.54
8	BARI Aam-11	35	490	14	0.12
9	Mollika	44	530	12	0.15
10	Katimon	138.25	1225	8.9	0.46
11	Gourmoti	141.25	1840	13	0.47
12	Haribhanga	34.50	435	12.5	0.12
13	Banana mango	113.25	1235	11	0.38
14	Arshini	2326	3140	13.5	7.75
15	Kumrajali	13	180	14	0.04
16	GutiAam/ local	625	5625	9	2.08
	Total	30000	378000		100

Table 4. Area and production of different mango varieties in Rajshahi district

SL No.	Variety Name	Area (ha)	No. of mango tree	Production (MT)	Yield (MT/ha)	% of total area
1	Laksmanbhog	6535	981605	84952.89	13.00	33.38
2	Gupalbhog	883	151789	11488.99	13.01	4.51
3	Khirshapat	2770	422970	36189.70	13.06	14.15
4	Langra	1650	263926	21460.75	13.01	8.43
5	Fazli	2433	345846	36033.85	14.81	12.43
6	Arshini	1816	333752	27273.25	15.02	9.28
7	BARI Aam-3	1271	501447	15814.20	12.44	6.49
8	Guti/others	1917	290419	21897.87	11.42	9.79
9	BARI Aam-11	9	1350	87.90	9.77	0.05
10	BARI-4	169	52665	2199.50	13.01	0.86
11	Ranipochondo	49	5632	594.43	12.13	0.25
12	Katimon	71.5	10305	434.45	6.08	0.37
13	Banana mango	0.8	365	3.5	4.67	0.004
14	Gourmoti	4	1800	24.40	6.10	0.02
15	Miajaki	0.3	115	0.00	0.00	0.00
	Total	19578.8	3363986		13.201	100

Table 5.Varity-wise area (ha) coverage in different Upazilas of Natore district

SL No.	Variety Name	Sodor	Noldanga	Singra	Guru-daspur	Borai-gram	Lalpur	Bagati-para	District total	% of total
1	Gupalbhog	110	35	30	25	22	110	30	362	6.30
2	Langra	100	45	10	30	50	100	25	360	6.26
3	Khirshapat	150	65	20	30	80	100	95	540	9.40
4	Laksmanbhog	190	85	0	10	110	690	475	1560	27.14
5	Fazli	80	50	52	90	80	120	45	517	9
6	BARI Aam-3	220	85	200	60	100	335	340	1340	23.32
7	Mollika	55	5	10	22	0	10	12	114	1.98
8	Arshini	75	20	0	10	10	240	85	440	7.66
9	Ranipochondo	25	3	0	0	17	0	7	35	0.61
10	Mohonbhog	0	2	3	0	2	0	0	22	0.38
11	BARI Aam-4	14	1	15	0	11	7	20	59	1.03
12	Haribhanga	15	2	10	10	0	1	10	59	1.03
13	Kachamita	0	0	0	0	0	0	5	5	0.09
14	Dudhsor	0	0	0	0	7	0	7	7	0.12
15	krishanbhog	0	0	0	0	6	0	6	13	0.23
16	Gourmoti	10	2	0	0	0	3	3	24	0.42
17	Katimon	1	0	0	0	0	1	1	3	0.05
18	Mohanonda	0	0	0	0	0	0	1	1	0.02
19	Beishaki	0	0	0	0	0	2	3	5	0.09
20	Guti	90	15	20	5	500	91	55	281	4.89
	Total	1135	415	370	292	100	1810	1225	5747	100

Reasons for choosing BARI Aam-3 variety: Table 6 shows that 85% of the respondents cultivated the variety as it is free from alternate bearing. At the same time, 77% of farmers told that BARI Aam-3 cultivation makes them financially profitable, and 73% of farmers mentioned that they obtained a good yield. Besides, several reasons were responsible for adopting this variety such as sweetness and taste (76%) and can plant a lot of saplings on a small piece of land (62%).

Table 6. Percent of responses on the reasons for choosing BARI Aam-3 variety for cultivation

Reasons	Natore	Naogaon	Chapainawabganj	All area
1. No alternate bearing	80	90	85	85
2. Higher profitability	79	75	77	77
3. Higher demand	60	60	62	60
4. Higher yield	75	71	75	73
5. Sweet and tasty	75	78	75	76
6. Needs smaller space to grow	65	60	62	62
7. Higher consumer preference	40	30	35	35
8. Availability of saplings	30	40	32	34

Source: Field survey 2023

Reasons for choosing BARI Aam-4 variety: Table 7 shows that 85% of the respondents cultivated BARI Aam-4 as it is high yielding hybrid variety. At the same time, 77% of farmers also told that BARI Aam-4 cultivation makes them financially profitable because the market price is higher for late harvest and 60% of farmers mentioned that the variety has good potential for export. Besides, several other reasons such as vibrant merun colour at the ripening stage (47%), large size (622%), and low disease and pest attack (35%) were responsible for adopting this variety.

Table 7. Percent of responses on the reasons for choosing BARI Aam-4 variety for cultivation

Reasons	Natore	Naogaon	Chapainawabganj	All area
1. Hybrid variety	80	90	85	85
2. Vibrant merun colour at the ripening stage	50	47	44	47
3.Getting higher price for late harvest	79	75	77	77
4. Export potentialities	60	60	62	60
5. Higher yield	75	71	75	73
6. Large size	65	60	62	62
7. Less disease and pest attack	40	30	35	35

Level of extension contact: Extension agents play a vital role in technology dissemination. DAE is the key agent of the Bangladesh government in the dissemination of crop-related agricultural technologies from research organizations to farmer's levels. Table 8 reveals that the adopters of BARI Aam-3 and BARI Aam-4 had frequent contact with the extension personnel and neighboring farmers and their level of contacts were more than non-adopters in the study areas. It was opined that about 49% of farmers contacted extension personnel regarding BARI Aam-3 and BARI Aam-4 cultivation which was much higher than that of non-adopters. Contact with mass media (i.e. radio, television, newspaper) was also higher than non-adopters in the study areas.

Table 8: Level of extension contact of mango farmers with different extension Medias

Extension medias	Farmer's responses (%)			
	Frequently	Often	Rarely	Never
A. Adopter				
Extension personnel	27	49	18	6
Neighbor farmer	23	58	17	2
Demonstration plot	3	7	14	76
Participating agricultural fair	-	3	13	84
Television	-	2	4	94
Attend in the field day		8	18	74
Research organization visit	-	4	10	86
Radio	-	6	8	86
Read Newspaper	-	-	4	96
Agriculture booklet/leaflet	-	-	1	99
B.Non-adopter				
Extension personnel	18	34	23	25
Neighbor farmer	17	44	7	32
Demonstration plot	6	29	18	47
Participating agricultural fair	2	3	11	84
Television	2	4	17	77
Attend in the field day	-	-	6	94
Research organization visit	--	12	17	71
Radio	-	-	-	100
Read Newspaper	-	-	-8	92
Agriculture booklet/leaflet	-	-	-	100

Influencing persons in variety adoption:

At the beginning stage of the adoption of the BARI Aam-3 and BARI Aam-4 varieties, most farmers were influenced by different persons at different levels. Table 9 shows that the overall influence of SAAO in adopting BARI Aam-3 and BARI Aam-4 was higher than the influence of others. Neighboring farmers and Agriculture Officer also influenced farmers in adopting BARI Aam-3 and BARI Aam-4 varieties in the study areas.

Table 9. Percent distribution of BARI Aam-3 BARI Aam-4 adopters by influencing persons

Persons	Level of influence (%)				
	Very high	High	Medium	Low	No influence
1.Family member	-	-	8	16	76
2.Neighboring farmer	15	41	27	5	12
3.SAAO	82	6	-	4	8
4.Agriculture Officer	13	18	13	42	16
5.IPM/ICM club			14	13	73
6.Scientists of OFRD	-	-	-	7	93

Profitability of BARI Aam-3 and BARI Aam-4 Variety Mango Farming

Profit is a basic criterion for selecting an enterprise. The study was found that farmers planted 120-140 trees per acre (100 decimal) of land in the sense that after 8 years they cut one of every three of the line to clear it for air and light. Farmers have the option to do intercrop with BARI Aam-3 and BARI Aam-4 mango orchards up to 3-4 years of age. Generally, after 4 years of mango tree, farmers did not cultivate any kind of intercrop. The study also found that only 18% of the respondents cultivated intercrop in one or two years in their orchard with different crops. Due to the complexity of accounting costs and return of intercropping, the present study does not cover costs and return of intercropping in determining the profitability of BARI Aam-3 and BARI Aam-4 mango farming.

It is evident from Table 10 that hired labor ranked the highest variable cost item of BARI Aam-3 mango orchard. The lowest-used variable input was manures followed by gypsum among the chemical fertilizers. The per hectare total cost of BARI Aam-3 mango orchard from the establishment year was estimated to be Tk. 210000 ha^{-1} where the cost of saplings was the major cost item. Fixed cost does not depend on the level of production. Only three items were considered as a fixed cost for the mango orchard viz., family labour, interest on operating capital (IOC), and the rental value of land in the study areas as indicated in Table 10. Considering all variable and fixed costs, total cost and net return were determined in the study areas.

The average return of BARI Aam-3 mango orchards in the study areas are depicted in Table 11. Farmers obtained fruits just after one year of plantation and this is unique only for BARI Aam-3 variety. So, the gross margin was negative in the first year of plant age as total cost was high enough at that time. The net return increased substantially over the years. Respondent farmer opined that the fruit yield of BARI Aam-3 started decreasing drastically after 15 years of plant age. The highest yield was found 12560 kg/ha and gross return and net return was Tk. 6,90,800/ha and Tk. 5,58,474/ha, respectively from 8 years above to 8 years of mango orchard.

Table 10. Cost of BARI Aam-3 cultivation in the study areas

Items	1st year	2nd year	3rd year	4-8th year	8th year & above	All years
Sample number	n=6	n=6	n=6	n=6	n=6	n=30
A. Variable Cost	95759	84000	78000	75550	78000	82262
Hired labour	13916	9876	13452	17678	31456	17276
Land preparation	4901	0	0	0	0	980
Saplings	30000	0	0	0	0	6000
Manures	9691	0	4689	4325	0	3741
Fertilizers						
Urea	1575	1649	1275	1326	1513	1468
TSP	3500	4080	3146	2834	2262	3164
MoP	2800	1072	1216	944	1152	1437
Gypsum	1235	150	0	165	115	333
Insecticides	5700	6750	7546	8787	17769	9310
Irrigation	7103	7103	7103	7103	7103	7103
Bamboo stick	8245	0	0	0	0	1649
Intercrop	-	15586	19258	18936	15326	17277
Interest on operating capital	7093	2454	3074	3453	4910	4197
B. Fixed cost	54282	50514	55641	56079	61630	55629
Family labour	11628	7860	12987	13425	18976	12975
Land use cost	90000	90000	90000	90000	90000	90000
C. Total Cost (A+B)	210000	174000	168000	165550	168000	177110

Table 11. Profitability of BARI Aam-3 cultivation in the study areas

Items	1st year	2nd year	3rd year	4-8th year	8th year & above	All years
Sample number	n=6	n=6	n=6	n=6	n=6	n=30
A. Total cost (Tk/ha)	210000	174000	168000	165550	168000	166094
Variable cost	120000	84000	78000	75550	78000	76094
Fixed cost	90000	90000	90000	90000	90000	90000
B. Yield of mango (kg/ha)	0	3000	5400	9600	12560	6112
C. Selling Price (Tk/kg)		55	55	55	55	55
D. Gross return frommango (Tk/ha)	0	165000	297000	528000	690800	336160
E. Gross return from intercrop (Tk/ha)	0	31546	35980	31546	35674	29267
F. Total gross return (Tk/ha)	0	196546	332980	559546	726474	365427
G. Gross margin (Tk/ha)	-120000	112546	254980	483996	648474	289333
H. Net return (Tk/ha)	-210000	22546	164980	393996	558474	199333
I. Per unit production cost (Tk/kg)	-	58	31	17	13	27
		BCR				2.05
		NPV				830354
		IRR				72%

The average per hectare return of BARI Aam-4 mango orchards in the study areas is depicted in Table 13. Respondent farmers opined that they got fruits just after one year of plantation and this is unique only for BARI Aam-4 variety. So, the gross margin was negative in the first year of plant age as the total cost was high enough at that time. The net return increased substantially over the years. The fruit yield of BARI Aam-4started decreasing drastically after 15 years of plant age. The per hectare highest fruit yield was found at 13,460 kg and gross return and net return was Tk. 7,26,474 and Tk. 6,24,474, respectively from above to 8 years of mango orchard.

Table 12: Cost of BARI Aam-4 cultivation in the study areas

Items	1st year	2nd year	3rd year	4-8th year	8th year & above	All years
Sample number	n=6	n=6	n=6	n=6	n=6	n=30
A.Variable cost	140000	80500	95300	115300	12000	88620
Hired labour	13916	9876	13452	17678	31456	17276
Land preparation	4901	0	0	0	0	980.2
Saplings	30000	0	0	0	0	6000
Manures	9691	0	4689	4325	0	3741
Fertilizers						
Urea	1575	1649	1275	1326	1513	1468
TSP	3500	4080	3146	2834	2262	3164
MoP	2800	1072	1216	944	1152	1437
Gypsum	1235	150	0	165	115	333
Insecticides	5700	6750	7546	8787	17769	9310
Irrigation	7103	7103	7103	7103	7103	7103
Bamboo stick	8245	0	0	0	0	1649
Intercrop	-	15586	19258	18936	15326	17277
Interest on operating capital	7093	2454	3074	3453	4910	4197
B. Fixed cost	54282	50514	55641	56079	61630	55629
Family labour	11628	7860	12987	13425	18976	12975
Land use cost	90000	90000	90000	90000	90000	90000
A. Total cost (A+B)	230000	170500	185300	205300	102000	178620

Table 13. Profitability of BARI Aam-4 cultivation in the study areas

Items	1st year	2nd year	3rd year	4-8th year	8thyear & above	All years
Sample number	n=6	n=6	n=6	n=6	n=6	n=30
A. Total cost (Tk/ha)	230000	170500	185300	205300	102000	178620
Variable cost	140000	80500	95300	115300	12000	88620
Fixed cost	90000	90000	90000	90000	90000	90000
B. Yield of mango (kg/ha)	0	1830	4580	14560	13460	6886
C. Price (Tk/kg)		90	90	90	90	90
F. Total gross return (Tk/ha)	0	196546	332980	559546	726474	365427
G. Gross margin (Tk/ha)	0	112546	254980	483996	648474	289333
H. Net return (Tk/ha)	-230000	26046	147680	354246	624474	186807
I. Per unit production cost (Tk/kg)	-	93.17	40.46	14.10	7.58	25.94
		BCR				2.63
		NPV				1005630
		IRR				84%

Returns to investment on BARI Aam-3 and BARI Aam-4 cultivation: To calculate the benefit-cost ratio (BCR), net present value (NPV), and internal rate of return (IRR), the cost and returns were discounted at a 12% rate of interest. The calculated BCR was found to be 2.05 at a 12% discount rate which is greater than unity and acceptable. The estimated NPV of the BARI Aam-3 orchard was Tk.830354 per hectare which indicates that BARI Aam-3 cultivation was highly profitable in the study areas. The IRR was found to be 72% which is highly acceptable because it is much higher than the opportunity cost of capital.

The estimated BCR was found to be 2.63 at a 12% discount rate which is greater than unity and acceptable. The estimated NPV of the BARI Aam-4 orchard was Tk.1005630 per hectare which indicates that BARI Aam-4 cultivation was highly profitable in the study areas. The IRR was found to be 84% which is highly acceptable because it is much higher than the opportunity cost of capital.

Problems and Constraints of BARI Aam-3 and BARI Aam-4 Cultivation

Although mango farming is a profitable enterprise, respondent farmers encountered several problems and constraints regarding mango farming (Table 13). About 93% of the respondents mentioned that very low bearing after 15 years of the trees (82%) are the main hindrance to this mango variety adoption followed by unequal size (87%). Besides, colour is not attractive (42%), short shelf life (73%), and susceptibility to diseases (56%) are some of the constraints towards disseminating this variety. The higher price of saplings (35%) and lack of quality saplings (63%) were found to be the main problem in BARI Aam-4 to dissemination at farmers' fields. Some common problems were found in the study areas such as adulteration of pesticides (55%), market controlled by a business syndicate (71%), no credit facilities for mango cultivation (79%), and lack of knowledge about modern cultivation (60%) etc.

Table 13. Problems and constraints of BARI Aam-3cultivation in the study areas

Problems and constraints	Farmer's responses (%)			
	Natore	Naogaon	Chapainawabganj	All area
A. BARI Aam-3				
Colour not attractive	42	40	45	42
Very low bearing after 15 years	80	82	84	82
Unequal size	90	86	87	87
Short shelf life	75	74	72	73
Susceptibility to disease	56	60	52	56
B. BARI Aam-4				
Higher price of sapling	50	30	25	35
Lack of quality saplings	60	64	67	63
C. Common problems				
Adulterate of pesticide	56	64	45	55
Market controlled by a business syndicate	50	86	78	71
No credit facilities of mango cultivation	75	80	82	79
High prices of inputs	45	65	62	57
Lack of knowledge about modern cultivation	80	50	50	60
Lack of storage facilities	90	95	92	92
Lack of irrigation facilities	30	57	64	50

Conclusions

This study examines the adoption status, profitability and bottlenecks of mango var. BARI Amm-3 and BARI Amm-4 cultivation at Rajshahi region. Above fifty percent farmers in Naogoan district adopt BARI Amm-3 in their gardens. Overall, adoption status of BARI Amm-3 has been increasing in Rajshahi region. Farmers shifted their garden through BARI Amm-3 because it's much more profitable than other varieties. Although adoption rate of BARI Amm-4 was found very low in Rajshahi region, but the establishment of orchard is showing increasing trend. But only a few farmers are informed that those varieties were released by BARI, especially BARI Aam-3. Gradually BARI Aam-3 and BARI Amm-4 occupy the rice and vegetable field as a best alternative in the survey areas. More than fifty percent respondents practiced intercropping different crop up to 4-5 years along with BARI Aam-3 and BARI Amm-4 mango orchard. The cultivation of BARI Aam-3 and BARI Aam-4 mango is profitable because it gives substantially higher gross return and net return. Human labor, cost of saplings, and insecticides were the major cost items for mango production in the study areas. The rate of returns (i.e. BCR, NPV, and IRR) indicated that BARI Aam-3 ad BARI Aam-4 cultivation in the study areas is profitable for the farmers. After adopting these varieties, all categories of farmers could change their livelihood. It signifies that BARI Aam-3 ad BARI Aam-4 mango production has good potential in Bangladesh.

Recommendations

- The saplings of BARI Amm-3 and BARI Amm_4 variety should be made available at farmers level. Government should encourage DAE, BADC and nursery owners to produce more BARI Amm-3 and BARI Amm-4 saplings and supply to the interested farmers at reasonable price.
- Regular training program should be arranged for the farmers to develop their knowledge about improved cultivation practices of BARI Amm-3 and BARI Amm-4 cultivation.
- A proper initiative should be taken to record the information on cost and return and to disseminate this information throughout the different stakeholders.
- At the same time, government, research institution and agriculture extension should work together to make a sustainable market environment for the growers so that they can get fair price of mango.

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EVALUATION OF DIFFERENT ROOTSTOCKS FOR IMPROVING GROWTH AND YIELD OF WATERMELON

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Abstract

Grafting method of propagation is a standard practice in developed countries to increase plant tolerance to several biotic and abiotic stresses and to improve fruit yield of watermelon. The technique is still remained unexplored especially for watermelon cultivation in Bangladesh. Hence, an experiment was conducted at the research field of Horticulture Research Centre, Bangladesh Agricultural Research Institute, Gazipur during late winter to early summer season of 2020-21 to find out the suitable rootstock as a grafting agent compatible better with watermelon and to study the effect of such rootstocks on the growth, yield and sweetness of watermelon. The seedlings of watermelon (*Citrullus lanatus*) cv. Black Jumbo F_1 was grafted onto four different rootstocks namely, Bottle gourd, Sweet gourd, Ash gourd and Snake gourd using the single cotyledon hole insertion method. The result indicated that grafting improved growth and development of watermelon influencing the length of main stem and numbers of lateral stems. Grafted plant showed significantly lower prevalence of plant wilting in watermelon over non-grafted plants. As such, the plant survival at fruit maturity stage (70 Days after planting) was higher in grafted treatments than non-grafted treatment. The result indicated that grafting of watermelon onto bottle gourd and sweet gourd rootstock significantly influenced vegetative and reproductive growth, decreased the wilt disease, which ultimately contributed to higher numbers of fruit/plant, greater fruit size and higher yield. However, fruit quality in terms of TSS was statistically similar under different grafting and non-grafting treatments. Grafting of water melon onto bottle gourd or sweet gourd could be suggested as an alternative effective method to control plant wilting and improve growth and yield of watermelon.

Keywords: Watermelon, Rootstock, Scion, Grafting, *Fusarium* wilt, Yield

Introduction

Watermelon (*Citrullus lanatus*) is a popular fruit crop in Bangladesh especially during the–summer season. In 2019-2020, about 255,000 metric tons of watermelon was produced from 12,140 hectares of land in the country with an average yield of 21.0 t/ha (BBS, 2020). The crop, watermelon is known as highly susceptible to soil borne diseases especially *Fusarium* wilt causing drastic yield reduction worldwide (Taylor *et al*., 2008).

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The technique of grafting in watermelon (*Citrullus lanatus*) onto different root-stocks proved to be a standard production practice in Japan for the management of *Fusarium* wilt. The grafted watermelons also showed higher water use efficiency compared to non-grafted plant under water deficit condition (Rouphael *et al.*, 2008). The benefit of using grafted watermelon seedlings has been increasingly popularized to address production challenges with the ban of the broad-spectrum soil fumigant like Methyl Bromide (Davis *et al*., 2008). Thus, the technique is described as an alternative to soil fumigate Methyl Bromide in controlling *Fusarium* wilt in watermelon (Miguel *et al*., 2004, Yetisir *et al*., 2007). Generally, the outbreak of wilting occurs during the late production stage after spending all the production costs involved in melon production (Taylor *et al*., 2008) and thus the growers face serious loss. Grafting of watermelon onto other cucurbitaceous crops tends to reduce the damage caused by several soil-borne pathogens (Louws *et al*., 2010) resulted in higher yield. The technique also increased the plant tolerance to biotic stress such as viral, fungal and bacterial infection (Rivero *et al*., 2003; Edelstein *et al.*, 2004; Cohen *et al*., 2007). The use of watermelon seedlings grafted on Cucurbita and Lagenaria rootstocks have an acquired resistance to soil borne diseases and thus suggested by several other researchers to achieve higher productivity of watermelon (Miguel *et al.*, 2004; Boughalleb *et al.*, 2008). The use of resistant rootstock to susceptible scions prevents the primary sources of infection resulting in reduced disease incidence (Davis *et al*., 2008) having better growth and yield of water melon. Now a days, the use of resistant rootstock to protect the susceptible scions from pest and diseases and to ensure sustainable production are the common practices in developed countries but rare in Bangladesh. Therefore, the present experiment was undertaken to find out the suitable rootstock as a grafting agent compatible better with watermelon and to study the effect of such rootstocks on the growth, yield and sweetness of watermelon.

Materials and Methods

Study site, weather and soil characteristics

The experiment was carried out at the research field of Olriculture Division, Horticulture Research Centre, Bangladesh Agricultural Research Institute, Gazipur (latitude: 23.988^o N, longitude: 90.407^o E, at 8.4 m above sea level) during late winter to early summer season of 2020-21. The soil of experimental site belongs to the Agro-Ecological Zone (AEZ)-28 namely Madhupur Tract. The soil was clay loam in texture with an average field capacity of 29.7% and mean bulk density was 1.49 and 1.65 g/cm^3 at the surface (0-15cm) and subsurface (15-30cm) layers, respectively. The fertility status of the soil reported to be medium level in general. The experimental area is characterized by moderately monsoon rainfall, high humidity, high temperature during March to June and heavy rainfall (about 80% of the total rainfall) during June to September. In general, the site having scanty rainfall, low humidity, low temperature, short day and clear sunshine during October to March. During the cropping period of 2020-21 the experimental area was completely rain free from the months of January to March and had showers of rain in April and May but had heavy rain in June (Fig 2). The mean data of maximum and minimum temperature, pan evaporation, relative humidity, sun shine hour and monthly rainfall were recorded during the crop growing period from the Meteorological Station located about 350 m apart from the experimental field and the relevant data are presented in Figure 1 and 2.

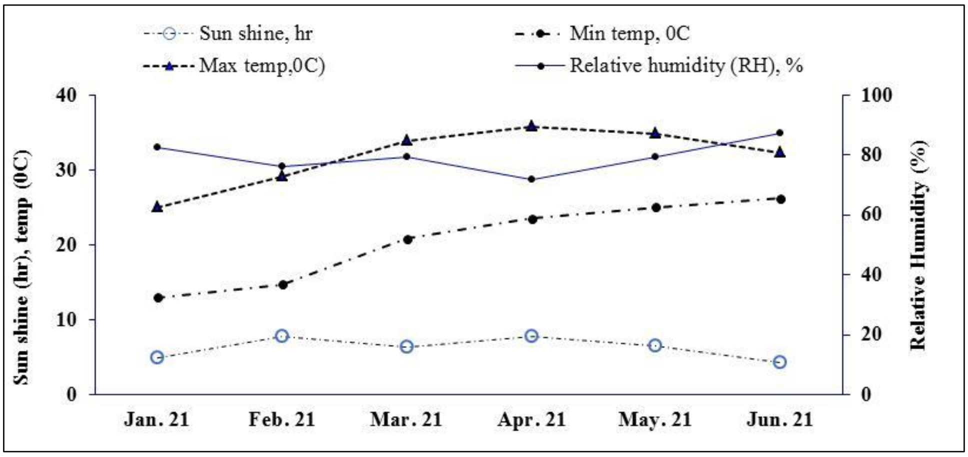

Fig. 1. Mean maximum and minimum air temperature (ºC), relative humidity (RH %) and sun shine (hrs) during the crop growing season in 2020-21.

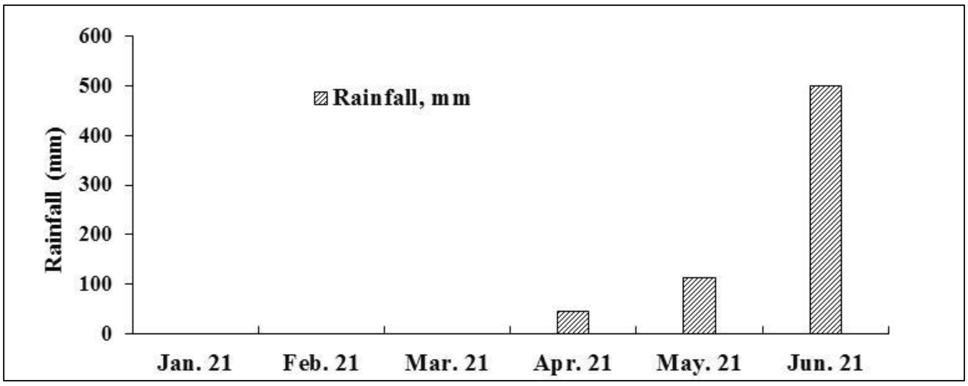

Fig. 2: Monthly rainfall during the crop growing season in 2020-21.

Raising seedlings of scion and rootstocks and grafting method

Seeds of scion (Black Jumbo F_1 watermelon) were sown in small size plastic pots filled with mixture of soil and decomposed cow-dung (l:1) during the first week of February 2021. After a couple of days, the seeds of different rootstocks were sown in the similar pot and media as mentioned above. Two seeds were sown in each pot; water was applied in each day to maintain the soil moisture level near to field capacity that was favorable for seed germination. About 90% seeds were germinated and after germination, one healthy seedling was allowed to grow in each pot. A total of one hundred seedlings of each root stock and 500 seedlings of scion were grown. Two weeks old seedlings of scion were grafted carefully onto the rootstocks on 16 February, 2021 using the hole insertion method (Fig 3). The true leaf, apical meristem and the axillary buds were removed from the topmost growing point of the root stocks. Due care was taken to remove all the apical meristem and buds to prevent future shoot growth of the root stock. A pointed probe was used to create a hole on the top of the root-stock from where the apical part was removed. At the same time, cuttings of scions were made below the cotyledon at 45° angle on two sides to form a wedge (Fig 3d). Then the cutting of scion was inserted into the hole of root stock just after removing the probe. The diameter of scion cutting was similar to diameter of vascular bundle of root stock thus no any grafting clip was required to hold the two plants together. Water was applied as mist to the grafted plants and they were placed in the healing chamber for 7 days. Non-grafted 'Black Jumbo' seedlings were grown in same place and condition as grafted plants.

Experimental design, treatments, land preparation and transplanting

The grafted plants of 'Black Jumbo' F_1 watermelon along with non-grafted plants were used as the test crops. Four different rootstocks such as Bottle gourd, Sweet gourd, Ash gourd and Snake gourd were tested. The land was cross ploughed by chisel and rotavator to ensure the well tilth soil condition. Then beds were formed with the size of 16 m x 2 m, which was considered as unit plot. The experiment was laid out in randomized complete block design replicating three times. Each replication consists of 5 beds where the root stock treatments were randomly distributed. Thirty days old seedlings were transplanted in each bed digging small pits in two rows 1.2m apart in the middle of beds. Due care was taken during transplanting so that the entire soil ball with seedling was removed from the pots without braking of soil ball. The seedlings were placed into the pit in such a depth thus the base of seedlings remained at the soil surface level.

Agronomic management and data collection

The main land was fertilized with decomposed cow-dung @ 5 t/ha prior to final land preparation. The fertilizers @ N_{80} P_{40} K_{60} S_{20} Zn_4 and $B_{1.5}$ kg/ha, were applied in the forms of urea, triple super phosphate, muriate of potash, gypsum, zinc oxide and boric acid, respectively (BARC, 2018). All fertilizers including half of urea and muriate of potash were uniformly applied in the field during final land preparation and the rest half of N and K fertilizers were applied as banding at 30 DAP (days after planting). The early crop growing period was rain free, thus, irrigation water was applied at a regular interval of 7 days starting from the day of transplanting. The crop was weeded only once at 20 DAP and thereafter weeding was not required due to rapid growth of vines covering the ground. Sex pheromone trap was used for controlling fruit fly. All the agronomic practices including irrigation, weeding and top dressing were conducted uniformly in all plots. Five sample plants were randomly selected from the middle of each plot and tagged for data collection. Length of the main stem was measured from the root collar level to the tip and branch length was measured from the base of the first node to the tip. The total number of branches, nodes formed on the main stem was counted and internode length of the middle node was measured. All the measurements were taken from the tagged plants at fruit maturity stage. The number of fruits per plant was recorded at fruit maturity stage. The watermelon fruit was considered as matured and harvestable when the 3 tendrils adjacent to the fruit had dried. The harvested fruits from the tagged plants were weighed separately at each time of harvest to determine the yield per plant and to estimate yield/ha. The recorded data on different characters was statistically analyzed using Statistix10.

Fig. 3a. Root-stock seedling

Fig.3b. Removal of apical meristem of Root-stock

Fig. 3c. Use of probe to create a hole in the top of the root-stock

Fig. 3d. Cutting of scion at 45° angle

Fig. 3e. Scion insertion into the rootstock.

Fig. 3f. Grafted seedling

Fig. 3. Different steps of grafting following hole insertion method.

Results and Discussion

Vegetative growth and plant survival

The growth and development of watermelon plants (cultivar- Black Jumbo) varied due to different rootstocks used as grafting agent (Table 1). The length of main stem of watermelon plant was the shortest (2.19 m) in non-grafted plants (BJ) and the plants grafted onto bottle gourd (BG + BJ) resulted in the longest main stem (2.95 m) at fruiting stage (70 DAP), which was significantly higher over rest of the treatments. Other watermelon plants grafted onto Sweet gourd (StG + BJ), Ash gourd (AG + BJ) and Snake gourd (SG + BJ) had statistically similar length in main stem but significantly higher than non-grafted control (BJ). Similarly, the number of lateral stems was the maximum (6.96) in plants grafted with bottle gourd (BG + BJ) which was significantly higher over rest of the treatments. Ash gourd and snake gourd grafted plants exhibited similar performance as was observed in non-grafted control (BJ). Grafting of water melon onto bottle gourd showed vigorous plant growth and development in terms of higher length of main stem and maximum number of lateral stems.

The onset of flowering was earlier in non-grafted plants both for male (46.8 DAP) and female (47.4 DAP) flower. Flowering was delayed in grafted plants compared to control and grafting with four different root stocks resulted in statistically similar numbers of days to male flowering. Female flower appeared 10-12 days earlier in non-grafted plants (BJ) and all the grafted plants resulted in statistically similar days to first female flowering (Table1). The maximum number of male flower per plant (39.2) as well as the minimum number of female flower (16.3) was recorded in non-grafted BJ plants. The lowest number of male flower (26.27/plant) was observed from SG which was identical to AG grafted plants. On the other hand, the highest number of female flower (24.52/plant) was observed from bottle gourd grafted plants (BG + BJ), which was significantly higher over rest of the treatments. The rest of the grafted plants showed identical number of female flowers which were significantly higher over non-grafted control (BJ). Thus, grafting of watermelon contributed to increase the number of female flower significantly over non-grafted plants.

The appearance of plant wilting varied significantly due to use of different rootstocks (Fig.4). At 15 DAP, 6.6 plants out of 16 died and the wilting damage continued till 45 DAP under non-grafted (BJ) water melon plants (Fig. 4). Grafting onto different rootstocks resulted in significant reduction of wilting damage in watermelon compared to control. However, periodical data exhibits the variation among the rootstock treatments in controlling wilting damage of water melon plants. The BG grafted plants showed the best result in controlling wilting followed by StG and AG as revealed from plant survival rate observed at 70 DAP (Table 1). During fruiting stage (70 DAP) the plant survival varied from 58.7% in control (BJ) to 78.7% in treatment grafted onto bottle gourd (BG + BJ). Grafting of water melon onto sweet gourd (StG + BJ) resulted in the second highest plant survival (73.5%) followed by (AG+BJ) and (StG + BJ). Similar findings were reported by Salam *et al*. (2002), Yetisir *et al*. (2007), Taylor *et al.* (2008) and Mohammed *et al.* (2012). Better plant growth in grafted watermelon compared to non-grafted plant was reported in several other studies (Yetisir *et al*., 2006; Boughalleb *et al.*, 2008; Mohammed *et al.*, 2012). More extensive root growth of bottle gourd rootstock due to augmented endogenous hormone production (Zijlstra *et al*., 1994) and enhanced uptake of nutrients (Huang *et al.*, 2016) might have contributed to more growth and elongation of main stems with higher number of lateral stems of grafted watermelon.

Yield and quality of water melon

Grafted watermelon plants produced higher numbers of female flowers and less male flowers comparing to non-grafted plants which resulted in higher sex ratio (Female/Male) and thus finally contributed to higher fruits/plant and higher yield (Table 1 and Table 2). The maximum number of fruits/plant (3.88) was recorded from bottle gourd grafted plants (BG+BJ) followed by sweet gourd grafted (StG+BJ) but these were statistically similar. Watermelon plant grafted onto ash gourd (AG+BJ) and snake gourd (SG+BJ) produced statistically similar numbers of fruit/plant which was higher than non-grafted plant (BJ) but less than (BG+BJ) and (StG+BJ) (Table 2). Similar kind of result of watermelon under grafting was reported by Salam *et al* (2002). Moreover, there were significant differences in fruit length, fruit circumference and fruit yield of watermelon among the rootstocks used as grafting agent in present study (Table 2). The grafting treatment (BG + BJ) gave the highest fruit length (39.71 cm) and maximum fruit circumference (56.25 cm) followed by (StG + BJ), (AG + BJ) and (SG + BJ). On the other hand, non-grafted plants resulted in significantly the lowest fruit length, fruit circumference and fruit yield. The maximum yield (17.85 kg plant^{-1} equivalent to 44.63 t ha^{-1}) was obtained when watermelon was grafted onto bottle gourd, which was statistically identical to sweet gourd grafted plants (StG+BJ) (Table 2). Ash gourd and snake gourd grafted plants of watermelon also produced significantly higher yields over non-grafted control. The grafted watermelon plant onto snake gourd, ash gourd, sweet gourd and bottle gourd contributed to yield increase of 28, 33, 53 and 74%, respectively, over the non-grafted plant. Plant growth and development was favoured by grafting resulted in more fruit set and bigger fruit size which finally contributed to higher yield compared to control. However, bottle gourd grafted watermelon plants were found superior to all other grafted plants followed by sweet gourd grafted watermelon plant. Rouphael *et al.* (2008) reported that both total fruit yield and marketable fruit yield were significantly higher in grafted watermelon than non-grafted plant. Luis Romero *et al.* (1997) also observed significant fruit yield improvement in response to grafted plant than respective non-grafted melon plants (*Cucumis melo* L.). Alan *et al.* (2007) reported that grafting improved growth and yield of water melon without any harmful effect on fruit quality. Present study revealed that the rootstocks like bottle gourd and sweet gourd were found to be more effective grafting agents in improving growth and yield of watermelon. The fruit quality measured as sweetness (TSS) of the fruits from grafted plant was almost similar to the fruit of non-grafted watermelon plant. In particular, the TSS of fruits of non-grafted plants was 11.2%, while for grafted plants it varied from 10.5 to 10.8% but such tiny variation was statistically non-significant. This result is in agreement with the findings of Alan *et al* (2007).

Table-1. Growth and flowering behavior of watermelon grafted onto different root stocks

Treatment	Length of main stem (m)	No. of lateral stems	Days to 1st male flower	Days to 1st female flower	No. of male flower/plant	No. of female flower/plant	Plant Survival (%) 70 DAP
Control (BJ)	2.19 c	5.51 c	46.8 b	47.4 b	39.24 a	16.28 c	58.7 b
BG + BJ	2.95 a	6.96 a	53.3 a	58.8 a	35.33 b	24.52 a	76.8 a
StG + BJ	2.55 b	6.39 b	53.5 a	61.9 a	32.13 b	20.82 b	73.5 a
AG + BJ	2.59 b	5.83 c	52.2 a	62.8 a	28.14 c	20.80 b	71.8 a
SG + BJ	2.49 b	5.69 c	54.8 a	60.2 a	26.27 c	19.62 b	74.7 a
CV%	11.38	7.39	6.59	7.15	9.26	8.53	8.76
Sig. Level	*	*	**	**	**	**	**

T_1= Control (BJ = Black Jumbo, Watermelon F_1), AG= Ash Gourd, StG = Sweet gourd, BG= Bottle gourd, SG = Snake gourd, *Significanct at P≤0.05, **Significanct at P≤0.01

Table-2. Yield and yield contributing characters of watermelon grafted with different root stocks

Treatment	Fruit length (cm)	Fruit circum-ference (cm)	Fruits /plant	Fruit wt. (kg/Fruit)	Sweetness TSS (%)	Yield (kg/ plant)	Yield (t/ha)
Control (BJ)	26.85 c	39.61 c	3.08 c	3.98	11.2	10.24 c	25.61 c
BG + BJ	39.71 a	56.25 a	3.88 a	4.03	10.8	17.85 a	44.63 a
StG + BJ	34.45 b	53.88 a	3.48 ab	4.12	10.7	15.68 ab	39.30 ab
AG + BJ	30.60 b	42.81 b	3.23 b	4.09	10.5	13.26 b	34.05 b
SG + BJ	31.08 b	45.54 b	3.27 b	4.03	10.5	13.12 b	32.80 b
CV%	3.68	4.46	8.13	6.55	7.22	12.55	12.54
Sig. Level	**	**	**	NS	NS	**	*

T_1= Control (BJ = Black Jumbo, Watermelon F_1), AG= Ash Gourd, StG = Sweet Gourd, BG= Bottle gourd, SG = Snake gourd, *Significanct at P≤0.05, **Significanct at P≤0.01.

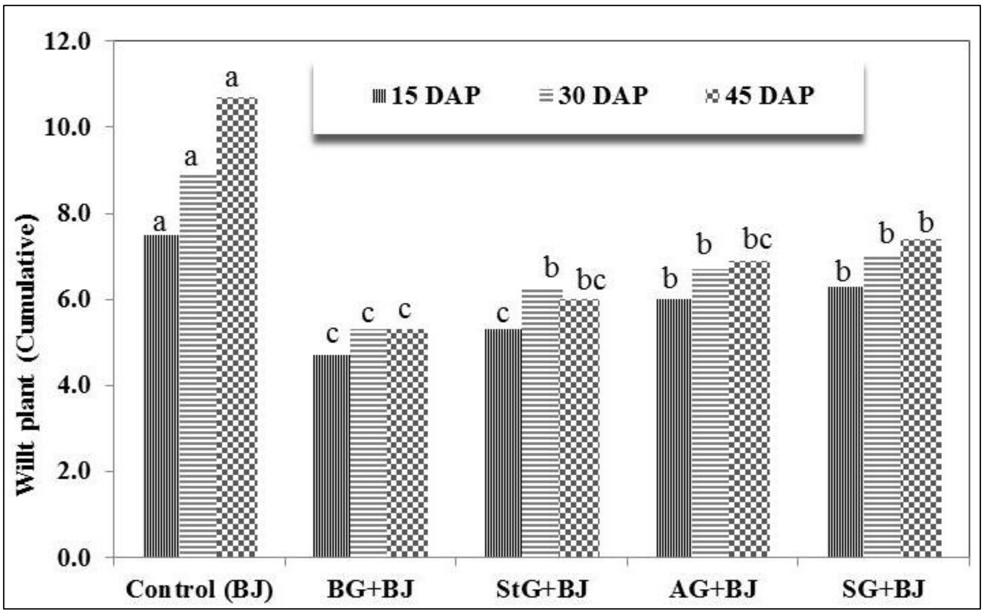

Fig. 4: Plant wilting (No.) at different stages under root stock-scion combinations

BJ = Black Jambo (F_1), BG = Bottle Gourd, StG = Sweet Gourd, AG = Ash Gourd, SG = Snake Gourd, DAP = Days after planting; Lettering on the basis of LSD value at 5% level of significance

Conclusion

Grafting is an effective tool generally used in propagation of fruits crops to improve yield and quality but the technique is rarely used for watermelon in Bangladesh. The present research demonstrated that grafting has the potentials in improving growth, development and finally fruit yield of watermelon. Among the four different root stocks, bottle gourd was found to be the superior grafting agent followed by sweet gourd. Thus, the grafting technique of watermelon onto bottle gourd or sweet gourd could be promoted at grower’s level for controlling wilting, getting better growth and higher fruit yield.

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GROWTH AND INSTABILITY ANALYSIS OF LENTIL IN BANGLADESH

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Abstract

lentil stands out as a major contributor, essential not only for human nutrition but also for sustaining both human health and agricultural soil quality. A comprehensive investigation spanning four decades (1981-2020) was conducted to analyze growth and instability of lentil in Bangladesh using secondary data. Understanding these dynamics is crucial for enhancing agricultural resilience, food security, and sustainable practices to meet increasing demands and challenges precipitated by erratic weather patterns. The study divided the period into four sub-periods (1981-1990, 1991-2000, 2001-2010 & 2011-2020) and utilized statistical tools for analysis. The application of exponential growth functions facilitated the determination of growth rates, while the Cuddy-Della Valle index assessed instability. The analysis revealed that the production and yield of lentils had increased. The change in area and yield contributed respectively 41.7% and 55.5% to the changes in the mean production of lentils at the national level. Though the lentil's yields increased significantly, the rate of growth is slow and inadequate to meet our country's demand. The analysis also revealed that the area, production, and yield of lentils were not stable during the study period.

Keywords: Lentil, growth rate, instability, Bangladesh.

Introduction

Pulses hold significant importance within the agricultural landscape of Bangladesh, serving as essential and valuable sources of nutrition for human consumption (Das et al., 2016). Pulses play a vital role in agricultural and environmental sustainability through nitrogen fixation, carbon sequestration, and organic matter enrichment (Senanayake et al., 1987; Zapata et al., 1987; Sarker and Kumar, 2011). The congenial climatic conditions prevailing across Bangladesh facilitate the cultivation of diverse pulse varieties all over the country, making pulses, often referred to for low-income populations. Among the pulses, lentils are one of the oldest and most popular food legumes in Bangladesh. Lentil is cultivated during the winter (November-March.) in Bangladesh. It occupied 40.23% of the cultivation of pulse crops, getting the first position in Bangladesh (BBS, 2019). They are frequently mixed with rice to produce more complete proteins. It contains carbohydrates, mainly starches (55-65%); proteins, including essential amino acids (24-28%), and fat (1-4 %) (Rahman et al.,2012). Different studies (Miah et al., 2021; Sarker et al., 2020; Matin et al., 2018; Tithi and Barmon,

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2018) revealed that lentil production is profitable to farmers. Hence, it is cultivated in different parts of the country covering 146.03 thousand hectares of land and producing 185.50 thousand metric tons of seed per year with a productivity of 1.27 t/ha (BBS, 2022). but imported 455298.43 tons of lentils valued at 284.952 million USD in 2021 (FAO Stat, 2021).

Instability is a very important characteristic of agriculture. Since agriculture is dependent on weather conditions, the area, production, and yield of crops are subject to significant variations over time. Instability also exists in the area, production and yield of lentils. There has been a dearth of studies dealing with the growth and instability of lentils in Bangladesh. Hence, the present study was undertaken to analyze the growth and instability in the area, production, and yield of lentil along with the contributory factors affecting the growth and instability of lentil production. The findings of this study could provide useful guidelines for the relevant researchers, policymakers, and planners of the country. The specific objectives of this study were given below.

(i) To determine the growth rates of area, production, and yield of lentils in Bangladesh;

(ii) To measure the change and instability in area, production, and yield of lentils; and

(iii) To derive some policy guidelines for the development of lentils in Bangladesh.

Materials and Methods

The study was based on secondary data collected from various published sources. Times series data on the area, production, and yield of lentils for 40 years period from 1981 to 2020 were collected from different issues of the *Yearbook of Agricultural Statistics of Bangladesh*.

Various statistical measures were used to analyze the data to examine the nature of change, instability, and degree of relationship in area, production, and yield of lentils in Bangladesh.

***Trend analysis*:** Trend analysis aims to find out the extent and causes of instability of area and production of lentils over time. This information may lead research managers as well as policymakers to prepare appropriate policy documents for the improvement of lentils for the country. A simple line graph and bar diagram were used to show the trends in area, production, and yield of lentils in Bangladesh.

***Index number*:** The relative changes in area, production, and yield of lentils that occur within a specified timeframe can be quantified using an index number. At first, the entire study period is divided into four sub-periods such as: 1981-1990, 1991-2000, 2001-2010, and 2011-2020. The reason for the division was to know the changes that occurred in the area, production, and yield of lentils in every 10 years period. The average value of area, production, and yield of first 10 years sub-period (e.g. 1981-1990) provided the base information.

Annual growth rates: Growth rates are *the percent change of a variable over time*. It is important because it can help researchers and policymakers predict future growth. For simplicity and widely used even in the recent past (Das and Mishra 2020; Chaudhary et al., 2016) the compound growth rates of area, production, yield, and price of lentils were worked out by fitting an exponential function of the following type:

$$Y = ae^{bt} \quad \text{or } LnY = Lna + bt \text{ -- } (1)$$

Where, Y is the area/production/yield of lentil, 't' is the time in a year, and 'a' is the constant, e^b -1 be the compound growth rate which is expressed in percentage.

The component analysis model has been used to measure the relative contributions of area and yield toward the overall output change with regard to individual crops. The growth performance of the crops has been studied using this model by numerous researchers in the literature (Gupta and Saraswat, 1997; Singh and Ranjan, 1998; Siju and Kombairaju, 2001; Kakali and Basu, 2006).

$$\Delta P = A\circ\Delta Y + Y\circ\Delta A + \Delta A\,\Delta Y \text{------------------------ } (2)$$

Change in production = Yield effect + Area effect + Interaction effect.

Thus, the total change in production is attributed due to area and yield that can be decomposed into three effects viz; yield, area and interaction effects.

Instability index: Instability means the quality or state of being unstable or lack of stability. Agricultural instability can be measured by different methods, such as the coefficient of variation (CV), dispersion, Cuddy Della Valle Index (CDI), Coppock Instability index, etc. The present study applied the Cuddy and Valle (1978) Index for examining the nature and degree of instability in the area, production, and yield of lentils in Bangladesh. The use of CV as a measure to show the instability in any time series data has some limitations. It does not explain properly the trend component inherent in the time series data. If the time series data exhibit any trend, the variation measured by CV can be overestimated, i.e. the region which has growing production at a constant rate will score high in instability of production if the CV is applied for measuring instability. As against that, CDI first attempts to de-trend the CV by using the coefficient of determination (R^2). Thus it is a better measure to capture instability in agricultural production. A low value of this index indicates low instability in farm production and vice-versa. The estimable form of the equation is as follows:

$$CV_t = (CV)\times\sqrt{1-R^2} \text{ -- } (2)$$

Where CV_t is the coefficient of variation around the trend; CV is the coefficient of variation around the mean in percent; and R^2 is the coefficient of determination from time trend regression adjusted by the number of degrees of freedom.

$$CV = \frac{Standard\ deviation}{Mean}\times 100$$

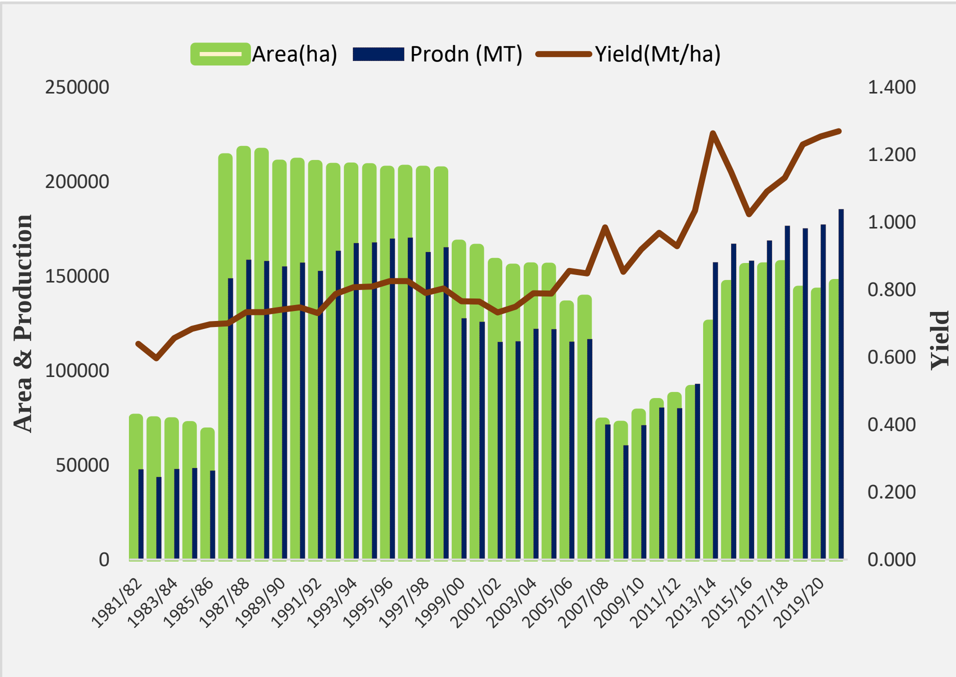
$$R^2 = 1 - \frac{Unexplained\ variation}{Total\ variation}$$

Results and Discussion

Trends of Area and Production of Lentil in Bangladesh

The area and production of lentils were found to fluctuate in nature, but the yield registered an increasing trend over the years. Figure 1 shows that the area and production of lentils for the period from 1981/82 to 1985/86 were very low compared to the succeeding years. Despite various positive sides, the crop faced competition in the recent past from cereals, particularly wheat and *Boro* rice, due to the expansion of irrigation facilities, and the availability of high-yielding varieties (Sarker et al., 1989). Bangladesh Agricultural Research Institute (BARI) and Bangladesh Institute of Nuclear Agricultural (BINA) disseminated some improved lentil varieties and popularized them through different projects. The inclusion of these improved varieties in the cropping patterns replacing local varieties might be the cause of the increase in area, production, and yield of lentils. Therefore, both area and production of lentils were very high for the period of 1986/87-1998/99. Again in the period 1999/00-2008/09, the overall area and production of lentils in the country were found decreasing trend which might be due to susceptible crops and less remunerative in production. After that period the area, production, and yield of lentils further increased steadily from 2009/10 to 2019/20.

Fig. 1. Trend of area, production, and yield of lentil during 1981/82-1999/20



Source: Using data from various issues of BBS

The overall indices reveal a substantial increase in both the area under cultivation and the production of lentils, indicating notable growth from the base period of 1981-1990 to the subsequent decade of 1991-2000 (Table 1). But the overall indices of area and production show a decreasing trend from 2001 to 2010. On the other hand, the productivity indices revealed an increasing trend during the period from 1981-1990 to 2011-2020. Despite the decrease in area, the per hectare yield of lentils has rise up in that period which was mainly due to the adoption of improved variety and management technologies.

Table 1. Index of area, production, and yield of lentil

Time Period	Area (%)	Production (%)	Yield (%)
1981-1990	100 (142345)	100 (101307)	100 (0.69)
1991-2000	139.6	155.4	114.1
2001-2010	84.1	97.8	122.5
2011-2020	94.3	152.0	164.1

Note: Figures within parentheses indicate 10 (ten) year average value of the base period. .

Source: Various issues of BBS

Annual Growth of Lentil Production The analysis of the overall annual growth rates over a period of 40 years (1981-2020) highlights a positive and significant growth in the production and yield of lentils. However, in contrast, the growth rate of cultivated area exhibited a non-significant negative trend during the same period (Table 2). The growth rates of different periods show that some growth rates registered in area and production were found positive and significant from 1981-1990 and 2011-2020. The period of 1991-2000 and 2001-2010 witnessed markedly adverse trends in both area and production growth rates, with values of (-2.27 & -2.2) and (-10.13 & -7.113), respectively. This decline can be attributed to a combination of factors, including delayed sowing, yield instability stemming from biotic and abiotic stresses, the heightened vulnerability of local cultivars to significant diseases, and the inherent low yield potential of local cultivars (Sarker et al., 2004). However, the growth rates of yield were positive and highly significant for all periods except the period 1991-2000. The highly significant growth rates of yield were mainly due to the adoption of improved lentil variety and technology. This indicates that more adoption of the modern varieties of lentil is needed in the farmers' fields.

Table 2. Annual growth rates of area, production and yield of lentil, 1981-2020

Time period	Area	Production	Yield
1981-1990	16.10***	18.34***	2.24***
1991-2000	-2.27***	-2.20*	0.08
2001-2010	-10.13***	-7.11***	3.02***
2011-2020	5.32**	7.77***	2.45**
1981-2020	-0.21	1.36**	1.57***

Note: ‘***’ ‘**’ and ‘*’ represent 1%, 5% and 10% level of significant

Sources of Growth of Lentil Production

Change in the mean area appeared to be the largest source of change in the mean production of lentil in all the periods. At the national level, changes in the mean area and yield were the main two sources of changes in lentil production in Bangladesh. The change in area and yield contributed respectively 41.7% and 55.5% to the changes in the mean production of lentils at the national level. This means that the positive change in production has been attributed to the positive change in area and yield (Table 3).

Table 3: Growth decomposition in the production of Lentil during 1981-2020

Time period	Effect (%)				
	Area (A)	Yield (Y)	Interaction	Residual	Total
	ΔA*Y	A*ΔY	ΔA*ΔY	ΔCOV(A,Y)	ΔQ
1981-1990	86.76	13.62	0.38	-0.76	100
1991-2000	111.60	-16.09	-4.50	8.99	100
2001-2010	167.15	-47.13	20.03	-40.05	100
2011-2020	69.90	33.38	3.28	-6.56	100
1981-2020	41.70	55.50	-2.78	5.58	100

Source: Author's calculation using BBS data of different years

Instability of Lentil Cultivation

The estimates of instability in the area, production, and yield of lentil are presented in Table 4. The instabilities of the lentil area (3.43%) and production (3.62%) at the national level were not so high, but the instability of production was a little bit higher than the area instability. On the other side, the instability related to productivity was about -48.68% during 1981-2020 meaning that lentil productivity was almost stable over the stipulated period.

Table 4: Instability indices for area, production and yield of lentil, 1981-2020

Time period	Instability (%)		
	Area (ha)	Production (t)	Yield (t/ha)
1981-1990	2.59	2.67	-7.79
1991-2000	0.52	0.79	-16.31
2001-2010	1.52	1.47	-26.82
2011-2020	1.25	1.48	61.44
1981-2020	3.43	3.62	-48.68

Source: Author's calculation using BBS data

Conclusions

During the study period (1981–2020), the trend analysis of lentils revealed a notable fluctuation in both their cultivation area and production levels. However, a more detailed examination of the last decade provides a distinct perspective, revealing a constant upward trajectory in both the cultivation area and production of lentil. This recent trend signifies a positive shift in lentil cultivation practices and management. This increase in yield can be attributed to the widespread adoption of improved varieties and management technologies. These advancements have likely played a crucial role in boosting the overall productivity of lentil cultivation and contributing to its upward trend in both area and production.

Sustainable agricultural performance hinges on the combination of a high growth rate and low production instability, a crucial consideration with profound implications for policymakers. In the context of this study, a comprehensive exploration of lentil production in Bangladesh has been undertaken, revealing noteworthy insights. The examination of lentil production growth underscores a positive and significant increase in both yield and production for lentil. However, it is important to note that this growth is modest in nature, indicating room for further advancement. Conversely, a contrasting trend emerges in the case of lentil cultivation area, with observed negative growth rates. This decline suggests a potential concern, suggesting the possibility of shrinking cultivable land or waning farmer interest in lentil cultivation. The inherent susceptibility of agricultural production to natural calamities is an enduring reality in Bangladesh, introducing a layer of instability. When scrutinizing the scope of instability in lentil cultivation, the national level showcases a comparatively lower degree of instability. This underlines the resilience of lentil cultivation in the face of natural fluctuations, offering a glimmer of stability within the broader agricultural landscape.

Recommendations

Based on the findings of this study, a number of recommendations can be made to promote sustainable growth in lentil production in Bangladesh. Firstly, agricultural scientists should develop more climate-friendly and high-yielding varieties of lentil to meet the increasing demand. Secondly, existing BARI-developed improved lentil varieties and technologies should be disseminated among farmers through pilot projects. Thirdly, research and policy support is needed to increase the acreage and yield of lentil which will help increase the per capita availability of lentil, reduce import dependence, and to some extent stabilize lentil prices. Finally, collaboration between government agencies, NGOs, and private sector stakeholders should be given priority for increasing the growth and stability of lentil in Bangladesh.

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ASSESSMENT OF NUTRITIONAL COMPOSITION, BIOACTIVE COMPOUNDS AND ANTIOXIDANT ACTIVITY OF CHIA SEEDS GROWN IN BANGLADESH

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Abstract

The present study was conducted to determine the proximate, fatty acids, minerals and antioxidants content in chia seeds. The results showed that fat ranged from 28.85-29.53%, protein 18.63-18.71%, moisture 5.27-5.28 %, ash 4.45-4.47 %, total carbohydrate 25.21-27.86 %, fiber 17.35-19.12% and energy obtained 428.11-436.70 kcal/100g. Fatty acid profile indicated the presence of linolenic (56.76-57.65 %), linoleic (20.49-21.47 %), palmitic (9.38-11.34 %) and oleic (7.47-7.99 %) were abundant, while less amount of saturated fatty acids myristic (0.09-0.15 %), lauric (0.11-0.33 %) and stearic (2.52-3.13 %) were found all the tested samples. Mineral content in all the tested samples showed that high amount of calcium (578.62-589.71 mg/100g), potassium (518.95-535.42 mg/100g), phosphorous (668.62-697.61 mg/100g), magnesium (334.24-344.51 mg/100g), iron (7.13-7.56 mg/100g), zinc (4.23-4.57), manganese (2.19-2.95 mg/100g), and copper (1.59 -1.83 mg/100g) were found. The total phenolic (1.71-1.92 mg GAE/g), total flavonoids (1.07-1.16 mg QE/g), DPPH (2,2-diphenyl-1-picrylhydrazyl) (2.51-2.81 mg GAE/g), and Ferric-reducing Antioxidant Power, FRAP (1.86-2.22 mg TE/g) were found in the seeds.

Keywords: Antioxidant capacity; Bioactive compound; Chia seeds; Minerals; Fatty acid profile; Phenolic compound; Proximate composition.

Introduction

Chia (*Salvia hispanica* L.) is a tropical and subtropical climates herbaceous plant that belongs to the family *Lamiceae,* originated from Mexico and Guatemala. Chia is oilseed crop oval in shape, smooth and shiny with black, brown, gray, black-spotted or white in colour. Chia seed contains high amount of omega-3 fatty acids, high quality protein, high amount of dietary fibres, minerals, vitamins and also contains wide range of polyphenolic antioxidants which protect the chia seeds from chemical and microbial oxidation (Cahill, 2003). The chia seed also contains mucilage inside the epidermal cells of mature chia seeds. On the other hand, chia seeds play an important role as a functional food and nutritional supplement.

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However, its nutritional composition and concentration of bioactive compounds such as high level of poly unsaturated fatty acid or high amount of the essential fatty acid alpha-linolenic acid (ALA $C_{18:3}$ (n-3), which is required for maintaining certain physiological functions (Meester *et al.* 2008). Moreover, another nutrient protein of chia seeds are promising food additives which can help in improving food quality and extend the shelf-life of food products (Valdivia-Lopez and Tecante, 2015). The dietary fibers present in chia seeds and mainly in whole grains is an important bioactive component because it has potential health benefits. Chia seeds are rich in vitamins such as niacin (8.83 mg/100 g), thiamine (0.62mg/100 g), riboflavin (0.17 mg/100 g) and vitamin C (Munoz *et al*. 2012). Chia seeds are also high in concentrations of phosphorus, iron, calcium, magnesium, zinc and potassium. Chia seeds and its oil is widely used in bread such as pasta, breakfast cereals, fruit juices, yoghurt, biscuits, snacks, cake, and cereal bars in the food industry of various countries around the world. According to recent research chia seeds contain high antioxidants (phenolic compounds) and these compounds may reduce the growth of cancer cells and improve the health (Valdivia and Tecante, 2015). Chia is a new crop in Bangladesh. However, so far know no study their nutritional and chemical composition and bioactive compound of chia seeds has yet been done. This study was therefore aimed at determining the proximal chemical composition, fatty acids, nutritional quality, antioxidant capacity and functional properties of chia seeds.

Materials and Methods

This study was implemented in the Central laboratory, Bangladesh Agricultural Research Institute (BARI), Gazipur. Chia seed line-1 was collected from Agronomy Division, BARI, Gazipur and another Chia line-2 was purchased from one commercial retail shop at Gazipur city, Bangladesh in October 2022. The seeds of chia was taken and ground to powdered form by using a grinder.

Determination of proximate composition of chia seed:

The proximate composition of chia seeds were determined according to the Association of Official Analytical Chemists (AOAC, 1995) methodology: crude oil/fat (AOAC, 920.39c), crude protein (AOAC, 928.08), moisture (AOAC, 950.46), carbohydrate (AOAC, 935.08), fibre (AOAC, 921.13), and ashes (AOAC, 923.03).

Energy Value: The energy values (Kcal/100 g samples) of chia seeds were estimated using the factors for protein (4 Kcal/g), fat (9 Kcal/g) and carbohydrate (4 Kcal/g). The equation is

Food energy = (% Crude protein × 4) + (% Fat content × 9) + (% Carbohydrate × 4).

Mineral determination:

Mineral contents of chia seeds were determined by spectrophotometer and atomic absorption spectrometer. The each sample was dissolved in 100 ml 1N nitric acid, the solution mixture filtered through Whatman paper no. 42 and stored in 150 ml plastic bottles with tight lids. All minerals determination was done in triplicate. Calcium, magnesium, potassium, iron, zinc, manganese and copper were analyzed by atomic absorption spectrophotometer (AAS) with appropriate standards (Garcia, R. and P. Baez, 2012). Total phosphorus was determined by a pH adjustment method (ammonium molybdate/ ammonium vanadate mixed reagent) (Okalebo *et al.* 1993).

Chromatographic determination of fatty acid composition by gas chromatography (GC):

Fatty acid composition in chia seeds was determined by colorometric method. One gram chia seeds were crashed and were taken in a 15 ml tube. Then 5 ml of ethylate reagent (sodium hydroxide, ethanol and petroleum ether mixed) was added and vortex the sample tube 1 min. The plastic tubes were put in 10-12 h overnight at room temperature. Then 5 ml salt solution (sodium hydrogen sulphate and sodium chloride mixed) was added in each tube and shaken after 30 second. After then the ether content was evaporated and remaining oily surface was injected into gas chromatography for fatty acid profile. Chromatographic analysis of the fatty acid methyl esters (FAMEs) from the seeds extracted from the samples was conducted using a gas chromatograph (Thermo Scientific Company) apparatus equipped with an auto sampler, flame–ionization detector (FID) and supelco wax column (30 m x 0.25 μm film coating). The samples (1 μl) were injected with helium (1.2 mL/min) as a carrier gas onto the column, which was programmed for operating conditions such as column oven temperature 160°C @ with subsequent increase of 3°C/min until 180°C. The column oven temperature was increased from 180°C to 220°C @ 1°C/min and was held for 7.5 min at 220°C. Split ratio was 50% with injector 240°C and detector 250°C temperatures. The peak areas and total fatty acids composition were calculated for each sample by retention time using Shimadzu Chemical Station software. The standards of fatty acids methyl esters purchased from Sigma-Aldrich were also run under the same conditions for comparison with experimental samples. FAMEs were identified by comparing their retention times with a standard retention time supelco 37 component FAME Mix (Sigma-Aldrich, MO, USA). For calculation, the fatty acids were normalized to 100%, considering the composition (moles %) from fatty acid composition data (area %) and was expressed as percentage of total fatty acid methyl ester in the oil. Each of the experiment was repeated three times.

Determination of total DPPH-RSA (2, 2-diphenyl-1-picrylhydrazyl) content:

The total DPPH content was determined by a colorimetric method as in (Ao *et al.* 2008) with some modifications. The defatted 0.5 g of chia flour was soaked in 10 ml of 80% methanol and shaken in room temperature. The extracts were centrifuged for 15 min and supernatant were collected. The extract was mixed with methanol and DPPH (100 µmol/L in ethanol) and shaken vigorously. The mixture was incubated for 30 min at room temperature in the dark. The absorbance was read at 515 nm in a spectrophotometer (Shimadzu UV-2401PC, Japan). The total DPPH content are expressed as mg gallic acid equivalent (GAE)/g DW.

Determination of Total Phenolic content (TPC):

The total phenolic content was measured the Folin-Ciocalteau method (Velioglu, *et al.* 1998) with some modifications. The defatted 0.5 g of chia flour was soaked in 10 mL of 80% methanol and shaken in room temperature. The extracts were centrifuged for 15 min and supernatant/methanol extracts were collected. The methanol extract (50 µL) was mixed with Folin-Ciocalteu reagent, distilled water and incubated at room temperature for 5 min. Then, 7.5% of sodium carbonate solution was added to the mixture and incubated for 20 min at room temperature. Absorbance was read at 750 nm in a spectrophotometer. The total phenolic content is expressed as mg gallic acid equivalent (GAE)/g DW.

Measurement of total flavonoids content

The total flavonoid content was determined by a colorimetric method as in Barreira *et al.* (2010) with some modifications. The defatted 0.5 g of chia flour was soaked in 10 mL of 80% methanol and was shacked in room temperature. The extracts were centrifuged for 15 min and supernatant/methanol extracts were collected. The methanol extract (400 µL) was mixed with 1600 µL distilled water and 150 µL $NaNO_2$ (5%) solution and the mixture was incubated for 5 min. At the end of the reaction, 75 µL of $AlCl_3.6H_2O$ (10%) solution were added, and the mixture was allowed to stand for 5 min. Finally, 0.5 mL NaOH (1 M) was added to the reaction mixture and absorbance was read at 510 nm in a spectrophotometer. The total flavonoid content is expressed as mg catechin equivalent (CE)/g DW.

Determination of Ferric-reducing Antioxidant Power (FRAP):

In order to determine the FRAP of the extracts, the method described by Benzie and Strain (1996) was used. The FRAP reagent (Fe (III) solution-TPTZ) was obtained from the combination of 25 mL of 0.3 M acetate buffer, 2.5 ml of a solution TPTZ (tri-piridiltriazina) 10 mM and 2.5 ml of a 20 mM aqueous solution of ferric chloride. In a test tube was added 200 µL of pre-diluted sample and 1.8 mL of the FRAP reagent and kept in a water bath at 37^0C for 30 min. Afterwards, the absorbance of the colored complex formed with Fe^{2+} and TPTZ was measured at 593 nm in a spectrophotometer. FRAP reagent was used as a blank. The compound trolox was used as standard for the calibration curve and expressed in milimol the trolox equivalents per kilogram of dry sample (mmol/kg ET dry sample).

Results and Discussions

Proximate composition of chia genotypes

The average oil/fat, protein, fiber, moisture, ash, carbohydrate and energy in Chia Line-1 and Chia Line-2 ranged from 28.85-29.53%, 18.63-18.71%, 17.35-19.12%, 5.27-5.28%, 4.45-4.47%, 25.21-27.86% and 428.11-436.70 kcal/100g, respectively (Table 1). As shown in the Table 1, proximate composition of chia seeds such as fat, fibers, ash, carbohydrate data, there is no difference between Chia Line-1 and Chia Line-2. The crude fat represents the major component in the chia seed, followed by crude protein, fiber and total carbohydrate. The moisture, ash and protein contents were close to 7.86%, 3.63% and 21.52%, respectively as reported by Sargi *et al.* (2013). Chia seed is a good source of fibers. Ullah *et al.* (2016) showed that defatted chia seed contains from 34-40% of dietary fiber. This amount is equal to 100% of the daily recommendations for adults to decrease the risk of coronary heart disease, diabetics' mellitus type 2 and several types of cancer (Amato *et al.* 2015). Carbohydrate that was obtained by a difference ranged from 25.21-25.86% and corroborates the findings as reported by Marineli *et al.* (2014) and Owaga *et al.* (2018). However, many factors that can affect its nutritional composition and concentration of bioactive compounds, such as climatic conditions, soil type, geographical area, agricultural practices and to some extent the extraction methods (Suri *et al.* 2016). Indeed, results on proximate composition support other findings that chia seeds are rich in proteins, fats and fiber particularly insoluble fiber Sargi *et al.* (2013) and Ullah *et al.* (2016).

Table 1. Proximate composition of chia seeds

Nutrients	% of seeds	
	Chia Line-1	Chia Line-2
Crude Oil/Fat	29.53± 0.93	28.85 ± 0.59
Crude Protein	18.71 ± 1.08	18.63 ± 0.71
Crude Fibre	17.35 ± 1.59	19.12 ± 1.23
Carbohydrate	25.86 ± 1.25	25.21 ± 1.02
Ash	4.47 ± 0.42	4.45 ± 0.59
Moisture	5.27 ± 0.65	5.28 ± 0.46
Energy	436.70 ± 3.99 Kcal/100g	428.11 ± 3.08 Kcal/100g

Values are means SD ± (standard deviation) of triplicate determinations (n = 3).

Fatty Acid Profile of chia seeds

Results on fatty acid composition indicated the presence of lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic (LA), and linolenic (ALA) in all the tested samples (Table 2). However, linolenic (56.76-57.65 %), linoleic (20.49-21.47 %), palmitic (9.38-11.34 %) and oleic (7.47-7.99 %) were abundant, while low content of saturated fatty acids myristic (0.09-0.15 %), lauric (0.11-0.33 %) and stearic (2.52-3.13 %) were found in Chia Line-1 and Chia Line-2. As shown in the Table 2, fatty acid composition of chia seeds, there is no difference between Chia Line-1 and Chia Line-2. The amount of ALA (ω-3, 56.76-57.65%) was similar to that reported by Ding *et al.* (2018). Furthermore, the amount of LA (ω-6) and ALA (ω-3) in chia seeds was close to 17-26% and 50-57% respectively (Fig. 1 and Fig. 2) as cited by Saphier *et al.* (2017). Chia seeds had the highest amount of ALA 57.65 % but this was lower (56.76 %) reported by Owaga *et al.* (2018). High levels of ALA implies the beneficial effects of chia seeds have on human health, for example, the anti-hyperglycemia, anti-hyperlipidemia, anti-hypercholesterolemia, anti-inflmatory and anti-cancer properties (de Ramzi and Sharada, 2014). On the other hand, the average ratios of ω-6 PUFA to ω-3 PUFA were 1:3 (Fig. 1 and Fig. 2). This observation is an important hint towards lowering the risk of coronary heart disease (CHD) as cited by Mozaffarian *et al.* (2011).

Table 2. Fatty acid composition of chia seeds

Fatty acids	Chia Line-1	Chia Line-2
Unsaturated fatty acids	**(%)**	
Lenolenic/ω-3 ($C_{18:2}$)	56.76 ± 0.48	57.65 ± 0.32
Lenoleic/ω-6 ($C_{18:2}$)	20.49 ± 0.40	21.47 ± 0.08
Oleic/ω-9 ($C_{18:1}$)	7.84 ± 0.56	7.99 ± 0.48
Palmitolic ($C_{16:1}$)	0.16 ± 0.01	0.20 ± 0.04
TSFA	14.31 ± 0.56	13.94 ± 0.49
MUFA	8.56 ± 0.57	8.29 ± 0.66
PUFA	77.25 ± 0.88	79.05 ± 0.88
ω-3/ ω-6	2.77 ±1.19	2.68 ±1.66
ω-6 /ω-3	0.36 ± 0.84	0.36 ± 0.33
Saturated fatty acids		
Lauric ($C_{12:0}$)	0.32 ± 0.11	0.33 ± 0.02
Myristic ($C_{14:0}$)	0.15 ± 0. 02	0.11 ± 0.02
Palmitic ($C_{16:0}$)	11.34 ± 0.36	10.76 ± 0.24
Stearic ($C_{18:0}$)	2.52 ± 0.11	2.74 ± 0.22

** TSFA = Total saturated fatty acid; TUFA = Total unsaturated fatty acid; PUFA = Poly unsaturated fatty acid; MUFA = Mono unsaturated fatty acid. Values are means SD ± (standard deviation) of triplicate determinations.

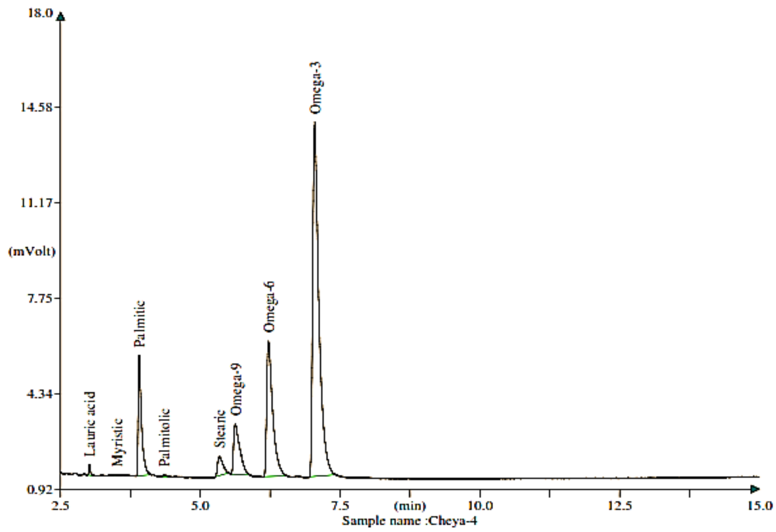

Fig. 1. Chromatogram of fatty acid composition of Chia line-1 seeds

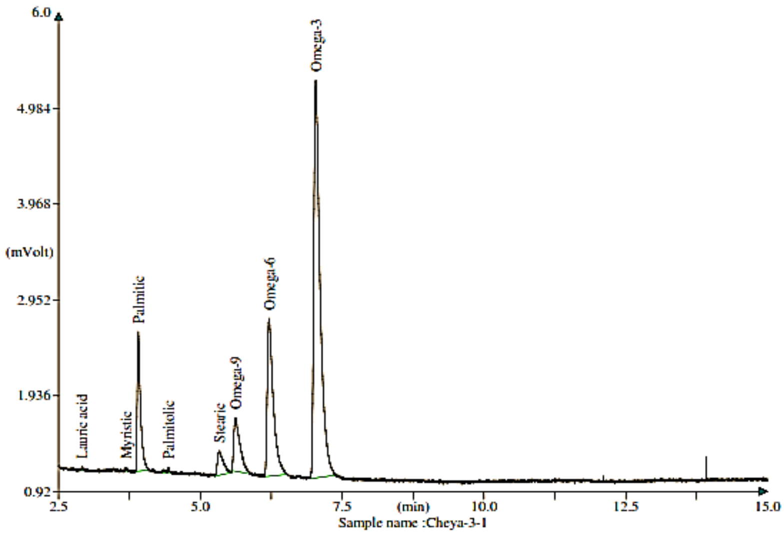

Fig. 2. Chromatogram of fatty acid composition of Chia line-2 seeds

Mineral Content of Chia seeds

Minerals found in chia seeds were high amount of calcium (578.62-589.71 mg/100g), potassium (518.95-535.42 mg/100g), phosphorous (668.62-697.61 mg/100g) and magnesium (334.24-344.51 mg/100g) in Chia Line-1 and Chia

Line-2 (Table 3). Furthermore, the amount of iron (7.13-7.56 mg/100g), zinc (4.23-4.57), manganese (2.19-2.95 mg/100g), and copper (1.59 -1.83 mg/100g) were also considerable (Table 3). The USDA, (2016) also reported that chia seed rich in calcium concentration (631 mg), phosphorus (860 mg), potassium (407 mg), magnesium (335 mg), iron (7.72 mg) and zinc (4.58 mg) per 100g chia. As shown in the Table 3, minerals composition of chia seeds, there is no difference between Chia Line-1 and Chia Line-2. The amount of phosphorus, calcium and potassium in chia seeds is 6-9 times higher than that contained in wheat, rice, oats and corn, while iron content is higher than that of spinach and lentils (Ullah *et al.* 2016). Kon *et al.* (2013) reported that mineral content of 100 g chia seed as calcium (536 mg), magnesium (350 mg), potassium (564 mg), phosphorus (751 mg), iron (6.3 mg), copper (1.4 mg), and zinc (4.4 mg). Mineral deficiency decreases enzyme activity affecting the human body and resulting in functional disorders of individual organs and the immune system thereby leading to frequent depressions. Munoz *et al.* (2012) analyzed chia seeds and contained six times more calcium, eleven times more phosphorus and four times more potassium than 100 ml of milk. Moreover, Beltran-Orozco and Romero, (2003) reported that chia contained 13 to 15 times more calcium, 6 to 9 times more phosphorus and 1.6 to 9 times more potassium than 100g of wheat, rice, oats and corn. The iron content of chia was also quite high compared to most other seeds. It contains six times more iron than spinach, 1.8 times more than lentils and 2.4 times more than liver.

Table 3. Mineral composition of chia seeds

Minerals	Amount in (mg/100g)	
	Chia Line-1	Chia Line-2
Macro-elements		
Calcium	589.71 ± 2.64	578.62 ± 2.08
Magnesium	344.51 ±2.16	334.24 ± 1.68
Phosphorus	697.61 ±2.02	668.62 ± 1.91
Potassium	535.42 ± 1.61	518.95 ± 2.15
Micro-elements		
Iron	7.56 ± 0.39	7.13 ± 0.19
Copper	1.59 ± 0.05	1.83 ± 0.13
Zinc	4.57 ± 0.51	4.23 ± 0.23
Manganese	2.19 ± 0.33	2.95 ± 0.71

Values are means SD ± (standard deviation) of triplicate determinations.

Phenolic and flavonoid content

Total phenolic and flavonoid contents of the defatted ground chia seeds are given in Table 4. The total phenolic and flavonoid contents of Chia Line-1and Chia Line-2 seeds were found to be 1.92 ± 0.11, 1.71 ± 0.15 mg GAE/g mg GAE/g and 1.07

± 0.13, 1.16 ± 0.12 mg GAE/g, respectively. Phenolic and flavonoid content of chia seeds, there is no difference between Chia Line-1 and Chia Line-2. It was reported that the total phenolic content of Chilean chia seeds to be 0.94 ± 0.06 mg GAE/g sample (Marineli *et al.* 2014), and Mexican chia seeds to be 1.64 ± 0.08 mg GAE/g sample (Martinez-Cruz and Paredes-Lopez, 2014). The differences in total phenolic content between this study and the others could be due to two factors; i) the samples used in our study and in the others are harvested from different locations, and it has been proven that growing locations significantly impact the composition of chia seeds (Ayerza, 2013); ii) the methods used for the extraction of phenolic compounds in different studies vary, and different extraction methods have been shown to dramatically influence the total phenolic contents of chia seeds measured (Scapin *et al.* 2016). Beside, Lin and Tang (2007) reported that the content of total flavonoids was obtained 0.75 mg EQ/g for green peppers, 0.41 mg EQ/g for yellow pepper.

Table 4. Extractable phenolic, flavonoids and antioxidant capacity of chia seeds

Elements	Amount (mg g^{-1})	
	Chia Line-1	Chia Line-2
Phenolic compounds		
Total phenolic (mg GAE/g)	1.92 ± 0.11	1.71 ± 0.15
Total flavonoids (mg EQ/g)	1.07 ± 0.13	1.16 ± 0.12
Antioxidant capacity		
DPPH (mg GAE/g)	2.81 ± 0.20	2.51 ± 0.46
FRAP (mg TE/g)	2.22 ± 0.39	1.86 ± 0.16

Values are means SD ± (standard deviation) of triplicate determinations.

Antioxidant activity

Total antioxidant activities of chia seeds were determined by testing the DPPH free radical scavenging activity of the defatted extracts. DPPH radical scavenging activities and antioxidant power of iron reduction, FRAP of chia seed were found 2.81 ± 0.20, 2.51 ± 0.462.51 ± 0.46 mg GAE/g and 2.22 ± 0.39, 1.86 ± 0.16 mg TE/g, respectively (Table 4). The results are lower than those reported for defatted Chilean seed flour (4.26±9.7 mg TE/g) (Marineli *et al.* 2014), Argentinian chia fibrous fraction (4.46±19.8 mg TE/g) (Capitani *et al.* 2012), and Mexican chia fibrous fraction (4.88 TE/g) (Vazquez-Ovando *et al.* 2009). The study showed that chia seed has higher antioxidant activity than other highly edible cereals such as sorghum, millet, barley, rye, and wheat, which have 1.95 ± 8.82, 2.38 ± 0.67, 2.10 ± 0.83, 1.21 ± 0.50, and 4.33 ± 0.17 mgTE/g of sample, respectively (Ragaee *et al.* 2006). Jeong *et al.* (2004) reported that increased chia seed consumption the antioxidants influence oxidation of free radicals there by providing better health and preservation of food lipid systems in the body.

Conclusion

Chia is now a high value crop for its unique nutritional and potential medicinal properties. Chia seeds have high contents of dietary fibre and proteins, high contents of polyunsaturated fatty acids, mainly alfa-linolenic acid ($C_{18:2}$), belonging to the group of omega-3 fatty acids. These seeds are also a good source of many minerals and vitamins, as well as bioactive compounds of high antioxidant activity. The research confirmed the cardioprotective, antihypertensive, antidiabetic, antiatherosclerotic, nephroprotective, anti-inflammatory, as well as antioxidant properties and its valuable chemical composition and biological activity with their availability. The results from this study support other findings that Chia Line-1 is the best-known plant source with the highest content of quality fats, fibers, proteins, alfa-linolenic acid, ω-3 and ω-6, antioxidant capacity and functional properties of chia. Finally, chia seeds are the best source of nutrients and are suggested as super foods or novel foods (EU food safety authority) and can be widely used in the food industry.

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Bangladesh J. Agril. Res. 48(2): 271-274, June 2023 **Short Communication**

INFESTATION AND DAMAGE SEVERITY OF COCONUT MITE, *ACERIA GUERRERONIS* KEIFER AT DIFFERENT LOCATIONS OF BARISHAL DISTRICT IN BANGLADESH

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Keywords: Infestation, Pest, Status, Coconuts, Coconut mite, Grade.

Coconut (*Cocos nucifera* Beccari) is the main source of cash income for farmers in the coastal belt/southern region in Bangladesh. The coconut palm and its fruit are regarded as the most important plant to human being around the world. Approximately 100 million coconuts are produced in the country in an area about 35 thousand hectares. South and southwestern parts of the country contributes 80% of total coconut production (BBS, 2009). The yield of coconut is about 21 nuts per plant per year which is very low, compared to those of other coconut-growing countries (Keifer, 1965). This poor yield is due to lack of high-yielding varieties, inadequate nourishment, insect pests and diseases as well as management practices. At least 750 insect pests of coconut have been recorded from around the world (Lever 1969). These pests attack the leaves, stems, flowers, nuts and roots of the coconut plant. In the Caribbean over 26 major pests have been recorded in coconut orchard of which two species are mite, 15 species are insect and three species are rodent (Krantz *et. al.* 1978). Recently, coconut palms are found to suffer from the attack of mite in Bangladesh. Coconut eriophyid mite is microscopic, slender, vermiform organism and creamy white in color. Although the mites are small measuring 200-250 Â (microns) in length and 36-52 Â (microns) in breadth, they can cause severe damage to palm and fruits. These minute arthropods cannot be seen with the naked eye. Massive colonies of the mites and individual mites can be detected with a 10 x hand lens. At this magnification, the colonies appear as vague silvery patches. Individual coconut mites appear small even when viewed under standard stereo microscopes. Like typical eriophyid mites, they are elongate body, worm like appearance have two pairs of legs, instead of four pairs as is typical of most mites. They are white and translucent. Mite injures the tender portion of young nuts and suck sap from the nut. The injury ultimately leads to warring and longitudinal fissures on the nut surface. Due to their extensive feeding on young buds resulted in reduction in size followed by immature bud drop. Considering theimportance of coconut and the potentiality of this mite to cause damage, the present study was undertaken to study pest status of coconut mite at different locations of Barishal district.

Study locations and observation of pest status: The study was conducted at 10 coconut plantations at in Patuakhali district from February to May 2019 and February to May 2020. In February to May 2019 first year first investigative pest

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status was conducted in Patuakhali sador, Lebukhali, Mahipur, Kuakata, Pakhimara, Kalapara, Subidkhali, Mirzagong, Dumki, Galachipa. Similarly, second year second investigative pest status from February to May 2020 in same plantations places were conducted in Patuakhali District. The studies were made with the emphasis on the documentation of damage potential and incidence of the promising pests. The places were selected randomly for recording observations and data on eriophyid mite infestation (%) and intensity of eriophyid mite infestation. Pest status of coconut mite was done by visual observation of scoring method as prescribed by Muralidharan *et al.* (2001) and Girisha **(**2005). Three coconut plants were selected from each place for collecting sample coconuts. In each plant five matured bunches of coconut from selected three plants were observed to record the infested coconut due to coconut eriophyid mite infestation. Similarly, in the month of February-May every year, total number of harvested coconuts and number of infested coconuts were recorded for calculated per cent infestation. Data recoded on different parameters were processed for statistical analysis. Mean comparisons for treatment parameters were compared using Duncan's Multiple Range Test (DMRT). Average values of mite infestation were worked out every pest status. Later the observations subjected to (ANOVAs) during statistical analysis Panse and Sukhatme (1985).

Incidence and severity of fruit damage caused by *A. guerreronis* were assessed on three randomly selected plants per plantation by classifying all coconut fruits on each plant on the basis of the extent of characteristic *A. guerreronis* damage visible on fruit surfaces. Amongst the harvested coconuts, the infested coconuts were also graded on the basis of visual scoring method given by Murlidharan *et al*., (2001). Coconut fruits were grouped into three grades – based on the percentage of fruit surface damaged by *A. guerreronis*.

Grade	Surface damage
Free	No symptoms of coconut mite
Grade I	1-25% of coconut surface damage by coconut mite
Grade II	26-50% of coconut surface damage by coconut mite
Grade III	Above 50% of coconut surface damage by coconut mite

Infestation rate of coconut mite *A. gurrerronis* at different locations of Patuakhal in Bangladesh

The infestation of coconut due to coconut eriophyid mites on the basis of per cent coconut infestation ranged between 71.49 - 90.26%, during 2018 - 2019. The highest per cent infested coconuts observed at Patuakhali Sador (90.26%) which was followed by Mirzagonj (89.88%), Kalapara (89.02%), Kuakata (87.98%, Galachipa (87.84%), Dumki (87.65%) at Lebukhali. During the year 2019, coconut infestation due to coconut eriophyid mites on the basis of per cent coconuts infestation ranged between 87.85 - 91.39%. The maximum per cent coconuts infestation level observed at Lebukhali (91.39%) which was followed by Subidkhali (90.95%), Galachipa (90.27%), Kalapara (89.91%), Kuakata (89.62%, Mirzagong (89.23%), Pakhimara (88.58%), Patuakhali Sador (87.97%), Dumki (87.92%) and the lowest at Mahipur (87.85%). The pooled mean of both years data was presented in Table 1 on pest status of coconuts infestation due to coconut eriophyid mite in different places of Patuakhali district. The infestation of coconut eriophyid mite based on per cent coconuts infestation. The maximum per cent infested coconut observed (90.34%) at Mirzagong which was followed by Subidkhali (89.52%), Kalapara (89.47%), Patuakhali Sador (89.12%), Koakata (88.80%), Mahipur (86.76%), Pakhimara (83.48 %), Dumki (82.65%), Galachipa (81.61%) and the lowest (81.44%) at Lebukhali. The variations in the range of infestation may be due to changing environmental condition as well as biotic stresses. Desai *et al*. (2009) also observed that the intensity of infestation of coconut mite and scale index was low in Ratnagiri and Raigadh districts.

Table 1. Infestation rate of coconut mite *A. gurreronis* at different locations of Patuakhal in Bangladesh during 2018, 2019 and pooled

Location	Year 2018			Year 2019			Pooled infestation
	Total count	Infestation (%)	Damage grade	Total count	Infestation (%)	Damage grade	
Patuakhali Sador	195	90.26	Grade-III	241	87.97	Grade-III	89.12
Lebukhali	235	71.49	Grade-III	244	91.39	Grade-III	81.44
Pakhimara	282	78.37	Grade-III	219	88.58	Grade-III	83.48
Kalapara	255	89.02	Grade-III	218	89.91	Grade-III	89.47
Mahipur	251	85.66	Grade-III	214	87.85	Grade-III	86.76
Kuakata	233	87.98	Grade-III	260	89.62	Grade-III	88.80
Subidkhali	299	87.63	Grade-III	221	90.95	Grade-III	89.52
Mirzagong	257	89.88	Grade-III	250	89.23	Grade-III	90.34
Dumki	243	87.65	Grade-III	240	87.92	Grade-III	82.65
Galachipa	255	87.84	Grade-III	226	90.27	Grade-III	81.61

Coconut eriophyid mite, *Aceria guerreronis* Keifer is one of the potential invasive pests of coconut in Bangladesh. Coconuts are rarely grown on large plantation in Bangladesh except for few in the coastal areas. They are mainly grown in the homestead in almost all parts of the country. Infestation of eriophyid mites on coconut occurred at all places of Patuakhali districts in Bangladesh and was found to have started much earlier at Pakhimara compare to other locations in the Patuakhali districts. The infestation of eriophyid mites on the basis of per cent coconut infestation was more at Mirzagong which was followed by Subidkhali, Kalapara, Patuakhali Sador, Koakata, Mahipur, Pakhimara, Dumki, Galachipa and the lowest Lebukhali.

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