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Original scientific paper

MORPHO-BIOCHEMICAL CHRACTERIZATION OF AMLA (*Phyllanthus emblica* L.) AND TAMARIND (*Tamarindus indica* L.) GERMPLASM FROM PAKISTAN

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Rabbani Ashiq M., S. Ahmad Jan, S. Uddin Siddiqui, A. Ghafoor and Z. Ahmad (2019): *Morpho-biochemical chracterization of amla* (*Phyllanthus emblica* L.) *and tamarind* (*Tamarindus indica* L.) *germplasm from Pakistan*.- Genetika, Vol 51, No.2, 539-549.

Amla (*Phyllanthus emblica* L.) and Tamarind (*Tamarindus indica* L.) are the least researched food plants in Pakistan although they have ample uses in food and herbal industry. Amla is being cultivated and observed as wild in most parts of the country including Sindh, Punjab and Khyber Pakhtunkhwa, whereas Tamarind is mostly confined to tropical areas mainly at Karachi. This study was focused to identify and collect the germplasm of both the species. Among all the collecting sites two types (Banarasi and Sheesha) of Amla were observed at farmers' fields, whereas wild or Desi types were under natural cultivation. Various important qualitative and yield related important quantitative traits were recorded. For biochemical characterization standard Sodium Dodecyl Sulphate- Polacrylamide Gel Electrophoresis (SDS-PAGE) protocol was used. The seed proteins were separated by using 11.25% polyacrylamide gel. The plants at farmers' fields were observed with a considerable level of variation. Low variation within Banarasi and Sheesha types might be due to limited number of mother plants by few commercial growers that needed to broaden through more germplasm collection/acquisition. High level of morphological diversity was observed among Amla and Tamarind

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genotypes. The protocol for total proteins in Amla did not exhibit good quality electrophoregram that is needed to refine for its practical utilization. The SDS-PAGE conducted for tamarind gave 11 protein bands and out of these six were polymorphic. The phylogenetic analysis classified all twenty Tamarind genotypes into seven diverged groups. Although most of the plants were similar on their phenotypic basis but the total proteins gave significant differences for most of the plants that indicated the diversity for genetic material and acclimatization to local environment.

*Keywords:*Collection, Local germplasm, Morpho-biochemical evaluation, Polymorphism, Propagation, SDS-PAGE

INTRODUCTION

Amla or Indian gooseberry (*Phyllanthus emblica* L.) is an indigenous to the Indian subcontinent. It is being cultivated and observed as wild in most parts of the Pakistan including Sindh, Punjab and Khyber Pakhtunkhwa, whereas tamarind is confined to tropical areas mainly at Karachi in the province of Sindh which might be indigenous or imported from neighbouring countries. Amla is a subtropical plant and prefers dry subtropical climate (ANONYMOUS, 2007). It grows both in plains and sub mountain land all over the Indian sub-content from 200-300 m altitude (PATHAK, 2003). For attractive prices, the fruits are graded on the basis of size and colour. People in Pakistan collect mature Amla fruits by shaking the trees when they are just ready to drop on the ground. In addition, they also collect the fruits that have already dropped on the ground and are sold in the market. The fruits remain in good condition for a longer period, provided they are handled properly. Amla fruits are a very rich source of vitamin C having ascorbic acid content of 0.9 to 1.3 percent, Jam (*Murabba)* is prepared from Amla fruits by keeping them in sugar syrup, a famous fruit product of Pakistan (OUDHIA, 2007). Other products include oil and shampoos and are reported to be effective. Besides fruits, leaves bark and seeds are being used for various medicinal purposes. Amla is of immense value in the traditional medicines in various Asian countries. Dried fruits have been reported to be useful in haemorrhages, diarrhea, dysentery, anemia, jaundice, dyspepsia and cough. Amla fruit is important ingredient of many formulations in herbal medicines. Most of the local practitioners consider indigenous types best for medicinal uses (OUDHIA, 2007). ZHO *et al.* (2015) reported its anticancer activities. According to GANESAN (2003), the marble green colour with less dark spots (fungal infections).

Tamarind (*Tamarindus indica* L.) is a multi-purpose tropical tree used primarily for its fruits, which are eaten fresh or processed for non-food uses. It belongs to the family Leguminosae (Fabaceae). The species has a wide geographical distribution in the subtropics and semiarid tropics, and cultivated in numerous regions (HAQ, 2001; KHANZADA *et al.*, 2008). It is commonly known as 'Imli' in Pakistan. Its fruit is marketed worldwide and used in sauces, syrups and processed foods (ICUC, 1999). It is full of pectin and is used in jams and jellies preparation. The pulp of the fruit is used as a spice in Asian cuisine having sour taste when young, whereas ripened fruit is sweet and can be used in desserts and drinks (KHANZADA *et al.*, 2008). Tamarind is also used as an alternative to tomatoes (BHADORIYA *et al.*, 2011).

Genetic diversity study through morphological and biochemical methods help in the identification and characterization of improved genotypes. The yield and yield related traits response varies with respect to plant species used (HLADNI *et al.*, 2016; JAN *et al.*, 2018; JAN *et al.*, 2017a ; SALEEM *et al.*, 2017). The SDS-PAGE is one of the key biochemical methods to

study protein based variability among different crop species. This method is quick and efficient and is used for the phylogenetic and taxonomic relationship among different plant species. It provides highly polymorphic protein bands (JAN *et al.,* 2017b; SHAH *et al.,* 2018; IBRAHIM *et al.,* 2017; QADIR *et al.,* 2017).

The plants growing wild are mostly used for medicinal purposes (mainly for lever disorder, hair tonic and stomach problems). Some improved clones are available; however planting material as well as knowledge about their production technology is limited. Although fruit of tamarind are used as condiment and spices in various preparations besides its medicinal uses, its production remained limited due to non-availability of suitable genotypes as well as access to its production technology. Both of these plant species can be grown even on marginal lands and have great potential for commercial scale cultivation. Therefore, the present study was conducted to study morpho-biochemical based variation among Amla and Tamarind germplasm for screening elite genotypes for further utilization.

MATERIALS AND METHODS

The present study was conducted to collect the germplasm of both species and preliminary evaluation for plant/fruit and protein profiling. The areas under planting of both the species were explored as a first step and germplasm of Amla and tamarind was collected for establishing field genebank for further utilization either for propagation or crop improvement. The germplasm of Amla was collected from nurseries and farmers' fields from Kamalia, Mian Channu, Patoki, Pir Mahal, Rajana, Vehari areas of Punjab province, Pakistan. The collected plants were planted under field conditions at National Agricultural Research Centre for further characterization.

As tamarind is mainly confined to tropical areas of Pakistan, therefore germplasm was collected from the vicinity of Karachi including Coastal Research Station, Gandhi Gardens, Gadap area, Malir, Koonker and from road side of super highway. During the collection expedition growers were also interviewed for sharing information on cultivation practices, harvesting, processing and uses of tamarind. In the surveyed area, commercial plantations of tamarind rarely exist but some farmers have managed 200-250 trees of tamarind at their farms along with other plantations such as coconut. The seed of twenty plants with conspicuous variability was collected and was planted in pots under greenhouse conditions for their preliminary characterization.

The total proteins were analyzed using slab type gel electrophoresis and the proteins were extracted from leaves (Amla) and seeds (tamarind). For the extraction of proteins, single seed or newly growing leaves in either case was crushed with the help of mortar and pestle. The 0.01 g of seed flour was added to new eppendorf tube with addition protein extraction buffer (400 µl). The sample was properly mixed with buffer with using mini glass rod. The protein extraction buffer included 0.5 M Tris-HCl (pH 6.8), 5% 2-mercaptoethanol and 2.5% SDS, 10% glycerol and minute quantity of Bromophenol Blue (BPB). Seed protein was separated by using 11.25% polyacrylamide gel. For checking the reliability and reproducibility of the method, two different gels were run with same electrophoretic conditions. The standard SDS-PAGE method of JAN *et al.* (2016) was used in the discontinuous buffer system with minor modification to study protein based variability among genotypes.

Data analysis

The data was analyzed by using the computer software "Statistica version 7.0" and "NTSYS pc 2.1".

RESULTS AND DISCUSSION

Morpho-biochemical based variability among Amla germplasm

There was not much variation observed for tamarind except the age of plant, whereas three types of Amla were observed in almost all the collecting areas. Based on preliminary characterization, under natural vegetation of Amla wild types (Desi) with very small fruit size, big tree and low to medium fruit yield were observed. The fruit of this type is considered important for medicinal use especially by the local practitioners. The second type was termed as 'Sheesha' with slightly transparent fruits, medium sized tree with high production. Most of the farmers used to plant this tree for herbal use and in pickle industry. The third type mainly called 'Banarasi' was characterized as light green large fruit size, medium to high tree size with high productivity (Figure 1). A considerable variation in fruit size was observed in all the three types of fruit of Amla. These were characterized by small fruit size in Desi and Sheesha types but large one in Banarasi types (Figure 2). Likewise, fruit color varied from green to light green fruit and solid green in Desi, Sheesha and Banarasi types respectively. When comparing all the three types, various farmers had different observations, but Desi types were marked unanimously as trees having dark green leaves medium sized, bearing small solid fruit in low to medium yield and trees were characterized as compact. Other two types were intermixed for most of the morphological traits except fruit texture that was slightly transparent for the cultivar 'Sheesha' and solid green in 'Banarasi'.

Figure 1. Desi (left), Sheesha (middle) and Banarsi type (right) of Amla at farmers' fields in Punjab

Figure 2. Comparison of fruit morphology of three *Phyllanthus emblica* L. types in Pakistan

Although there are three distinct classes on the basis of fruit size but there is no clear cut demarcation for uses. Either of these could be used for any specified purpose. On the basis of observation it was concluded that Southern Punjab could be a potential area for cultivation of Amla and uniformity of orchid could facilitate picking and other agronomic practices. There is dire need to evolve plants of low to medium height that will facilitate harvesting of fruit. Variation in improved clones, i.e., Sheesha and Banarasi types was very limited because most of these are produced by few nurseries with limited number of mother plants having narrow genetic background. An increasing trend in Amla cultivation was observed that might be due to economic returns, multiple uses and free market excess. There is a need to collect more germplasm from the areas with larger populations of wild types having potential of grafting with improved types. In case of tamarind, although a comprehensive survey was conducted and the information was recorded for preliminary plant descriptors but low variation was observed that indicated collection of more germplasm from abroad. SHAH *et al.* (2018) observed maximum morphogenic differences in some important Fenugreek germplasm. Similarly, JAN *et al.* (2018) and SALEEM *et al.* (2017) identified and characterized important *Brassica* genotypes through similar methods.

The total proteins were extracted from very young leaves of Amla using the standard protocol. Although the gel quality was not appropriate but one band specific to various three types was observed. The protocol is yet to be refined for accurate investigation that either this technique if valuable for identification of various types of Amla or it could only be implied for investigation of variation. Due to poor gel quality and less number of polymorphic bands, further analyses could not be conducted. SDS-PAGE was conducted for tamarind in various combinations and it was revealed that 11.25% acrylamide gel concentration gave the best results for both the species, whereas 6 µl of sample gave the best resolution for tamarind and 12 µl for Amla. Based on biochemical analysis, Banarasi gave a weak band that needed further confirmation by increasing sample size and clear characterization based on other traits. The morphological traits and significance of total protein profile for identification of Banarasi type Amla is presented in Table 1.

SHAH *et al.* (2018) and QADIR *et al.* (2017) recorded maximum polymorphic bands in important medicinal plant species through SDS-PAGE method.

Table 1. Comparison of three types of Amla on morphological and biochemical basis

Type	Morphological traits	Biochemical traits
Desi	Leaves medium in size and dark green, fruit size small, compact tree, green fruit, trees large	Not clear
Banarsi	Leaves small to medium in size and green, fruit size large, solid green fruit, loose tree (antonym to compact), trees medium to large size	Clear indication of one band at approximately 24 kDa
Sheesha	Leaves large in size and light green, fruit size large, slightly transparent fruit, light green fruit, intermediate tree (not very compact), trees medium to large size	Not clear

Seed Morphology, Seed-Pulp Ratio, 100 Seed Weight and SDS-PAGE Based Differences in Tamarind

Variation in seed-pulp ratio in tamarind was observed in sixteen accessions. Two types of tamarind were observed either under natural population or at farmers' fields. These were classified as sweet and sour and both of these types can be classified on the basis of seed size and shape (Figure 3). A third type called 'Hindi' is also available in the market with very large seed size. There was distinct variation on the basis of seed size and shape among various collections. Similarly there was significant variation for seed-pulp ratio that varied from 35.76 (Tamarind 026) to 73.49% (Tamarind 031) as mentioned in the Table 2. The variance (116) was found for this important trait (Table 2). Similarly, 100 seed weight ranged from 47.0 to 99.3 g that is expected to be more attributed to genetic control rather than environmental factors. Maximum 100 seed weight was recorded in genotype Tamarind 022 followed by 92.7gm in Tamarind 028. While lowest 100 seed weight was noted in genotype Tamarind 029.

Figure 3. Variation in seed size of tamarind

Table 2. 100-Seed weight and seed/pulp ratio in twenty accessions of Tamarind

Sr. No.	Accession No.	100 Seed Weight (g)	Seed/Pulp ratio
1	Tamarind021	57.6	69.31
2	Tamarind022	99.3	36.30
3	Tamarind023	82.0	36.73
4	Tamarind024	86.1	49.88
5	Tamarind025	62.3	42.82
6	Tamarind026	67.1	35.76
7	Tamarind027	58.0	50.42
8	Tamarind028	92.7	45.02
9	Tamarind029	47.0	40.36
10	Tamarind030	76.9	44.03
11	Tamarind031	86.9	73.49
12	Tamarind032	74.5	51.51
13	Tamarind033	79.2	60.20
14	Tamarind034	69.0	67.10
15	Tamarind035	68.2	46.32
16	Tamarind036	75.9	49.53
17	Tamarind037	77.4	54.08
18	Tamarind038	71.5	46.57
19	Tamarind039	80.1	55.25
20	Tamarind040	66.7	43.77
	Mean	73.9	49.9
	Minimum	47	35.76
	Maximum	99.3	73.49
	SD	12.6	10.8
	CV (%)	17.0	21.6
	Variance	158.5	116.0

In total, 11 protein bands were recorded for tamarind ranging from the molecular weight (MW) of 14 to 66 kDa. Many weak bands were observed but these were not taken into consideration due to inconstancy in reproducibility. A total of 11 protein subunits were recorded in which six (54.5%) bands were polymorphic and rest of the five (45.5%) were monomorphic (Figure 4). Maximum variation was observed in the region ranging from 24 to 45 kDa. Although most of these plants were similar phenotypically but the total proteins gave significant differences indicating the diversity for genetic material. At 50 percent genetic distance, eight clusters were observed and it was noted that two accessions of similar background were grouped in seven different clusters, whereas six accessions were together in cluster 5 (Figure 5). Based on these results it could be concluded that almost 60 percent of the material shared the protein banding pattern, although all of these looked similar for morphological characteristics. The SDS-PAGE has been frequently used for food crops to resolve the genetic dissimilarities in a variety of crop species (BOGYO *et al.,* 1980; ERSKINE and MUEHLBAUER, 1991; CLEMENTS and COWLING, 1994; PEZZOTTI *et al.,* 1994; RUIZ *et al.,* 1997).

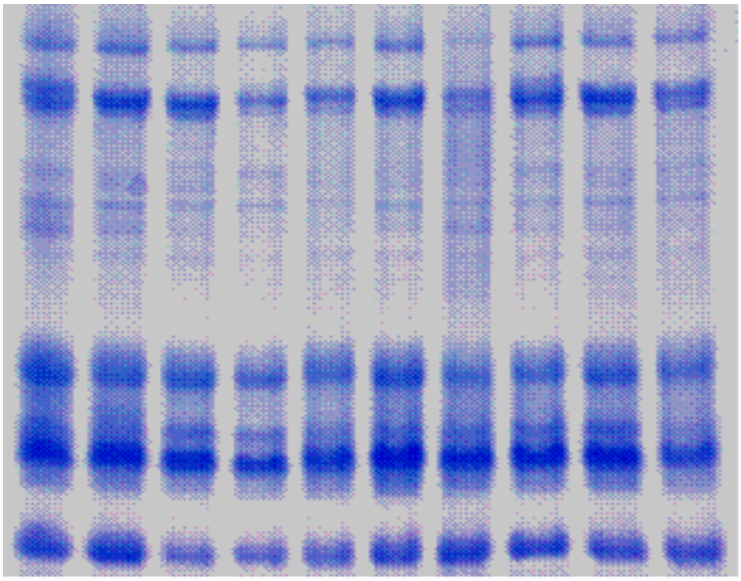

Figure 4. Variation in seed storage proteins of tamarind germplasm

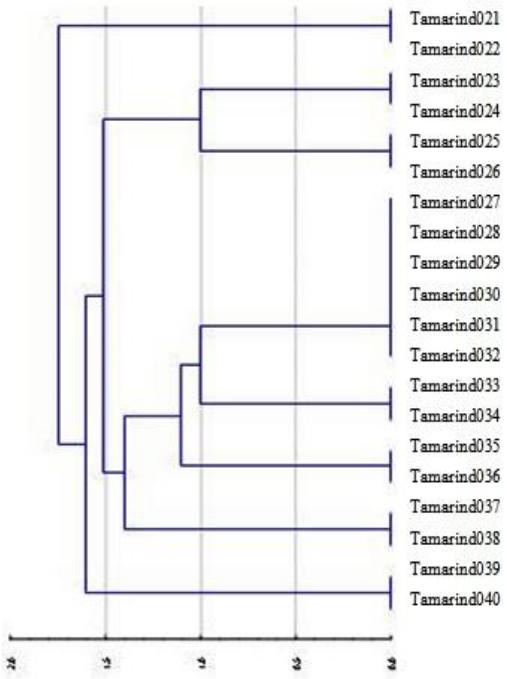

Figure 5. Clustering pattern of twenty accessions of tamarind based on UPGMA for total protein profile

CONCLUSION

Both the Amla and Tamarind genotypes showed maximum variability for both morphological and biochemical markers. However, the level of polymorphism was low in Amla as compared to Tamarind genotypes. This study provide basic information for genetic variation based on total protein profiles for tamarind and the data could be extended by including more germplasm and biochemical as well as molecular markers. Although most of the germplasm was collected from close vicinity but distinct clustering pattern indicated the genetic dissimilarities that could be due to different source of plant material that is yet to confirm either by having information from the growers and/or including the germplasm in the analyses from probable source.

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MORPHO-BIOHEMIJSKA KARAKTERIZACIJA GERMPLASME AMLE (*Phyllanthus emblica* L.) I TAMARINDA (*Tamarindus indica* L.) IZ PAKISTANA

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Izvod

Amla (*Phyllanthus emblica* L.) i tamarind (*Tamarindus indica* L.) su najmanje istraživane prehrambene biljke u Pakistanu, iako imaju široku primenu u prehrambenoj i biljnoj industriji. Amla se gaji i tretira kao divlja u većini delova zemlje, uključujući Sind, Pendžab i Khiber Pakhtunkhva, dok je tamarind ograničen na tropska područja uglavnom u Karačiju. Ovo istraživanje je bila fokusirano na identifikaciju i sakupljanje germplazme obe vrste. Među svim mestima sakupljanja dva tipa amle (Banarasi i Sheesha) su posmatrana na poljima farmera, dok su divlji ili Desi tipovi bili pod prirodnom kultivacijom. Zabeležena su različita važna kvalitativna i kvalitativna svojstva vezana za prinos. Za biohemijsku karakterizaciju korišćen je standardni SDS-PAGE protokol. Proteini semena razdvojeni su korišćenjem 11.25% poliakrilamidnog gela. Biljke na poljima farmera imale su značajan nivo varijacije. Niske varijacije unutar tipova Banarasi i Sheesha mogu biti uzrokovane ograničenim brojem biljaka majke kod nekoliko komercijalnih uzgajivača, što je potrebno proširiti kolekcionisanjem/ prikupljanjem germplazme. Visok nivo morfološke raznolikosti je uočen kod genotipova amle i tamarinda. Protokol za ukupne proteine kod amle nije pokazao elektroforegram dovoljno dobrog kvaliteta, što je potrebno poboljšati zbog njegove praktične upotrebe. SDS-PAGE sproveden za tamarind je dao 11 proteinskih traka od kojih je šest bilo polimorfno. Filogenetska analiza svrstala je svih 20 genotipova tamarinda u sedam različitih grupa. Iako je većina biljaka bila slična po svojoj fenotipskoj osnovi, ukupni proteini su dali značajne razlike za većinu biljaka koje su ukazale na diverzitet genetskog materijala i aklimatizaciju na lokalnu sredinu.

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