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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (6): 888-893, 2008
ISSN 1028-8880

Fruit Properties and Genetic Diversity of Five Ber (*Ziziphus mauritiana* Lamk) Cultivars

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Abstract: The present study was conducted on five ber (*Ziziphus mauritiana* Lamk) cultivars (Komethry, Pakstany, Um-sulaem, Toffahy and Peyuan) grown in Saudi Arabia during 2005 and 2006 seasons. The aim of the present study was to investigate fruit properties (fruit weight, length, diameter, shape, specific gravity, seed weight, pulp percentage, total soluble solids (TSS), acidity percentage, TSS/acid, vitamin C content total, reducing and non-reducing sugars). Peyuan cv. had the heaviest fruit weight, fruit volume and reducing sugar content however, it was the lowest in pulp percentage and non-reducing sugars among the five cultivars in both seasons. Toffahy cv. had highest fruit diameter and seed weight while, had lowest TSS %, vitamin C and total sugars values. Um-sulaem cv. had highest acidity percentage and vitamin C content and lowest fruit weight, length and TSS/acid. On the other hand, Pakstany cv. had highest percentage of both pulp percentage, TSS, total and non-reducing sugars. Finally, Komethry had the longest fruit. The molecular characterization and fingerprint identification of the ber cultivars was conducted using the ISSR (Inter-Simple Sequence Repeats) technique. The ISSR technique was able to uniquely characterize and differentiate between the five ber genotypes. Moreover, the genetic similarity tree showed that the cultivar Um-slaem is genetically distant from the other four cultivars and the two cultivars Pakstany and Komethry were genetically identical.

Key words: Fruit properties, ber, *Ziziphus mauritiana* Lamk, fingerprinting, genetic diversity, ISSR

INTRODUCTION

Ber (*Ziziphus mauritiana* Lamk) belongs to the Rhamnaceae family. Of the well-known species of the genus *Ziziphus*, ber (*Z. mauritiana*) is the most common in the tropical and sub-tropical regions, while *Z. jujuba* is well known in temperate parts of the world. These species are indigenous to North Africa, Afghanistan, North India, Southern China, Malaysia and Queensland in Australia. However, ber is now widely distributed and has become naturalized in tropical Africa, Iran, Syria, Sri Lanka and part of the Mediterranean (Kaarira, 1998). Ber can provide food security, due to sustained production of the fruit, irrespective of drought, as the tree is drought and saline tolerant and can grow on poor degraded land (Pareek, 2001). Ber fruits are very nutritious and usually eaten fresh. Fruits are also eaten in other forms, such as dried, candied, pickled, as juice, or as ber butter (Maydell, 1986). The fruits are a drupe, varying from round to elongate and from cherry-size to plum-size depending on cultivar (Reich, 1991). The ber cultivars were varying in fruit physio-chemical characteristics. Fruit weight ranged from 3.8 to 39.5 g; fruit length ranged from 1.82 to 5.80 cm, diameter 1.1 to 4.7 cm. Fresh mature ber fruits contains 81 to 97% pulp (Chovatia *et al*., 1993; Jawanda *et al*., 1981; Ghosh and Mathew, 2002). Ber pulp contains 12-23% TSS, 0.13-1.42% acidity, 3.1-14.5% total sugars, 1.4-9.7% reducing sugars, 5.6% sucrose, 1.5% glucose, 2.1% fructose and 1.0% starch (Bal, 1992; Ghosh and Mathew, 2002). Ber pulp is a rich source of vitamin C. Jawanda and Bal (1978) reported that ascorbic acid content in different ber cultivars ranged from 39-166 mg/100 g of pulp.

In recent years, a series of molecular markers techniques have been developed to analyze and estimate genetic diversity in plant species. Among the various marker systems, the randomly amplified polymorphic DNA (RAPD) is one of the most popular DNA-based approaches (Martin and Hernandez Bermejo, 2000; Bekessy *et al*., 2002; Abdel-Mawgood *et al*., 2005, 2006). The RAPD technique is a potentially simple, rapid, reliable and effective. In addition, it has the advantage of that there is no prior knowledge of DNA sequence information is required. However, there is a major disadvantage associated with it which is the lack of reproducibility between laboratories (Pooler and Scorza, 1995; Weeden *et al*., 1992). On the other hand, the ISSR is more reproducible than the RAPD technique and is preferred. The ISSR markers are generated from single-primer PCR reactions where the primer is designed from di-or

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trinucleotide repeat motifs with a 5 or 3 anchoring sequence of one to three nucleotides (Wolfe and Liston, 1998), without the requirement for prior sequence information (McGregor *et al*., 2000). Moreover, ISSR technique producing a high degree of polymorphism, generating reliable information for DNA analysis with the necessary sensibility to distinguish among individuals genetically related. ISSR analysis is technically simpler than many other marker systems. The method provides highly reproducible results and generates abundant polymorphisms in many systems.

The ISSR technique was successfully applied to study genetic diversity in *Astragalus oniciformi* populations (Alexander *et al*., 2004), *Penstemon* sp. (Wolfe *et al*., 1995) and in taxonomic studies of *Vigna* (Ajibade *et al*., 2000). In ornamental species ISSRs have been used in Escandon *et al*. (2005); *Nierembergia* (Escandón *et al*., 2005), *Pandorea* sp. (Jain *et al*., 1999) and *Chrysantemum* (Wolfe *et al*., 1995). The ISSR strategy was applied to generate fingerprints in newly developed inbred lines of wheat (Abdel-Mawgood, 2007).

The objectives of this research were to study the fruit properties and genetic diversity of five ber (*Ziziphus mauritiana* Lamk) cultivars (Komethry, Pakstany, Um-sulaem, Toffahy and Peyuan) grown in Saudi Arabia.

MATERIALS AND METHODS

Plant genotypes: The present study was conducted during 2005 and 2006 seasons in order to study the fruit properties and genetic diversity of five ber (*Ziziphus mauritiana* Lamk) cultivars grown in Saudi Arabia namely; Komethry, Pakstany, Um-sulaem, Toffahy and Peyuan (local names). Eight-year old orchard trees were budded on (*Ziziphus spina-christi* Lamk) rootstock, grown in the Agricultural Research and Experiment Station (Dirab), College of Food Sciences and Agriculture, King Saud University, Riyadh, Saudi Arabia.

Fruit properties: Fruits were harvested at the first week of April. Random samples of 50 fruits were harvested from each tested tree (five replicates in each cultivar) for fruit quality determinations. In each fruit sample, fruit weight; volume, length, diameter, fruit shape and seed weight were determined. The percentage of total soluble solids (TSS) was determined in fruit juice using BRX-242 digital refractometer. Juice acidity percentage (estimated as citric acid equivalent) was determined by titration with NaOH and phenolphthalein indicator. Vitamin C (Ascorbic acid) was determined by titration with 2, 6 dichlorophenol-endophenol blue dye and expressed as ascorbic acid (mg per 100 g pulp). Reducing, non-reducing and total sugars were determined according to AOAC (1986).

DNA extraction: DNA was extracted from lyophilized young leave powder. Twenty milligrams of lyophilized powder was used for genomic DNA extraction using the DNeasy Plant Mini Kit (Qiagen Inc., Mississauga, ON, Canada) according to the manufacturer's directions. Extracted DNA was quantified by spectrophotometer followed by dilution to 25 mg μL-L for ISSR analysis.

ISSR protocols: The Polymerase Chain Reaction (PCR) for the ISSR (Inter-Simple Sequence Repeats) was performed in a final volume of 25 μL containing 1xTaq polymerase buffer, 0.5 units of FastStart Taq polymerase (Qiagen), 200 mM of each dNTPs (Promega, USA), 15 ng of random primer (UBC, 2005), 2.5 mM $MgCl_2$ and 25 mg of genomic DNA. The reaction mixture was performed using the following cycling parameters: 1 cycle of 15 min at 94°C, followed by 45 cycles of 30 sec at 94°C, 1 min at 50°C, 2 min at 72°C and a final step of 10 min at 72°C. Amplified PCR products were separated on 1.5% agarose gel in 1xTBE buffer (100 mM Tris-HCl, pH 8.0, 83 mM boric acid, 1 mM EDTA) at 50 volts. The gels were stained with 0.5 μg mL^{-1} ethedium bromide solution and visualized by illumination under UV light. The sizes of the amplified products were determined by comparison with 100 bp ladder (Promega, Wisconsin, USA) (Sambrook *et al*., 1989).

Data analysis: The experimental design was completely randomized blocks. Each cultivar (five cultivars) involved five replications of 50 fruits each. Statistical analysis was performed with SAS software package version 6.03 (SAS, 1988). To examine the genetic relationship between the five cultivars, a dendrogram was constructed using a UPGMA analysis as implemented by NTSYS-pc, Version 2.02c (Rohlf, 1997). The PCR data generated from the eight different primers were scored into 0 and 1. For each genotype, the presence of a band (1) or its absence (0) was scored.

RESULTS AND DISCUSSION

Fruit properties: Fruits morphology of the five ber cultivars are clear difference in the morphological characters (Fig. 1). The fruit quality for five ber cultivars shown in Table 1 and 2 during 2005 and 2006 seasons.

Peyuan cultivar had the largest fruit weight (32.69 and 33.3 g) followed by the other cultivars: Toffahy (31.72 and 31.32 g), Komethry (21.51 and 22.61 g), Pakstany (14.98 and 15.68 g) and Um-sulaem (14.26 and 14.72 g) in both seasons, respectively. Regarding the average fruit length, Komethry cultivar fruit was the tallest (5.83 and 5.87 cm), while Um-sulaem cv. exhibited shortest (3.31 and 3.19 cm) and peyuan was intermediate. However, insignificant

Table 1: Fruit properties of five ber cultivars during 2005 growing seasons

	Cultivars					
Properties	Komethry	Pakstany	Um-sulaem	Toffahy	Peyuan	$LSD_{0.05}$
Fruit weight (g)	21.51	14.98	14.26	31.72	32.69	0.86
Fruit length (cm)	5.83	4.40	3.31	4.04	4.39	0.10
Fruit diameter (cm)	2.72	2.48	2.90	4.00	3.52	0.06
Fruit shape	2.14	1.77	1.14	1.01	1.25	0.05
Fruit volume (cm^3)	21.50	14.25	16.95	35.50	36.02	0.73
Specific gravity	1.00	1.05	0.84	0.89	0.91	0.04
Seed weight (g)	1.12	0.72	0.73	2.09	1.96	0.13
Pulp (%)	94.81	95.22	94.91	93.40	91.32	0.53
TSS (%)	10.75	12.55	10.50	9.00	10.86	0.85
Acidity (%)	0.48	0.35	0.82	0.62	0.44	0.04
TSS/Acid	22.52	36.15	12.90	14.58	24.62	1.95
Vitamin C (mg/100 g pulp)	120.63	62.88	135.28	43.20	56.45	2.60
Reducing sugar (%)	5.37	4.82	4.47	4.83	6.37	0.41
Non-reducing sugar (%)	4.04	5.68	3.77	2.85	2.22	0.41
Total sugar (%)	9.41	10.50	8.24	7.67	8.59	0.72

Table 2: Fruit properties of five ber cultivars during 2006 growing seasons

	Cultivars					
Properties	Komethry	Pakstany	Um-sulaem	Toffahy	Peyuan	$LSD_{0.05}$
Fruit weight (g)	22.61	15.68	14.72	31.32	33.03	1.33
Fruit length (cm)	5.87	4.34	3.19	3.96	4.41	0.09
Fruit diameter (cm)	2.87	2.44	2.92	4.01	3.53	0.08
Fruit shape	2.04	1.79	1.09	0.99	1.25	0.04
Fruit volume (cm^3)	22.10	14.51	17.22	35.63	36.33	0.87
Specific gravity	1.02	1.08	0.86	0.90	0.91	0.03
Seed weight (g)	1.17	0.72	0.73	1.98	1.96	0.13
Pulp (%)	95.00	94.00	95.00	93.75	91.00	1.15
TSS (%)	10.60	12.83	9.83	8.93	10.86	0.87
Acidity (%)	0.50	0.36	0.83	0.61	0.43	0.05
TSS/Acid	21.15	35.94	11.93	14.67	25.48	3.26
Vitamin C (mg/100 g pulp)	120.15	65.00	133.50	43.95	57.90	2.08
Reducing sugar (%)	5.77	5.12	4.77	5.03	6.55	0.52
Non-reducing sugar (%)	4.34	5.75	3.97	2.85	2.48	0.47
Total sugar (%)	10.01	10.87	8.64	7.88	9.03	0.76

Fig. 1: Fruit morphology of the five ber cultivars; A = Komethry, B = Pakstany, C = Um- sulaem, D = Toffahy and E = Peyuan

difference was found between Pakstany and Toffahy cultivars. Toffahy cultivar had the highest fruit diameter (4.0 and 4.01), while Pakstany had the least diameter (2.48 and 2.44 cm). It was noticed that Toffahy and Peyuan have a significant different in their diameters compared with other cultivars. However, cultivars Komethry, Pakstany and Um-sulaem showed no significant difference in their diameters (2.72, 2.48 and 2.90 cm), respectively. The data in Table 1 and 2 indicated that Komethry had the greatest value of fruit shape (2.14 and 2.04). This value differs significantly with other cultivars fruit shape. On the other hand, Toffahy cv. gave the least value of fruit shape (1.01 and 0.99).

Concerning the fruit volume there is a clear difference between the five cultivars. Toffahy and Peyuan have significantly larger fruit volume than the other three cultivars (35.53 and 36.33 cm^3, respectively). While the cultivar Um-slaem has the smallest fruit volume (16.85 and 17.22 cm^3) in both seasons, respectively. The highest specific gravity was achieved in fruits of Pakstany cv. (1.05 and 1.08) flowed by Komethry cv. (1.0 and 1.02), while the lowest specific gravity was found in Um-suleam cv. (0.84 and 0.86) in both seasons, respectively. For seed weight character, the cultivar Toffahy and Peyuan showed no significantly difference in seed weight. However, they differ significantly with the other three cultivars. The heaviest seed were collected form Toffahi cv. (2.0 g), while the lightest seeds were from Pakstany cv. (0.72 g). In all studied cultivars pulp represent a high percentage of fruits which exceeded 90%. The cultivars of Komethry, Pakstany and Um-suleam gave greater pulp percentage than other cultivars. Seemingly, pulp percentage showed a relationship with fruit weight and seed weight.

Regarding TSS percentage in fruits, it was observed that Pakstany cv. had the highest percentage (12.55 and 12.83%) while the least TSS percentage was obtained in Toffahy cv fruits (9.0 and 8.93%) for tow season, respectively. There was no significant difference in TSS percentage in Kemethry, Um-suleam and Peyuan cultivars in the both seasons. The fruit of Am-suleam cv. had a significantly high content of juice acidity percentage compared to the other two cultivars. On the other hand, Pakstany cv. contained the least percentage of acidity. Data in Table 1 and 2 indicated that fruits of Um-suleam cv. had a significantly higher content of V. C. (135 and 133.5 mg/100 g pulp) than other cultivars. There was a significant difference between the fruits of cultivars in their total sugar. The cultivar Pakstany has a significantly higher value of percentage of total sugar than the other cultivars, but lower percentage was obtained in the fruits of the cultivar Toffahy (7.67 and 7.88%). However, in terms of reduced sugar content the fruits of Peyuan were significantly higher than other cultivars, while the least percentage of reduced sugar was showed in the fruits of the cultivar Um-suleam (4.47 and 4.77%). Meanwhile, the greatest percent of non-reducing sugar was found in fruits of Pakstany cv. and the lowest was in Peyuan.

The data presented in this study which described the physical and chemical properties of fruits of different ber cultivars were in the range obtained by previous studies. For example the findings of fruit weight are in line with those reported by Chovatia *et al.* (1993) and Jawanda *et al.* (1981). The measurement of fruit length in the current study is in agreement with those reported by Morton (1987) and Pareek and Sharma (1991). However, fruit pulp and chemical contents measurement were varied than those reported earlier. For instant, in the current study, ascorbic acid content was ranged from 43 to 135. These results agree with those found by Jawandas and Bal (1978). The total sugar content and reducing sugar were in agreement with the data mentioned by Bal (1992) and Ghosh and Mathew (2002).

Genetic diversity: There is a complete lack of information on the extent of genetic diversity in ber (Singh *et al.*, 2006). For our knowledge, this is the first study to investigate the extent of genetic diversity of cultivated ber genotypes in Saudi Arabia. Total of 15 ISSR primers have been used in this study. Out of which only 8 ISSR primers gave good polymorphism with reproducible pattern. The sequence of the primers, the number of bands scored for each primer, the number of polymorphic bands and the percentage of polymorphism is described in Table 3. All the eight primers produced polymorphic bands. Two primers gave 100% polymorphism. The two primers are P2 $(AGAG)_4T$ and P7 $(CTCT)_4T$; they also gave a pattern that can be used for fingerprinting to distinguish between the five cultivars. The rest of the primers gave polymorphism ranged from 25 to 70%. The gel patterns using the ISSR primer (P2) $(AGAG)_4T$ are given in Fig. 3.

The percentage of polymorphism as an average of the eight primers is 53%. However, in another study (Singh *et al.*, 2006) using 11 primer pairs and AFLP technique, the percentage of polymorphic bands was 84%. The difference between the two studies in the percentage of polymorphic alleles could be due to the using of different genotypes and that our genotypes are cultivated which always have a less genetic diversity than the wild types (Singh *et al.*, 2006), this is an addition to the difference in both the ISSR and AFLP in showing the polymorphism.

Table 3: ISSR primer used in this study, number of bands from each primer, polymorphic bands and percentage of polymorphism

Primer sequence (5'- 3')	No. of bands	Polymorphic bands	Polymorphism (%)
ATA TAT ATA TAT ATA TT	14	7	50.0
AGA GAG AGA GAG AGA GT	10	10	100.0
AGA GAG AGA GAG AGA GC	10	7	70.0
AGA GAG AGA GAG AGA GG	8	5	62.5
GAG AGA GAG AGA GAG AT	10	3	30.0
GAG AGA GAG AGA GAG AA	12	3	25.0
CTC TCT CTC TCT CTC TT	10	10	100.0
CTC TCT CTC TCT CTC TA	8	7	87.5

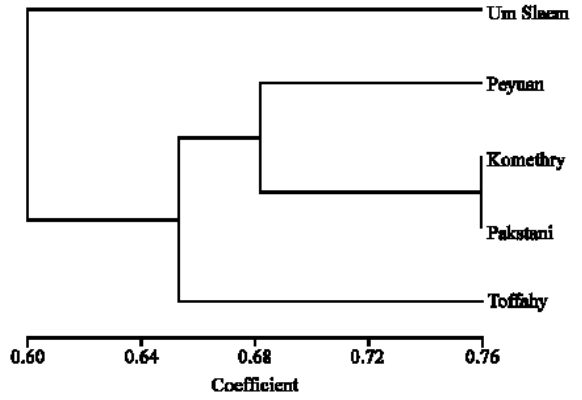

Fig. 2: Genetic similarity coefficient between the five ber cultivars

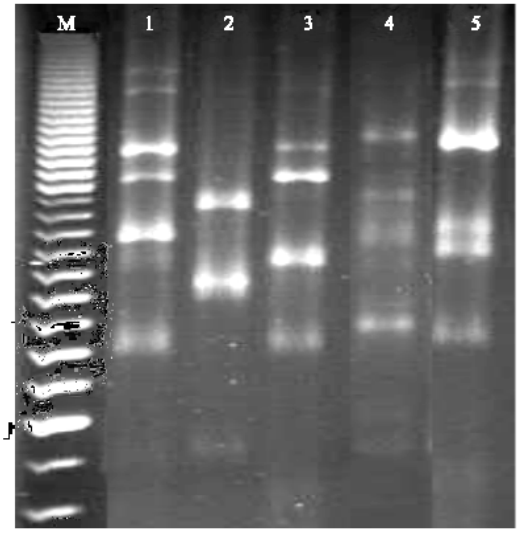

Fig. 3: Polymorphism based on the ISSR of the five genotypes using the ISSR primer $(AGAG)_4T$. M, is 100 bp molecular weight marker. lane 1: Komethry, 2: Toffahy, 3: Payuan, 4: Um-Sulaem and 5: Pakstany

The genetic similarity of tree produced from the above data is shown in Fig. 2. The cultivar Um-Sulaem was genetically distant from the other four cultivars with genetic similarity of 60%. The other four cultivars have genetic similarity coefficient close to 65.5%. The second branch was represented by the cultivar Toffahy and the rest of the three cultivars. The third branch was represented by Komethry, Pakstany and Payuan cvs. The two cultivars Komethry and Pakstany were very closely related with genetic similarity of 76%. The only published results so far on ber genetic diversity were by Singh *et al.* (2006), who used the AFLP technique to study genetic diversity in 33 cultivated ber accessions. The genetic diversity index between the 33 accessions ranged from 0.14 to 0.86 with an average of 0.62. They also stated that the genetic diversity between the cultivated species is a lot less than that in the wild relatives that are used as a rootstock. Their finding is in agreement with our data, which showed that genetic similarity between the cultivars under this study is high.

As indicated earlier the cultivar Um-Sulaem was genetically distant from the rest of the cultivars. It has the lowest value of fruit weight and length, seed weight, TSS/acid and reducing sugars. However, it has the highest vitamin C content, pulp percentage and acidity percentage. On the other hand, although both the Komethry and Pakstany cultivars were very closely related to each other based on the genetic tree, although there was no similarity between the two cultivars in their fruit properties.

From the present study it could be concluded that fruits of Peyuan cv. is preferable for the customer according to their physical and chemical properties. The molecular characterization and fingerprint identification of the ber cultivars using ISSR technique is able to uniquely characterize and differentiate between the five ber genotypes.

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