Bangladesh J. Plant Taxon. **27**(1): 195–199, 2020 (June) **- Short communication**

EVALUATION OF THE FIELD PERFORMANCE AND GENETIC DIVERSITY OF 23 VARIETIES OF OKRA FROM BANGLADESH USING RAPD MARKERS

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Keywords: Abelmoschus esculentus, Genetic diversity, Polymorphic Information Content, Yellow Vein Mosaic Virus, Enation Leaf Curl Virus.

Okra (*Abelmoschus esculentus* L.) is a globally cultivated, economically important vegetable, and the most used species of Malvaceae family. It is grown mostly in tropical, sub-tropical and mediterranean region of the world (Kumar *et al.*, 2015). Its cultivation is challenged due to severe attack by Yellow Vein Mosaic Virus (YVMV) and Enation Leaf Curl Virus (ELCV), through an insect vector namely white fly (*Bemisia tabaci*). The loss in marketable yield has been estimated at 50–94% depending upon the crop growing stage at which the infection occurs (Kumar *et al.*, 2015). The relationship among okra germplasm and their genetic variability study may play important role in plant breeding program for biotic and abiotic stress tolerance (Gulsel *et al*., 2007). RAPD (pronounced 'rapid'), for Random Amplification of Polymorphic DNA, is a type of molecular marker system in which random primers of short length (10 bp) are used to amplify the genomic DNA by Polymerase Chain Reaction (Hossain *et al*., 2020; Roslan *et al*., 2017). There are few reports on field performance and genetic diversity study of okra by RAPD on local and foreign germplasm cultivated in Bangladesh. As a part of genetic improvement program of okra we aimed to evaluate field performance against virus incidence and estimate genetic relatedness among a set of 23 okra genotypes using RAPD primers.

Twenty-three okra genotypes collected from different regions of Bangladesh were used to assess their performance in an open filed condition (Table 1). Plots were prepared for okra cultivation according to local agronomic practice and maintained (irrigation, weeds cleaning, plant enemies, environmental factors observation etc.) properly. Seeds were sowed with Randomized Complete Block Design with three replicas. Spacing, plant space: 30 cm × 50 cm was maintained. Each of the plot size was 3 m × 1 m and 45 cm was left for irrigation and drainage between two beds. Manures and fertilizers were applied as recommended by Bangladesh Agricultural Research Institute. No pesticide was applied during the experimental studies. Twenty plants of each plot from each variety were selected for data collection. The yield per plant and virus incidence was recorded every two weeks during the period of cultivation over 120 days.

Young and healthy 3–4 days aged leaves of 23 okra varieties were collected in aluminium foil and washed before air-drying. A total of 100-110 mg leaves of each variety was used to extract genomic DNA according to the modified protocol of Doyle and Doyle, 1987. PCR amplification of DNA extracted from all the 23 varieties of okra was carried out using 20 RAPD primers of OPA series (OPA-1 to OPA-20). PCR reaction was performed in a 10 µl volume containing a mixture of 2X GoTaq master mix (5 µl), template DNA 1 µl (approximately 40–50 ng/ µl), 10 mM RAPD single primer (0.5 µl), and 3.5 µl of nuclease-free water.

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Table 1. Morphological characters of 23 genotypes of okra.

Name of variety	Plant height (cm)	No of branches/ plant	No of leaves/ plant	No of flowers/ plant	No of fruits/ plant	Fruits weight (gm)	No of seeds/ plant	Per 100 seed weight	Yield (gm/ plant)
SB	102.42	3.00	30.53	1.0	24.00	19.93	70.52	6.89	478.32
MC	93.78	3.27	29.96	1.16	20.11	18.91	61.82	6.21	380.28
OA	81.88	3.51	33.82	1.0	19.56	17.03	60.57	4.98	333.10
SH	85 .44	3.00	33.12	1.12	18.72	17.25	48.21	5.76	322.92
OAI	86.00	3.24	32.52	1.33	20.51	16.43	55.75	5.98	336.97
SSD	85.58	3.86	31.94	1.13	19.98	18.63	59.29	5.87	372.22
IB	75.73	3.31	30.45	1.03	19.57	15.96	51.47	5.04	312.33
B1	81.31	3.52	32.18	1.31	8.28	19.78	60.68	5.91	401.13
KB	93.48	3.23	32.47	1.00	20.53	13.55	52.90	6.65	278.18
DC	80.66	3.72	31.51	1.13	19.34	15.82	59.33	6.54	305.95
HAD	79.65	3.25	25.67	1.23	10.23	17.67	52.89	6.34	320.98
HE	81.30	3.67	36.78	1.56	15.67	19.56	56.58	5.97	410.23
HHK	76.80	3.89	30.23	1.34	18.89	18.90	65.80	6.66	450.67
ND	83.60	3.12	37.98	1.54	15.90	16.89	59.10	6.12	390.61
HGG	70.62	2.60	26.89	1.67	17.91	19.01	66.89	6.73	399.89
HS	84.60	3.63	32.68	1.29	20.56	18.73	64.70	6.19	440.67
HA	79.90	2.90	25.70	1.56	21.00	16.34	60.80	6.33	401.90
HG	78.65	3.00	31.99	1.10	12.45	18.90	66.70	6.71	389.90
HP	82.56	3.36	35.58	1.35	18.90	16.60	62.79	6.77	420.56
HDS	85.68	2.99	38.90	1.50	16.78	18.45	67.70	6.57	410.56
WO	95.60	5.00	56.90	1.00	7.99	11.56	40.10	5.00	250.89
CH1	90.20	3.65	38.60	1.99	16.89	17.90	61.50	6.63	460.80
CH2	92.67	3.50	33.78	1.90	13.56	18.76	65.19	6.19	450.09
$LSD_{0.05}$	5.38	0.89	6.21	0.31	6.05	3.91	5.4	0.11	21.47
SD	7.290	0.480	6.246	0.285	4.196	2.011	7.06	0.56	61.328
SE (±)	0.086	0.141	0.1865	0.217	0.242	0.114	0.118	0.09	0.161
CV %	1.532	0.117	1.291	0.112	0.887	0.413	1.453	0.11	12.80

N.B; [SB, Shamol Bangla; MC, Mahira Cross; OA, Orka Anamika; SH, Shomy hybrid; OAI, Orka Anamika India; SSD, Sobuj Sathi; IB, Iron Bhendi; B1, BARI-1; KB, Kolatia Bhendi; DC, Dheros Chamak; HAD, Hybrid Dheros Alok; HGE, Hybrid Green Energy; HHK, Hybrid Hira Kamal; ND, Nowdapara Dheros; HGG, Hybrid Green Glowry; HS, Hybrid Sumi; HA, Hybrid Alif; HG, Hybrid Godhuli; HP, Hybrid Padma; HDS, Hybrid Dheros Sumona; WO, Wild Okra; CO1, Chinese Okra-1; CO2, Chinese Okra-2.]

During preparation, the mixtures were kept on ice. PCR amplification was performed in a thermocycler (Gene Atlas) under the following conditions: Initial denaturation at 94°C for 5 min followed by 46 cycles of denaturation at 94°C for 1 min, primer annealing at 36°C for 30 sec, elongation at 72°C for 3 min and final elongation at 72°C for 10 min. The reaction was then cooled and held at 4°C for 10 min. After the completion of PCR, the amplified products were run

using 1% agarose gel electrophoresis stained with ethidium bromide. The bands were viewed and photographed by gel documentation system.

The RAPD banding pattern for each primer was scored manually by visual observation. For phylogenetic analysis of all the RAPD bands, a binary matrix was prepared on the basis of presence or absence of bands in a particular locus of all the genotypes. The presence of band was scored as 1 and the absence was scored as 0. Thus the 0 and 1 binary matrices were used to produce a phylogenetic tree of all the 23 okra varieties. A dendrogram was prepared using an online software package called "Dendro UPGMA" and the clustering was done using the Jaccard coefficient index (Jaccard *et al.*, 1908).

The yield performance and virus incidence were calculated at 90 days which are as follows: i) Total Yield: Shamol Bangla was recorded as the highest yielding variety followed by Chinese 1, Hybrid Hira Kamol and Chinese okra 2. Inspite of having highest branch, leaves, and virus tolerances, Wild Okra yielded the lowest among the 23 varieties (Table 1). ii) YVMV incidence: No variety was found to be virus resistant or immune. Virus incidence was very high in some varieties. Dheros Chamak was found to be the most susceptible variety to YVMV followed by Sobuj Sathi and Chinese 2 (Data not shown). Wild Okra was observed as the most tolerant variety. iii) ELCV incidence: ELCV incidence was also observed in all the okra genotypes and it was found that virus incidence was very high in Hybrid Dheros Alok and Orka Anamika. On the other hand, ELCV incidence was found to be the lowest in Wild Okra (Data not shown).

Out of the 20 RAPD primers, used to analyze the genetic diversity among 23 okra genotypes 14 primers gave clear and scorable bands. A RAPD profile generated by OPA 1 is shown in Fig. 1. 80 RAPD alleles were amplified by the 14 RAPD primers and 66 of them were found as polymorphic. 82.50% polymorphism was obtained among the 23 okra varieties (Data not shown). Martinello *et al.* (2001) identified 103 amplified bands in okra by 31 Random decamer primers.

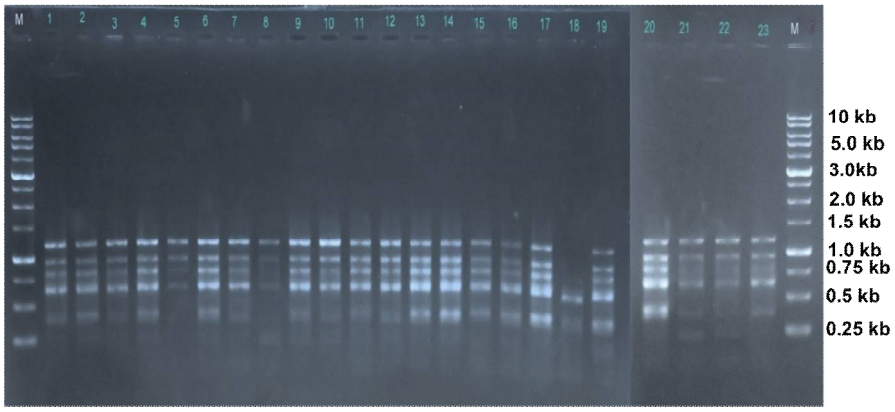

Fig. 1. RAPD profiles of 23 okra genotypes on 1% agarose gel electrophoresis using primer OPA01. Lane M, 1kb DNA marker; Lane 1-23 represents the genotypes in the same order as listed in Table 1.

The number of bands obtained per primer varied from 10 (OPA 03) to 3 (OPA 04 and OPA 15). In some other studies, amplified allele numbers have been reported from 2-6 (Gulsen *et al*., 2007), 7–9 (Saifullah *et al*., 2010) and 8–12 alleles (Aladele *et al*., 2008). Size of the amplified bands for all primers also varied from 200 bp to 1500 bp (Fig. 1). The Polymorphic Information Content (PIC) value ranged from 0.101 (OPA-2) to 0.429 (OPA-5) with an average of 0.289 (Data not shown).

Genetic dissimilarity value ranges from 10 to 56%, which suggests a narrow genetic distance within different okra varieties studied. Saifullah *et al*., 2010 observed genetic distance value from 0.00 to 0.66 among okra accessions while 86 to 100% genetic similarity was found by Gulsen *et al*., 2007 using Sequence Related Amplified Polymorphism. The highest genetic distance 0.56 was obtained between HG and OA, which indicated that these two varieties are genetically more distinct. The lowest genetic distance (0.10) was obtained between HE and HAD which is an indication that these two varieties are genetically more similar than any other varieties. Cross-pollination might be the reason for the narrow genetic distance of okra. Bertini *et al*. (2006) also reported a narrow genetic distance in cotton. Prakash *et al*. (2011) studied the genetic diversity of okra by RAPD marker and reported narrow genetic distances. The dendrogram for the 23 individuals of okra was constructed using an online software package called Dendro UPGMA and clustering was performed using Jaccard Index (Jaccard *et al*., 1908). The dendrogram placed the 23 okra genotypes into three main clusters depending on the basis of similarity (Fig. 2). These clusters included 3, 9 and 10 genotypes with an out-group. Cophenetic or correlation was found to be 0.94, which suggests that the cluster analysis strongly represents the similarity matrix. Similar correlation was obtained by other scientists (Gulsen *et al*., 2007; Kaur *et al*., 2013).

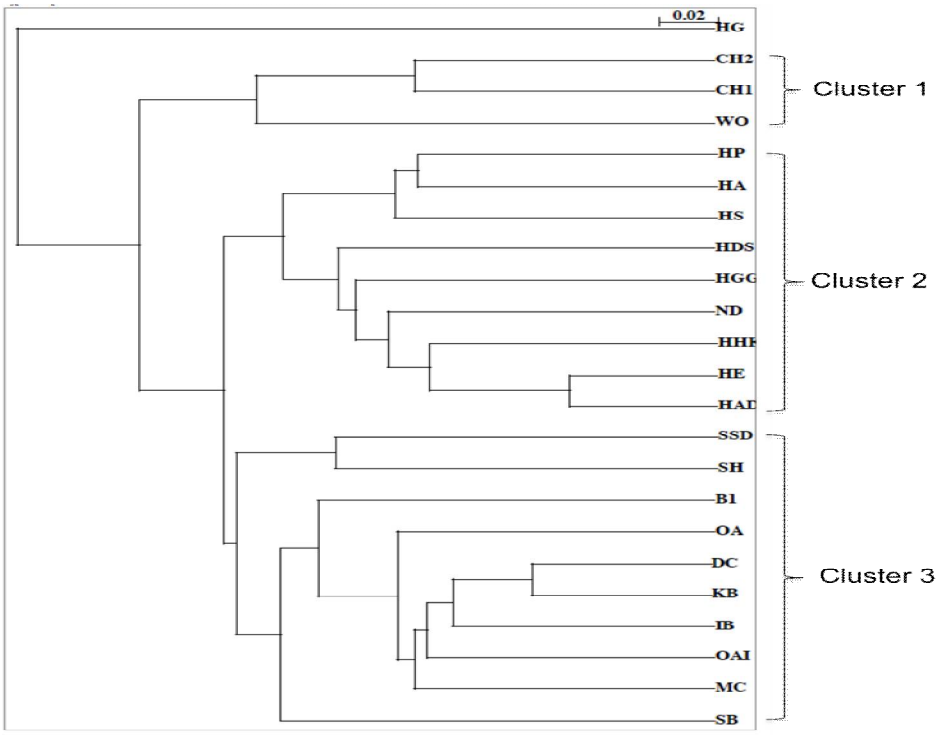

Fig. 2. UPGMA-Neighbour Joining unrootedphyogentic tree. Dendrogram showing the genetic diversity among 23 okra accessions using cluster analysis of RAPD data (for 1 to 23 accessions ref. Table 1).

Figure 2 shows the genetic relationship of 23 varieties of okra with an out group representative HG. Cluster 1 consisting of three okra genotypes including two Chinese okra and one wild type okra variety (Fig. 2). Nine genotypes included in cluster 2 which are HP, HA, HS, HDS, HGG, ND, HHP, HE and HAD. Subcluster included 2 genotypes from the dendrogram, it

was clear that HP and HA are more similar to each other than HS (Fig. 2). In the other subcluster 2, HE and HDA were found to be more similar than the other genotypes of this group (Fig. 2).Ten genotypes were found in cluster 3 and these varieties are SSD, SH, B1, OAL, DC, KB, IB, OAI, MC, and SB. Among the varieties SSD and SH were grouped into one subcluster and diverged from the other genotypes of this clusters. In another subcluster 3, SB variety was found to be the most diverged from the other variety of this subcluster. DC and KB are also found to be the most similar among all the varieties. The results obtained from this genetic diversity study will be useful to breed okra germplasm with desired traits for crop improvement program.

Acknowledgement

Authors are thankful to Grant for Advanced Research in Education (GARE), Ministry of Education, Government of Bangladesh for funding this research project (PCN No: LS201628).

References

Aladele, S.E., Ariyo, O.J. and de Lapena, R. 2008. Genetic relationships among West African okra (*Abelmoschus caillei*) and Asian genotypes (*Abelmoschus esculentus*) using RAPD. Afri. J. Biotech. **7**(10): 1426–1431.

Bertini, C.H., Schuster, I., Sediyama, T., Barros, E.G.D. and Moreira, M.A. 2006. Characterization and genetic diversity analysis of cotton cultivars using microsatellites. Gen. Mol Biol. **29**(2): 321-329.

Doyle, J.J., Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phyt. Bull. **19**: 11–15.

Gulsen, O., Karagul, S. and Abak, K. 2007. Diversity and relationships among Turkish okra germplasm by SRAP and phenotypic marker polymorphism. Biol. **62**(1): 41–45.

Hossain, M.A., Hossen, M.S. and Karim, M. R. 2020. Molecular markers: Indispensable tools for genetic diversity analysis and crop improvement biotechnology. Int. J. Plant Breed. Crop. Sci. 7(1): 613–623.

Jaccard, P. 1908. Nouvellesrecherchessur la distribution florale. Bull. Soc. Vaud. Sci. Nat. **44**: 223–270.

Kaur, A., Kaur, P., Singh, N., Virdi, A.S., Singh, P. and Rana, J.C. 2013. Grains, starch and protein characteristics of rice bean (*Vigna umbellata*) grown in Indian Himalaya regions. Food Res. Int. **54**(1): 102–110.

Kumar, A., Verma, R.B., Solankey, S.S. and Adarsh, A. 2015. Evaluation of okra (*Abelmoschus esculentus*) genotypes foryield and yellow vein mosaic disease. Indian Phytopath. **68**(2): 201–206

Martinello, G.E., Leal, N.R., Amaral, Jr. A.T, Pereira, M.G. and Daher, R.F. 2001. March. Comparison of morphological characteristics and RAPD for estimating genetic diversity in *Abelmoschus* spp. International Symposium on Molecular Markers for Characterizing Genotypes and Identifying Cultivars in Horticulture. **546**: 101–104.

Prakash, K., Pitchaimuthu, M. and Ravishankar, K.V. 2011. Assessment of genetic relatedness among okra genotypes [*abelmoschus esculentus* (l.) Moench] using RAPD markers. Electron. J. Plant. Breed. **2**(1): 80–86.

Roslan, H.A., Hossain, M.A., Othman, N.Q., Tawan, C.S. and Ipor, I. 2017. Sequence characterized amplified region markers for species-specific identification of three threatened aquilaria species. Chiang Mai. J. Sci. **44**(4):1304–1310.

Saifullah, M., Rabbani, M.G. and Garvey, E. J. 2010. Estimation of genetic diversity of okra (*Abelmoschus esculentus* L. Moench) using RAPD markers. SAARC. J. Agri. **8**(2): 19–28.

(Manuscript received on 3 March 2019; revised on 9 May 2020)