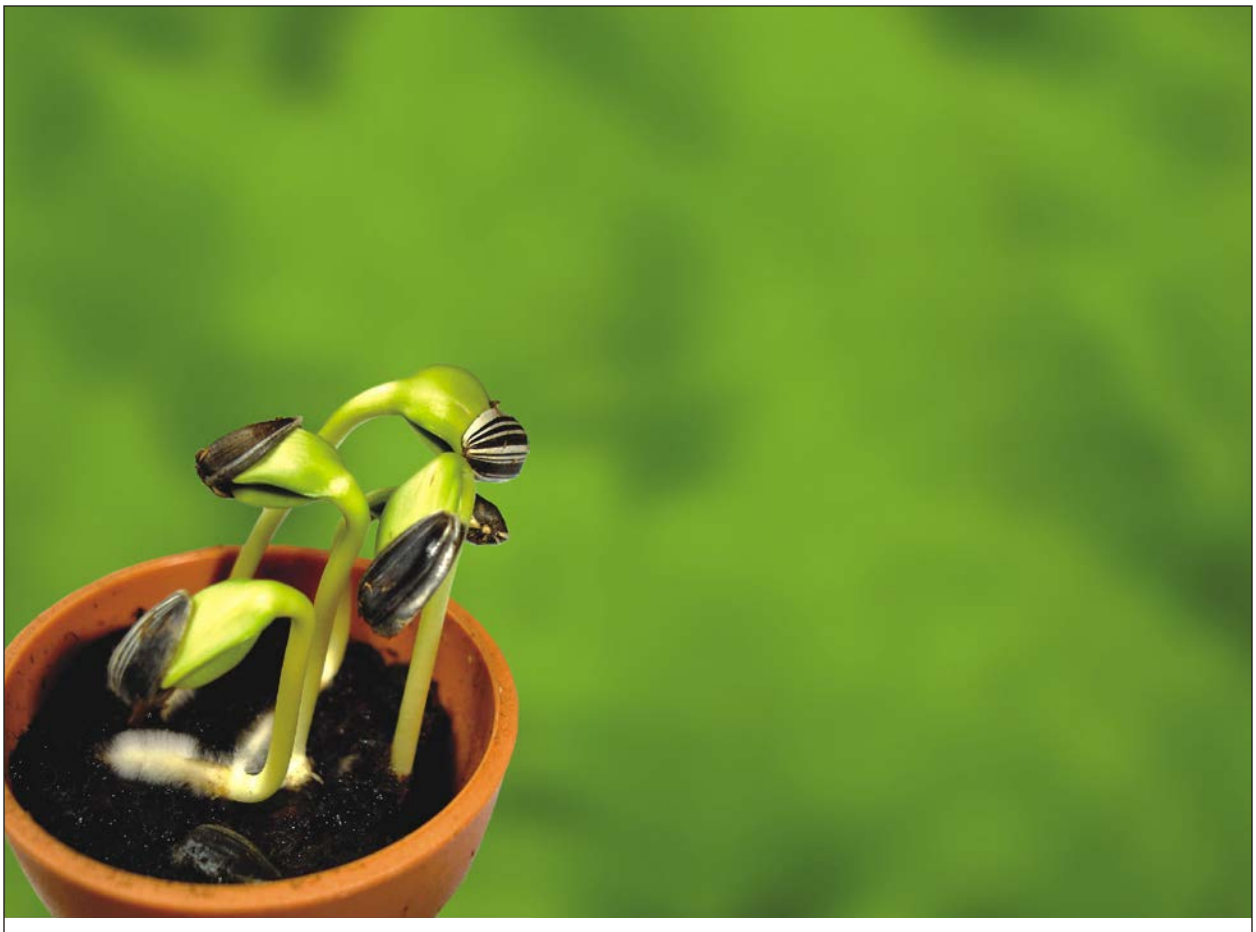

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Adaptability of Litchi Germplasm in Hilly Areas of Sylhet Agricultural University and Screening their Genetic Variation by Using RAPD Markers

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ABSTRACT

Ten germplasm of litchi were collected from different eco-geographic regions of Bangladesh to evaluate their adaptation in hilly areas and screening the genetic variability both in morphological and molecular characteristics by RAPD markers. The morphological data was conducted in Randomized Complete Block Design (RCBD) with three replications and found the significant differences at all stages. Among the germplasm, the maximum number of branches per plant (14.33) and number of leaves per plant (66.33) were found in Mongalbaria which were statistically similar to BARI litchi-2 13.67 and 61.67, respectively. The germplasm, Malaysian recorded the highest plant height (68.40 cm) and the lowest trunk diameter (1.77 cm), while China-3 gave the highest trunk diameter (3.23 cm). So, by assessing morphological parameters, Mongalbaria and BARI litchi-2 have a bright prospect for growing under the hilly areas in Sylhet region. In Random Amplified Polymorphic DNA (RAPD) markers analysis, primer OPB-04 showing good technical resolution and sufficient variation among germplasm and produced a total of 7 RAPD markers of which 6 (85.71%) were considered as polymorphic loci. The highest genetic distance (1.9459) was observed in Bombai vs. Malaysian varietals pair whereas, the lowest genetic distance (0.1234) was estimated in BARI litch-2 vs. BARI litch-3 varietals pair. The UPGMA dendrogram showed that 10 germplasm of litchi could be classified into different groups or clusters according to their genetic similarities and dissimilarities. Two germplasm Bombai and Malaysian were found the highest genetic distance than the others that ultimately helpful for further hybridization program.

Key words: Germplasm adaptation, hilly area, genetic variability, RAPD markers

INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) is one of the highly priced, popular and major table fruit in Bangladesh, although it is seasonal. It has a great demand among all classes of people. Litchi is a non-climacteric fruit (Wills *et al*., 2004) and it deteriorates very fast after harvest. The litchi is considered as one of the best fruits due to its high nutritive value. Besides a rich source of vitamin C, litchi contains a blond amount of phosphorus, calcium, iron, vitamins A and B (Islam *et al*., 2003). Though so many varieties of litchis are available in Bangladesh, only a few are well accepted

by consumers considering their taste quality. Litchi requires temperature around 25°C, dry weather, well drained loamy and slightly acidic soil having high organic matter for maximum production. But in Sylhet region, more acidic soil, high rainfalls about 5000 mm per annum, humid condition, inadequate irrigation facilities in dry season, mites and fruit borer infestation are obstacles for litchi cultivation. Thus, especial emphasis should be given for selecting the litchi germplasm, which can withstand stress conditions and use them for further to developing desired variety by means of breeding program suitable for hilly areas in Sylhet region of Bangladesh.

Litchi cultivars vary greatly in vegetative flushing pattern, flush colour and flowering ability (Mahajan and Dhillon, 2000). There has been wide range of confusion in the names of cultivars due to varying agro-climatic conditions, growth behavior, fruit colour, shape and size. For this reason, the same cultivar may be called by different names at different locations. Genetic diversity in litchi is indicated by a large number of cultivars in China and India, which provides the bases for development of new cultivars. The environment profoundly influenced the characteristics of litchi cultivars and this may explain why a large number of cultivars are available (Groff, 1921). There are different characteristics, which are used to identify the cultivars. The size, shape, length and colour of litchi fruits are characteristics for different cultivars (Rai *et al*., 2001; Khurshid *et al*., 2004). No systematic attempts for collecting and conservation of litchi germplasm have been made in Bangladesh. Only few cultivars are available, which perpetuated through vegetative methods of propagation.

Germplasm collection and their conservation is the effective method for the expansion of a plant by the utilization of selective plant breeding program. The variability among litchi cultivars is still unknown, since breeding for new cultivars is done by growers and is based on low number of parents (Aradhya *et al*., 1995). To identify genetic materials that may contain useful traits for germplasm enhancement, a systematic evaluation of genetic diversity is required to understand relationship among the accessions and their corresponding environment (Steiner and Greene, 1996). Understanding the genetic diversity within a germplasm collection facilitates their use and provided that information is available from characterizing these germplasm collections (Strauss *et al*., 1998). Comparison of parents using difference in DNA markers may be one of the methods by which breeders can increase the probability of selecting those parents with different gene sets. Examination of genetic variance is important for plant breeder in general and particularly in a new introduced crop like litchi, which is not a native and yet to be commercially cultivated in Bangladesh. Different cultivars have been separated through the use of DNA based markers, particularly Random Amplified Polymorphic DNA (RAPD) to measure genetic diversity in litchi cultivars (Ding *et al*., 2000; Tongpamnak *et al*., 2002; Kumar *et al*., 2006; Cheng *et al*., 2015). However, there is no report on genetic variability of litchi cultivar grown in Bangladesh. Regarding this, it is essential for an effective breeding programme, information concerning the extent and nature of genetic diversity within Bangladeshi varieties. It is particularly useful for characterizing individual varieties, as well as a general guideline in selecting the parents for hybridization. Literature indicates that different cultivars of litchi differ in their morphological characteristics depending upon the locations, climatic conditions and their existing genetic variability. Therefore, the present study was conducted to find out the adaptability (growing performance) and the genetic variation among the litchi germplasm in hilly areas of Sylhet Agricultural University, Sylhet, Bangladesh.

MATERIALS AND METHODS

Experimental materials: The present research study was carried out during the year 2013-2014 for collection and adaptation of litchi germplasm and find out the genetic diversity in different morphological characters and also for genetic traits among the germplasm by RAPD markers. Total ten varieties of *Litchi chinensis* were collected from two different geographical locations of Bangladesh. BARI litchi-2 and BARI litchi-3 were collected from BARI (Bangladesh Agricultural Research Institute) regional sub-station at Akbarpur, Moulvibazar and China-3, Bombai, Mongalbaria, Malaysian, Bedana, BAU litchi-1, BAU litchi-2 and BAU litchi-3 from Germplasm Center, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. Litchi germplasm were planted on 4th November 2013 in slightly hilly areas of Sylhet Agricultural University region in Randomized Complete Block Design (RCBD) with three replications. Each treatment comprised of three, thus involving total of thirty genotypes in the experiment. The research study was carried out in two different patterns such as morphological characteristics and molecular analysis of litchi germplasm.

Morphological characteristics: The experiment was laid out in a randomized complete block design with three replications. Spacing between rows was 2 and 1.7 m between plants in a row. Recommended production packages were followed to ensure normal plant growth and development. Data on various characters, such as; plant height, number of branches per plant, number of leaves per plant and trunk diameter was taken from 3 selected plants from each genotype. For all parameters, data was recorded in four occasions such as first day of planting, 90, 180 and 270 Days After Planting (DAP). Diameter of the trunk was measured in centimeter from three portion of the each plant viz. base, middle and top at the same occasion of data collection using Vernier Scalipers.

Statistical analysis: Appropriate statistical analyses (computer programme MSTAT-C v.2.10) were performed with the mean data of each character. Least Significant Difference (LSD) test at 5% probability level was applied to compare the differences among treatments means (Steel and Torrie, 1980).

Molecular analysis of litchi germplasm: Ten germplasm of litchi viz BARI litchi-2, BARI litchi-3, Malaysian, Mongalbaria, China-3, Bedana, BAU litchi-1, BAU itchi-2, BAU litchi-3 and Bombai were used in the study. Fresh and young leaf samples were collected from one out of three replications of each litchi germplasm and used as the source of genomic DNA.

Extraction of genomic DNA: Genomic DNA was extracted from the young leaf tissues following standard procedures at the Genetic Engineering Laboratory, Department of Genetics and Plant Breeding, Sylhet Agricultural University, Sylhet, Bangladesh. Approximately 0.3 g of leaf tissues was cut into small pieces, homogenized and digested in extraction buffer [50 mM Tris-HCl, 25 mM Ethylene Diamine Tetra Acetic Acid (EDTA), 300 mM NaCl and 1% SDS (Sodium Dodecyl Sulphate), pH = 8.0]. The DNA was purified by successive extraction with Phenol: Chloroform: Isoamyl alcohol = 25:24:1 (v/v/v); pH near 8.0 with TEN buffers. The DNA was precipitated first absolute (100%) ethanol, pelleted by centrifugation, then reprecipitated in 70% ethanol with 20 μL 3 M sodium acetate. The pellets were air-dried and re-suspended in 50 μL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH = 8.0).

PCR amplification: Polymerase Chain Reaction (PCR) reactions were performed on each DNA sample in a 10 μL reaction mix containing 1 μL of 10X Ampli *Taq* polymerase buffer, 0.25 μL of 0.4 μM Primer, 1 μL of 250 μL dNTPs, 0.5 unit of 0.2 μL Ampli *Taq* DNA polymerase (Takara, Japan) and 4 μL of 50 ng μL^{-1} plant genomic DNA and a suitable amount of sterile deionized water. The DNA amplification was performed in an oil-free thermal cycler (Master Cycler Gradient, Eppendorf). The reaction mix was preheated at 94°C for 3 min followed 44 cycles of 1 min denaturation at 94°C, 1 min annealing at 34°C and elongation or extension at 72°C for 2 min. After the last cycle, a final step of 5 min at 72°C was added to allow complete extension of all amplified fragments. The PCR products from each sample were confirmed by running 1.5% agarose gel containing 5 μL Ethidium bromide in 1X TBE buffer at 120 V for 1 h. Loading dye (2.5 μL) was added to the PCR products and loaded in the wells. Molecular weight marker DNA (1000 bp DNA ladder on right side) was also loaded on the one side of the gel. The RAPD bands were observed under ultra violet light on a transilluminator and documented by taking photograph using a Gel documentation system.

RAPD data analysis: Data from molecular marker techniques requires detailed analysis to establish a genetic relationship among the litchi germplasm. For each primer, polymorphic bands are scored for their presence (1) or absence (0) in all the accessions by visually assessing photographs of the gels. The size of amplification product was determined by comparison with gene ruler (1000 bp DNA ladder). Only distinct, reproducible, well-resolved fragments, in the size range from 200-600 bp, were scored as discrete variables for the 10 accessions. Bands or RAPD markers not identified by both the persons and readers were considered as non-scorable. The scores obtained using all primers in the RAPD analysis were then combined to create a single data matrix. From the band data, monomorphic and polymorphic bands were identified for each type of cultivar. This was used for estimating polymorphic loci (Nei, 1973) gene diversity, genetic identity, genetic distance and constructing a Unweighted Pair Group Method of Arithmetic (UPGMA) means dendrogram among the populations using computer program POPGENE (Version 1.31) (Francis *et al*., 1999).

RESULTS AND DISCUSSION

Morphological characteristics: Litchi germplasms showed significant variation in plant height (Fig. 1). Plant height increased gradually after the time of planting at the different stages. Result revealed that the germplasm Malaysian showed the tallest plant (68.40 cm) at all growth stage which was statistically similar to Bedana (63.77 cm) and BAU litchi-1 (61.63 cm). The shortest plant was found in BAU litchi-3 (37.33 cm) which was statistically similar to Mongalbaria (41.73 cm). These results were in agreement with the findings of Miao *et al*. (1998), Ghaffoor *et al*. (1999) and Rai *et al*. (2001) stated that plant height differed significantly among the studied litchi genotypes due to the time upto the maturity. The difference in observations can be attributed to the genetically and climatic variation.

The branch number was significantly differed among the genotypes at all growth stages in litchi germplasm (Table 1). Result revealed that the number of branches increased with the time up to 270 Days After Planting (DAP). The maximum number of branches (14.33) was found in Mongalbaria, which was statistically similar to that of BARI litchi-2 (13.67), while the minimum number was recorded in BAU litchi-1 (7.00), which was closely similar to that of Malaysian (8.00) and Bedana (8.67). The result is similar to the result of Singh *et al*. (1973) and

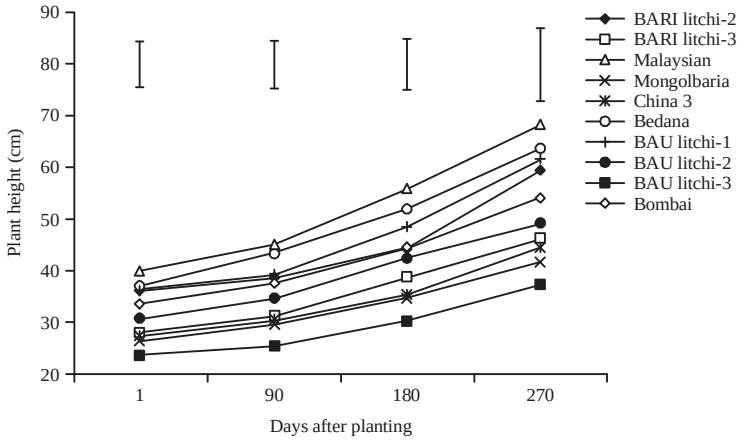

Fig. 1: Plant height in different litchi germplasm at different days after planting. Vertical bars represent $LSD_{(0.05)}$

Table 1: Branches per plant of litchi germplasm at different days after planting

	DAP			
Germplasm	1	90	180	270
BARI litchi-2	5.33^{cd}	7.00^{bc}	9.67^{abc}	13.67^{ab}
BARI litchi-3	6.00^{bcd}	7.33^{bc}	9.00^{bcd}	11.00^{c}
Malaysian	3.00^{fg}	4.00^{f}	5.67^{fg}	8.00^{de}
Mongalbaria	7.33^{a}	8.67^{a}	11.00^{a}	14.33^{a}
China-3	6.33^{abc}	8.00^{ab}	10.00^{ab}	12.67^{b}
Bedana	4.00^{ef}	5.33^{e}	7.00^{ef}	8.67^{d}
BAU litchi-1	2.67^{g}	3.67^{f}	5.33^{g}	7.00^{e}
BAU litchi-2	5.00^{de}	6.67^{cd}	8.33^{cde}	10.67^{c}
BAU litchi-3	6.67^{ab}	8.00^{ab}	10.00^{ab}	13.00^{b}
Bombai	4.00^{ef}	5.67^{de}	8.00^{de}	11.00^{c}

Values having same letter(s) in a column do not differ significantly at 5% level of significance, DAP: Days after planting

Table 2: Number of leaves per plant of litchi germplasm at different days after planting

	DAP			
Germplasm	1	90	180	270
BARI litchi-2	26.67^{ab}	35.33^{ab}	48.33^{ab}	61.67^{ab}
BARI litchi-3	23.33^{b-e}	29.67^{cd}	38.00^{cd}	46.33^{de}
Malaysian	20.33^{cde}	24.33^{e}	30.00^{e}	38.00^{g}
Mongalbaria	29.67^{a}	39.33^{a}	52.00^{a}	66.33^{a}
China-3	20.33^{cde}	28.33^{cde}	39.00^{c}	51.67^{c}
Bedana	18.33^{e}	23.67^{e}	31.67^{e}	40.00^{fg}
BAU litchi-1	20.00^{de}	26.33^{de}	34.00^{de}	43.33^{ef}
BAU litchi-2	25.33^{abc}	30.67^{bcd}	38.00^{cd}	47.67^{cde}
BAU litchi-3	24.33^{b-d}	33.00^{bc}	45.00^{b}	57.67^{b}
Bombai	21.00^{c-e}	27.00^{de}	37.00^{cd}	48.67^{cd}

Values having same letter(s) in, a column do not differ significantly at 5% level of significance, DAP: Days after planting

Khurshid *et al*. (2004) reported that branches number increased with increasing the plant age up to physiological maturity in litchi. From the planting date of air layering of litchi to 90 DAP, the branches emerged slowly due to the adaptation in slightly acidic soil as well as new environment and after that number of branches increased gradually up to the time.

Number of leaves per plant increased gradually with the advancement of plant growth up to 270 DAPS in all the germplasm at different rate. The number of leaves of ten litchi germplasm showed significant variation in every stage of growth of plant (Table 2). The maximum number of

Table 3: Trunk diameter of litchi germplasm at different days after planting

	DAP			
Germplasm	1	90	180	270
BARI litchi-2	1.17^{bc}	1.47^{cd}	1.83^{de}	2.20^{f}
BARI litchi-3	1.07^{cde}	1.37^{de}	1.73^{e}	2.37^{e}
Malaysian	0.90^{e}	1.13^{f}	1.47^{f}	1.77^{g}
Mongalbaria	1.10^{cd}	1.40^{cd}	1.97^{cd}	2.67^{cd}
China-3	1.50^{a}	2.07^{a}	2.67^{a}	3.23^{a}
Bedana	0.93^{de}	1.23^{ef}	1.50^{f}	1.90^{g}
BAU litchi-1	1.31^{b}	1.65^{b}	2.23^{b}	3.03^{b}
BAU litchi-2	1.20^{bc}	1.53^{bc}	1.97^{cd}	2.47^{e}
BAU litchi-3	1.30^{b}	1.63^{b}	2.10^{c}	2.80^{c}
Bombai	1.20^{bc}	1.53^{bc}	2.00^{c}	2.63^{d}

Values having same letter(s) in a column do not differ significantly at 5% level of significance, DAP: Days after planting

leaves (66.33) was observed in Mongalbaria, which was statistically similar to that of BARI litchi-2 (61.67), whereas, the minimum number of leaves was recorded by the germplasm Malaysian (38.00) at 270 DAP, which was closely similar to that of Bedana (40.00). Rai *et al*. (2001) have also reported genetic variation in 13 litchi cultivars for various traits including, leaf number. The leaf number is an important varietal character and is also used for cultivar identification (Singh *et al*., 1999). Leaf number increased with the increasing number of branches in each germplasm and also varietal characters probably responsible for the variations.

Among the cultivars trunk diameter differed significantly (Table 3). The maximum trunk diameter (3.23 cm) was recorded in China-3 closely followed by BAU litchi-1 (3.03), which statistically differed from all other germplasm. The minimum trunk diameter (1.77 cm) was found in Malaysian and that was statistically same in Bedana (1.90). Similar results also corroborated with the results found by Ghaffoor *et al*. (1999) and Khurshid *et al*. (2004). The difference might be due to their different genetic make up and response to soil and climatic conditions. Mahajan and Dhillon (2000) stated that trunk diameter is a genetic character and may differ from cultivar to cultivar under similar soil and environmental conditions. The growth behaviour of a germplasm may vary in particular climate other than it originated.

From the morphological parameters, it could be sum up that among the litchi germplasm, Mongalbaria and BARI litchi-2 were found to be the best in respect of number of branches per plant, number of leaves per plant and trunk diameter with the shortest plant height. Considering the overall performance of all the germplasm, it appeared that Mongalbaria and BARI litchi-2 have a bright prospect for growing under the hilly areas in Sylhet, Bangladesh.

Molecular analysis of litchi germplasm: The RAPD is one of the frontline techniques and in recent years, a number of plants or other organisms have been characterized by standard and improved RAPD analysis (Cheng *et al*., 2015; Long *et al*., 2014). Three 5'-3' sequences primers namely OPG-02 (GGCACTGAGG), OPB-01 (GTTTCG CTCC) and OPB-04 (GGACTGGAGT) were initially screened for their ability to produce polymorphic patterns and only one primer OPB-04, which gave reproducible and distinct polymorphic amplified products were selected. This primer showing good technical resolution and sufficient variation among germplasm and produced a total of 7 RAPD markers of which 6 (85.71%) were considered as polymorphic loci (Fig. 2). The RAPD analyses yield similar results and expressed great potential to identify and establish genetic relationship among litchi germplasm. These results more or less agreed with RAPD in litchi accessions (Ding *et al*., 2000; Kumar *et al*., 2006). Different combinations of the banding patterns

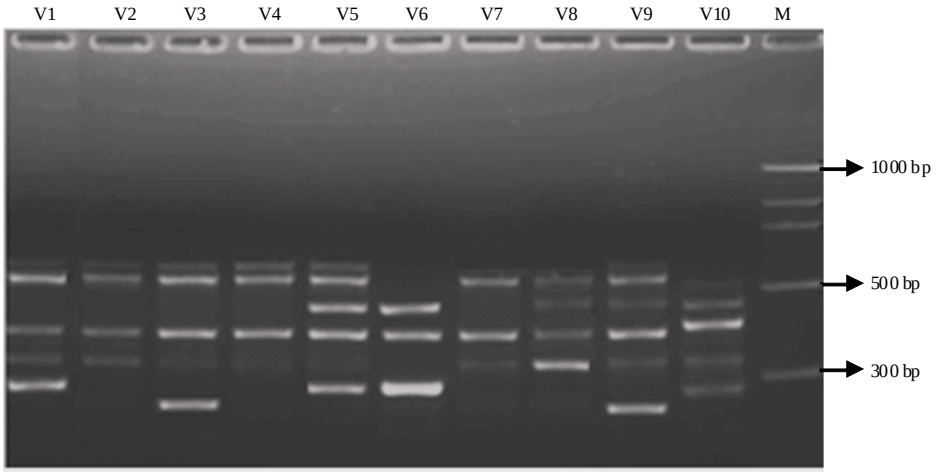

Fig. 2: RAPD profile of ten different germplasm of litchi using OPB-04 primer Lane V1: BARI litchi-2, V2: BARI litchi-3, V3: Malaysian, V4: Mongalbaria, V5: China-3, V6: Bedana, V7: BAU litchi-1, V8: BAU litchi-2, V9: BAU litchi-3, V10: Bombai, M: Molecular weight marker (1000 bp DNA ladder)

Table 4: Summary of Nei's genetic identity (above diagonal) and genetic distance (below diagonal) values between ten litchi varieties

	BARI						BAU			
Germplasm	Litchi-2	Litchi-3	Malaysian	Monga-lbaria	China-3	Bedana	Litchi-1	Litchi-2	Litchi-3	Bombai
BARI litchi-2	****	0.8839	0.5714	0.7143	0.7143	0.4286	0.7143	0.5714	0.4286	0.5714
BARI litchi-3	0.1234	****	0.7143	0.8571	0.5714	0.2857	0.8571	0.7143	0.5714	0.4286
Malaysian	0.5596	0.3365	****	0.8571	0.5714	0.2857	0.5714	0.4286	0.5714	0.1161
Mongalbaria	0.3365	0.1542	0.1542	****	0.7143	0.4286	0.7143	0.5714	0.4286	0.2857
China-3	0.3365	0.5596	0.5596	0.3365	****	0.7143	0.4286	0.5714	0.4286	0.5714
Bedana	0.8473	1.2528	1.2528	0.8473	0.3365	****	0.4286	0.5714	0.4286	0.8571
BAU litchi-1	0.3365	0.1542	0.5596	0.3365	0.8473	0.8473	****	0.8571	0.7143	0.5714
BAU litchi-2	0.5596	0.3365	0.8473	0.5596	0.5596	0.5596	0.1542	****	0.8571	0.7143
BAU litchi-3	0.8473	0.5596	0.5596	0.8473	0.8473	0.8473	0.3365	0.1542	****	0.5714
Bombai	0.5596	0.8473	1.9459	1.2528	0.5596	0.1542	0.5596	0.3365	0.5596	****

provided by the primer, is a clear evidence of the high discrimination capacity of these markers. Estimation of genetic diversity is highly influenced by the genome selected for evaluation and by the number of markers assayed. Since, fruit tree cultivars are maintained by vegetative propagation, accurate identification of vegetative materials is crucial for growers and is required for plant breeder's rights. Thus, by using RAPD markers, most of these studies have been carried out in case of cross-pollinated plants and consequently, relatively higher estimates of genetic variability were obtained. The present study reveals that RAPD is informative for estimating the extent of genetic diversity as well as to determine the pattern of genetic relationships among different accessions of 10 litchi germplasm in Bangladesh.

Genetic identity and genetic distance: The highest Nei's genetic identity (0.8839) was observed in BARI litch-2 vs. BARI litch-3 varietals pair whereas, the lowest genetic identity (0.1161) was estimated in Bombai vs. Malaysian varietals pair. The highest genetic distance (1.9459) was observed in Bombai vs. Malaysian varietals pair whereas, the lowest genetic distance (0.1234) was estimated in BARI litch-2 vs. BARI litch-3 varietals pair (Table 4) (Nei, 1972). Furthermore, the same and high level of genetic distance was found in Bedana vs. BARI litchi-3, Bedana vs.

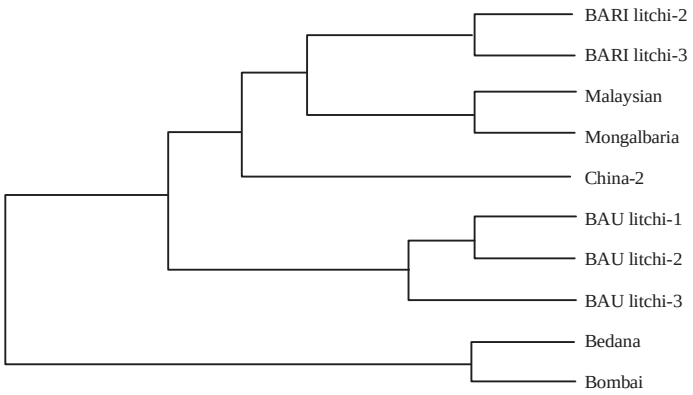

Fig. 3: UPGMA dendrogram based on Nei's genetic distance, summarizing the data on differentiation among ten litchi germplasm according to RAPD analysis

Malaysian and Bombai vs. Mongalbaria (1.2528) varietals pair. The UPGMA dendrogram based on Nei (1972) genetic distance indicated the segregation of 10 germplasm of litchi into two main clusters: BARI litchi-2, BARI litchi-3, Malaysian, Mongalbaria, China-3, BAU litchi-1, BAU litchi-2 and BAU Litchi-3 grouped in cluster 1 while Bedana, Bombai in cluster 2 (Fig. 3). In cluster 1, BAU litchi-1, BAU litchi-2 and BAU litchi-3 formed sub-cluster I; BARI litchi-2, BARI litchi-3, Malaysian, Mongalbaria and China-3 formed sub-cluster II. In sub cluster II, China-3 alone formed sub sub-cluster I while BARI litchi-2, BARI litchi-3, Malaysian and Mongalbaria formed sub-cluster II. In sub-cluster II; all four germplasm were found partially close to each other and grouped again BARI litchi-2, BARI litchi-3 in sub-cluster II(a) and Malaysian, Mongalbaria in sub-cluster II(b).

Sub-cluster I contains three accessions BAU litchi-1, BAU litchi-2, BAU litchi-3 and sub-cluster II(a) also contains two accessions BARI litchi-2, BARI litchi-3 which belongs to the same places separately, these accessions were found to be associated with each other according to their origin and habitat relatedness. While, cluster 2 contains only two accessions Bedana, Bombai and sub-cluster II(b) also contains two accessions Malaysian, Mongalbaria, which reflects a lower level of genetic diversity despite their different geographical locations. Tongpamnak *et al*. (2002) observed that there was no higher level of similarity among the cultivars originating from the same or nearby geographical locations by assessing RAPD markers. This DNA markers technique can be used to identify genetic variation and detect the relationship between DNA markers and horticultural traits of interest (Kumar *et al*., 2006). For this reason, RAPD technique has been employed to screen the germplasm in case of several higher plants.

The RAPD markers have been proved to be a powerful tool for molecular genetic analysis of litchi germplasm for plant breeding programmed to assess genetic diversity for the development of improved varieties that are able to withstand biotic and abiotic stresses. The present study was the preliminary study to assess genetic variation of litchi germplasm and it had some limitations in terms of limited number of individuals and germplasm, as well as number of primers used. The results of Bombai vs. Malaysian indicated that they are maintaining higher genetic variation than other germplasm. The present study might be used as a guideline for further study and used to be considered for sustaining the genetic qualities of litchi in Bangladesh. The study also provides a basis for litchi breeders to make informed choices on selection of parental material based on genetic diversity to help in overcoming the problems usually associated with a fruit tree crop improvement program.

CONCLUSION

It could be concluded that by assessing morphological characteristics among the litchi germplasm, Mongalbaria and BARI litchi-2 have a bright prospect for growing under the hilly areas in Sylhet, Bangladesh. On the other hand, two germplasm Bombai and Malaysian have the highest genetic distance than the others by using RAPD markers. The RAPD markers are the reliable and most effective tools for the plant breeders to reveal the genetic variation among the germplasm that ultimately helpful for further hybridization program. So, to get more clear findings, this experiment should be repeated for further verification up to the fruit settings of litchi germplasm.

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