MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL GENETIC STUDIES ON SOME INDIAN JUJUBE (*ZIZIPHUS MAURITIANA* LAMK.) VARIETIES GROWN AT EL NUBARIA

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Abstract

The present study was conducted in a private orchard during 2007 and 2008 seasons in order to evaluate the morphological, physiological characteristics and molecular marker in the two Indian jujube varieties, Balahy and Tofahy. The results showed that: Tofahy variety recorded the higher vigorous growth, leaf area, leaf length, leaf width and length of primary and secondary shoots, while Balahy variety recorded the higher leaf length/width ratio. Tofahy variety recorded the higher fruit set, fruit number/tree, fruit weight, yield, fruit width, fruit pulp thickness while Balahy variety recorded the higher fruit length, fruit length/width ratio, fruit firmness, seed weight and seed/fruit weight ratio. Tofahy variety recorded the higher fruit acidity, ascorbic acid while Balahy variety recorded the higher leaf and fruit total chlorophyll, fruit TSS/acidity ratio. Tofahy variety recorded the higher leaf and fruit nitrogen, phosphorus, calcium, magnesium, iron, manganese and zinc, leaf potassium and fruit sodium while, Balahy variety recorded the higher fruit potassium. No significant differences were recorded between both varieties concerning fruit drop, volume, TSS, reducing, non-reducing and total sugars, juice pH, chlorophyll A, B, carotene and leaf sodium. Genetic similarity was found for the two varieties in elecrophoresis analysis, but there were, little differences at the sequence of amino acids in protein and DNA bands.

INTRODUCTION

Ber or the Indian jujube belongs to the family *Rhamnaceae* that consists of about 45 genera and 550 species. It is widely distributed in tropical and sub-tropical climates in the world. Ber can be successfully cultivated even in the most marginal lands with few agriculture inputs and little attention. The Indian jujube has various names in different languages of the world there are, in Arabic, Aunnabe Hindi, Nabig, Sidr, while, in English, ber, Chinese Date, Indian Cherry, Indian Jujube, Indian Date, Indian Plum. The Indian jujube tree is a vigorous grown and has a rapidly developing tap root system within a short period and it can also withstand alkalinity and slightly water-logged condition. Ber tree may be a bushy shrub, or a tree, erect or wide – spreading, with gracefully drooping branches and downy, zigzag branchlets, thornless or set with short, sharp straight or hooked spines (Morton, 1987). This quick growing

tree starts producing fruits within three years. It will not set fruit by self –pollination, because in the ber varieties both self and cross incompatibility has been reported by Pareek (1983). The fruits are drupe, globose to ovoid, up to 6×4 cm in cultivation, usually much smaller when wild, skin smooth or rough, glossy, thin but tough, yellowish to reddish, flesh white, crisp, juicy, subacid to sweet, becoming mealy in fully ripe fruits. The average yield of this tree for the different varieties during the prime bearing age of about 10 to 20 years ranged between 80 to 200 Kg fruits per tree (Pareek, 1983). The different part of Indian jujube trees are used in different uses. The leaves and twigs of most species can be used as nutritious fodder for livestock, due to the high dry weight protein content (Ngwa and Mafeni, 2000). The ber tree also, can be used in medicinal uses as well as, the fruits are applied on cuts of ulcers, are employed in pulmonary ailments and fevers indigestion and biliousness. The dried ripe fruit is a mild laxative. They check diarrhea and are poultice on wounds. The leaves are helpful in liver troubles, asthma and fever. Juice of the root bark are used to alleviate gout and rheumatism. An infusion the flowers serve as an eye lotion (Morton, 1987). New plant varieties can now get protection in many more countries. With the ability of getting highly specific, DNA fingerprinting will provide an objective evaluation of genetic identity of plants based on species, cultivars or geographic origin. DNA fingerprint can prove that a new variety satisfies necessary criteria for granting protection. These criteria may include novelty, distinctiveness, uniformity and stability. For administrators of plant property rights, DNA fingerprinting can help in selecting most suitable reference varieties for morphological comparison and save cost. It is most effective in enforcing protection by proving infringement of property rights.

MATERIALS AND METHODS

The present investigation was conducted during 2007 and 2008 successive seasons in a private orchard (Nubaseed company farm) on the two Indian jujube *(Ziziphus mauritiana* Lamk) varieties namely, Balahy and Tofahy. The selected trees were twelve years old of uniform size, planted at 5×5 meters apart in sandy soil, grafting on *Ziziphus spina christi* rootstocks. Thirty trees (fifteen trees from each variety) were randomly selected. Five replicates and three trees for each replicate for each variety were used in this study. Experimental trees were arranged in complete block design randomized statistical analysis was carried out using LSD according to Snedecor (1980).

The following parameters were recorded

1- Morphological characteristics

1- During October, a sample of ten mature leaves were chosen, leaf area, length and width were recorded and leaf length/width ratios were calculated.

2- In January, tree height and length of primary and secondary shoots were recorded by graduated tape.

2- Fruit set and drop percentages

1- To study the fruit set %, number of flowers were chosen in four branches around each tree in August, then after one month the fruit set percentages were determined according to the following equation:

$$\text{Fruit set}\% = \frac{\text{No. of developing fruitlets}}{\text{No. of flowers at full bloom}} \times 100$$

3- To study the fruit drop percentages remainder fruits were calculated in October through the following equation:

4-

$$\text{Fruit drop}\% = \frac{\text{No. of developed fruitlets - No. of remained fruit}}{\text{No. of developed fruitlets}} \times 100$$

3- Yield

Average fruit weight was determined by weighting the fruit sample (10 fruits) and average weight per fruit was calculated. Moreover, number of fruits per tree was calculated and total yield was determined as follows:

Total yield (Kg/tree) = No. of fruits tree × average fruit weight.

4- Fruit characteristics

A sample of 10 mature fruits were taken during December from each tree to determine the physical and chemical characteristics

1. Physical fruit characters

- Fruit length and width (cm) were measured by the calliper, fruit length/width ratio were also calculated.
- Fruit volume was measured via water displacement by using graduated cylinder.
- Fruit firmness was measured by hand fruit pressure tester.
- Fruit pulp thickness (cm) was measured.
- Seed weight was determined by weighting sample of 10 seed, seeds weight/ fruit weight ratio was also calculated.

2. Chemical fruit characters

- Total Soluble Solids (TSS): were measured by hand refractometer in pulp juice.
- Acidity was determined as citric acid (%) in juice.
- Fruit juice pH was determined by pH meter.
- Ascorbic acid fruit content (V.C) was measured in juice (mg/100g) by titration with 2,6 Di-chlorophenol indophenol according to A.O.A.C. (1980).
- Fruit content of sugars was determined, the flesh of each fruit sample was cut into small pieces by a clean knife and mixed well. Five grams of the cut flesh were used for water extraction by distilled water according to A.O.A.C. (1980). The total sugars were determined colorimetically using phenol and sulphoric acid according to Malik and Singh (1980). The reducing sugars were determined by the Nelson arsenate-molybdate colorimetric method according to Dubios *et. al*. (1956). The non-reducing sugars were calculated by the difference between total sugars and reducing sugars.
- Fruit pigments: chlorophyll a, b, total chlorophyll and carotene (mg/100g) were determined in skin according to Wensttein (1957).

5- Leaf chlorophyll

The average of 10 reading was taken during October on the leaves of the middle of the shoots from allover the tree circumference to determine the total chlorophyll content as SPAD unit using non destructive chlorophyll meter according to Monje and Bugbee (1992).

6- Leaves and fruits mineral content

In both studied seasons, ten leaves, during October, were selected for each tree under study to determine the leaf mineral content. In addition, during December for each season, ten fruits were taken from each tree under study to determine the fruit mineral content. The dried leaves and fruits samples were digested by sulphoric acid and hydrogen peroxide according to Evenhuis and Dewaard (1980). In this digested, solution nitrogen, phosphorus, potassium, calcium, magnesium, sodium, iron, manganese, copper and zinc were determined. Total nitrogen and phosphorous were determined by using Spectophotometer. Calcium and magnesium were determined by versenate (EDTA) method according to Cheng and Bray (1951). Potassium and sodium were measured against a standard using Flamephotometer. Iron, manganese, copper and zinc were determined by Perkin Elmer Atomic Absorpation Spectophotometer. Nitrogen, phosphorous, potassium, calcium, magnesium and sodium were expressed as a percentage (%) on dry weight basis, while, iron, manganese, copper and zinc were expressed as part per of million (ppm).

7- Electrophoretic analysis

Young fresh leaf samples of two Ziziphus *mauritiana* (Balahy and Tofahy) varieties were kindly supplied to the Horticulture Research Institute, Agricultural Research Center, Giza. Egypt. Samples from the experimental trees were gathered separately from each variety and treated for both SDS-protein and isozymes extraction.

A- SDS-protein electrophoresis

About 0.2 g of the plant materials was homogenized separately in 200 µl buffer containing 1 M Tris-HCl pH 8.8 and 0.25 M EDTA. After centrifugation at 10.000 rpm for 10 minutes, 100 µl supernatant containing water soluble protein were transferred to a new Eppendorf tube and mixed with 800 µl acetone and then kept in a freezer for 15 minutes. After centrifugation at 10.000 rpm for 10 minutes, the pellets were re-suspended in 80 µl buffer containing 1 M Tris- HCl pH 8.8, 0.25 M EDTA, 10 % SDS and 10 % glycerol. Mercaptoethanol was added to each tube and boiled in water bath for 10 minutes, SDS- polyacrylamide gel electrophoresis was performed 12.5 % acrylamide slab gels following the system of Laemmli (1970). Electrophoresis was run at a current of 15 mA for 30 minutes followed by 25 mA till the tracing bromophenol blue dye reached the gel bottom. Molecular weights of different bands were calibrated with the following protein subunits as molecular weights standard: Isozyme (14.3 kDa), bovine serum albumin (66.2 KDa), trypsin in hibitor (21.5 kDa), carbonic anhydrase (30.0 kDa), ovalbumin(46.2 kDa), and phosphorylase b (97.4 kDa)

B- Isozymes electrophoresis

Isozyme extraction from the different treated trees of *Ziziphus mauritiana* varieties was performed separately for each variety and treated by homogenizing 0.5 g fresh leaf samples in 1 ml extraction buffer using a mortar and pestle. The extract was then transferred into clean Eppendorf tubes and centrifuged at 10000 rpm for 5 minutes. The supernatant was transferred to new clean Eppendorf tubes and kept at -20°C until using electrophoretic analysis.Vertical slab gel electrophoresis apparatus was used according to Stegemann *et. al.* (1985). A volume of 40 µl extract each sample was mixed with 20 µl sucrose and 10 µl bromophenol blue, then a volume of 50 µl from this mixture was applied to each well. The run was performed at 30 volt until the bromophenol blue dye has reached the separating gel and then the voltage was increased to 70 volt. After electrophoresis, the gels were stained according to their enzyme system with the appropriate substrate and chemical solutions then incubated at room temperature in the dark for complete staining. In most cases

incubation for about 1 to 2 hours was enough. Staining of the gels for esterase (Est) was performed as described by Scandalios (1964). The gel was soaked in 0.5 m borate buffer (pH 4.1) for 90 minutes at 4°C. The gel was stained for esterase activity by incubation at 37°C in a solution of 100 mg a-naphthyl acetate (as a substrate) and 100 mg fast blue RR salt in 200 ml of 0.1 M phosphate buffer pH 6.5. While staining of the gels for peroxidase (Px) was performed as described by Larsen and Benson (1970). The staining solution was composed of 50 ml of 1 M Na acetate, pH 4.7, (TMBZ) and 2ml of 30% H_2O_2.

C- Randomly amplified polymorphic DNA (RAPD)

In this study, RAPD was used for the identification of the two Indian jujube varieties according to Lu *et. al.* (1996) DNA extraction following the Dellaporta method (Dellaporta *et. al*., 1983) as follows:

About 0.1 gm (fresh weight) of young leaves was ground to fine powder in liquid N_2 in a mortar. Before the tissue thawed, 1 ml extraction buffer (100mM Tris-HCl pH 8.0, 50 mM EDTA and 0.5 M NaCl) and 0.2 ml 20 % SDS were added. The mixture was incubated at 65°C in water bath for 20 minutes. Then 1 ml of phenol, chloroform and isoamyl alcohol (25:24:1) was added. Centrifugation was performed at 10000 rpm for 10 minutes. The supernatants of each sample were transferred separately to a new tubes, then 1 ml of chloroform and isoamyl (24:1) was added. Centrifugation was performed at 10000 rpm for 10 minutes. The supernatants of each sample were transferred separately to a new tube, then 1 ml of isopropanol was added and kept overnight in freezer. Centrifugation was formed at 10000 rpm for 10 minutes. The resulted pellets containing DNA were re-suspended in 1 ml ethanol. Centrifugation was performed at 10000 rpm for 2 minutes. The DNA pellets were re-suspended in 200 ml TE (10 mM Tris-HCl pH 8.0 and 1 mM EDTA) buffer. DNA was quantitated by spectrophotometer and gel electrophoresis.

A total of fifteen 10-*mer* random DNA oligonucleotide primers were independently used in the PCR reactions according to Williams *et. al*. (1990). The primers are from operon kit (Operon Tech. Inc., USA). Only five primers were generated reproducible polymorphism in the DNA profiles. Each experiment was repeated two times and only stable products were scored. The following are the code and sequences of these primers.

No	Name	Nucleotide Sequence	No	Name	Nucleotide Sequence
1	OP-A03	5´ CTGCTGGGAC 3´	4	OP-B03	5` ACCGCGAAGC 3`
2	OP-A05	5´ TCGGCCATAG 3`	5	OP-B05	5` GGACCCAACC 3`
3	OP-Bo1	5` CTCACCGTCC 3`			

Amplification was performed in 25 ml reaction volume containing the following

Primer	2.5 µl
Template DNA	2.3 µl
Sterile water	7.7 µl
2 X Ready mix RED *Teq* PCR	12.5 µl

Reaction mix. It consists of the following

- 20mM Tris-HCl (pH 8.3)
- 100 mM KCl.
- 3mM $MgCl_2$.
- 0.002 % gelatin
- 0.4 mM dNTBs mix (dATP, dCTP, dGTP and dTTP).
- Stabilizers.
- 60 units Taq DNA polymerase/ml.

Each of the reaction mixtures was overlaid with a drop of light mineral oil per sample. Amplification was carried out in Perkin Elmer Gene Amp PCR thermocycler. The optimal conditions for PCR amplification was as follows: an initial 4 minutes denaturation step at 95°C followed by 35 cycles of 45 seconds at 94° C. 1 minute at 37°C and 2 minutes at 72°C, with a final extension step at 72° C for 12 minutes. A volume of 10 µl of the RAPD products were electrophoresed in 1.4 % agarose gel. The gel was prepared by adding 1.4 g agarose to 100 ml of 1X TBE (0.04 M Tris-aceate, 1 mM EDTA, pH 8), followed by boiling in water bath. Then 0.5 µg/ml ethidium bromide was added to the melted gel. The melted gel was poured in the try of mini-gel apparatus and the comb was inserted immediately. The comb was removed when the gel became hardened. The electrophoresis buffer (1 X TBE) was covered by the gel. About 10 µl of DNA amplified product was loaded in each well and run at 60 V for about 45-75 minutes. The gels were visualized and photographed by gel documentation system (Gel Doc Bio Rad 2000) under UV transilluminator.

RESULTS AND DISCUSSION

1- Vegetative growth characters

a- The tree height

The trees of Indian jujube were vigorous growing and had a wide spreading crown and a short bole. The results (Table 1) showed significant differences between the two varieties in both experimental seasons concerning tree height. Tofahy variety

was higher than that of Balahy variety by 8.13 % and 9.57 % in both seasons, respectively. These results were in agreement with Griffiths (1990), who reported that the tree height was 2-5 to 15 m.

b- Leaf characters

The leaf shape varied between varieties (figs. 1 & 2), as in Balahy variety, the leaves were oblong with dark green in the upper surface and hairy yellowish green in the under surface with 3 conspicuous, while in the Tofahy variety the leaves were oval with light green in the upper surface and hairy yellowish green in the under surface with 3 conspicuous. These results were in line with those found by Griffiths (1990), who reported that the leaves were alternate, distichous, elliptical, entire, or slightly toothed, distinctly–green and glabrous on the upper surface but cottony and pubescent on the under surface.

Data in Table (1) showed that there were, significant differences in leaf area, leaf length and leaf width among the two studied ber varieties. Tofahy variety was higher than Balahy variety in leaf area with 20.43 % and 20.99 %, leaf length with 11.48 % and 10.99 % and leaf width with 10.73 % and 15.85% in both seasons, respectively. These results were in line with Morton (1987), who reported that, the leaf length of Indian jujube was 2.5 to 6.25 cm and leaf width 2-4 cm. Moreover, Saran *et al.* (2007) reported that the maximum value of leaf area in Indian jujube genotypes was 45.72 cm^2. As for leaf length/width ratio, data in Table (1) indicated that, leaf length/width ratio of Balahy variety was more than Tofahy variety by 21.37 % in the second season. No significant differences between the two varieties in the first year of study were found.

C- Shoot characters

Ber shoots were pruned at the end of February or the beginning of March every year after harvesting. At the end of March, 1-10 shoots were developed on primary woody branches which bear leaves and flowers. These shoots take the shape of light zig-zag (Fig. 3). The shoots were selender, downy, bearing, paired of brown spines at the axils of the alternate leaves. One of the spines was straight and the other slightly hooked (Fig 7). The differences between both varieties in the shoots shape were not obvious.

Data in Table (1) revealed that the lengths of primary and secondary shoots of Tofahy variety were higher than Balahy variety. They were 13.49 % and 10.17 % for the length of primary shoots and by 11.18 % and 5.28 % for the length of secondary shoots in both seasons, respectively.

2- Flower morphology

The flowers of Indian jujube were small and yellowish green containing 5 petalled as shown in Figures (3 and 4) and they appeared in clusters in the leaf axils, each cluster contained 5-10 flowers. The period of flowering started in the first week of June and extended to the middle of September. These data were in line with Morton (1987) who reported that the 5-petalled flowers of Indian jujube were yellow, tiny, in 2 or 3 inches in the leaf axils. In addition Griffiths (1990) reported that the flowers arising in the leaf axils were small and greenish.

3- Yield, fruit set and fruit drop

The averages of fruit yield (Table 2) of Tofahy variety was significantly higher than Balahy variety by 24.8 % and 20.6 % in both seasons, respectively. Also, number of fruits/ tree (Table 1) of Tofahy was higher than Balahy variety by 15.5 %at the first season while, the differences between both varieties were un significant at the second season. In addition, the average of fruit weight of Tofahy was more than Balahy variety by 11.8 % and 18.7 % at both seasons respectively. These results were in line with Obeed *et al.* (2008), who reported that the averages fruit weight of Tofahy variety were 31.72 and 31.32 gm in both seasons, respectively. As for the fruit set percentage (Table 1) of Tofahy variety, it was significantly higher than Balahy variety by 3.16% in the second season while, the differences between the two varieties in the first season were not big enough to be significant. The fruit drop percentages of Balahy and Tofahy varieties were not big enough to be significant in both seasons.

4- Fruit characters

A- Physical fruit characters

The fruits are a drupe, varying from round to elongate and at size of plum (Figs. 5 & 6). The flesh was white, crispy, juicy, acid, or sub acid to sweet somewhat, astringent. Fully ripe fruits were wrinkled, the flesh buff-colored. The fruit contained one central brown stone (Figs. 7 & 8).

Data presented in Table (1) showed that the averages of fruit length of Balahy were higher than Tofahy by 6.7 % and 15.4 % in both seasons, respectively while, the average of fruit width of Tofahy was more than Balahy by 7 % in the second season and there were, not significant differences between both varieties in fruit width and fruit length/ width ratio in the first season. Fruit length/ width ratio was significantly higher in Balahy than Tofahy by 14 % in the second season. These results were in agreement with Obeed *et al.* (2008), who reported that Komethery 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 cv. was intermediate.

Data presented in Table (1) showed that fruit firmness of Balahy was higher than Tofahy variety by 16.06 and 12.63 % while, fruit pulp thickness of Tofahy was higher than Balahy by 12.88 % and 8.49 % in both seasons, respectively. As for fruit volume, there were not significant differences between both varieties in both seasons. These results were in agreement with Obeed *et al.* (2008). They reported that Tofahy and Peyuan cvs. had significantly larger fruit volume than the other three cultivars (35.53 and 36.33 cm^3), respectively. In addition, Ezhilarasi and Tamilmani (2009) reported that the fruit firmness of Indian jujube ranged from 3.5 to 12.5 kg/cm^2.

Data presented in Table (1) showed that seed weight and seed/fruit weight ratio of Balahy were higher than Tofahy variety by 12.25% and 13.60 % for seed weights and by 18.94 % and 29.43 % for seed/ fruit weight ratios in both seasons, respectively. These results were in line with Obeed *et al*. (2008). They reported that the heaviest seed weight was collected from Tofahy cv. (2.0 g) while the lightest seed weight was in Pakstany cv. (0.72g).

B- Chemical fruit contents

The averages of fruit acidity (Table 2) in Tofahy was higher than Balahy variety by 31.6 % in the first season while there were no significant differences between the two varieties in the second season. Also, there were no differences between both varieties in total soluble solids in the two experimental seasons. These results were in agreement with Obeed *et. al*. (2008). They reported that the fruit of Um-suleam cv. had high content of juice acidity percentage compared with the other cultivars. Also, it had the highest TSS percentage.

The averages of TSS/ acidity ratio (Table 2) of Balahy was more than in Tofahy variety by 33.83 % in the first season, while in the second year of study differences between the two varieties were not big enough to be significant. This explain that, the sweet taste of Balahy variety refer to the highest value of TSS/ acidity more than Tofahy variety. The average of ascorbic acid (Table 2) in Tofahy were higher than in Balahy variety by 16.16 % and 17.62% in both experimental seasons, respectively.

Data presented in Table (2) showed that there were no significant differences for reducing, non-reducing and total sugars between both varieties at both seasons. These results were in agreement with Ibrahim *et. al.* (2009). They reported that reducing sugars content ranged from 2.37 to 3.34 % and non-reducing sugars content from 4.15 to 6.38 % in both seasons, respectively. Also, there were no significant differences between the two varieties at fruit juice pH in both seasons. Ezhilarasi and Tamilmani (2009) reported that the pH gradually increased in peel and pulp of ber fruit.

5- Leaf and fruit pigments

A- Total leaf chlorophyll

The average of total leaf chlorophyll content (Table 2) of Balahy variety was more than in Tofahy variety by 6 % in the first year of study while there were no significant differences between both varieties in the second season.

B- Fruit chlorophyll

In both experimental seasons (Table 2), the differences between Balahy and Tofahy varieties were not big enough to be significant for fruit chlorophyll A and B while total fruit chlorophyll was significantly higher in Balahy variety than in Tofahy by 3.13% in the first year of study only. Ezhilarasi and Tamilmani (2009) reported that the means of chlorophyll A, B and total gradually decreased during the ripening in ber fruits.

C- Fruit carotene

Data in Table (2) showed that there were no significant differences between Balahy and Tofahy varieties in fruit carotene contents in both seasons.

6- Leaf and fruit mineral content

A- Leaf mineral content

1- Macro elements

The averages of leaf nitrogen, phosphorus, potassium, calcium and magnesium were significantly higher in Tofahy variety than Balahy at both seasons (Table 3). Tofahy variety was more than Balahy variety by 9.5 and 7.65 % for nitrogen, 18.18 and 20.69 % for phosphorous, 31.07 and 33.14 % for calcium and 11.59 and 12.31 % for magnesium in both seasons, respectively. These results were in agreement with Morton (1987) who reported that leaf phosphorous varied between 0.17-0.33 %, potassium was 0.47-1.57 %, calcium was 1.42-3.74% and magnesium was 0.46-0.83 % while, there were no significant difference between both varieties in leaf sodium contents in both seasons.

2- Micro elements

The averages of leaf iron, copper, manganese and zinc were significantly higher in Tofahy variety than Balahy in both seasons (Table 3). Tofahy variety was more than Balahy by 8.65 and 11.79% for iron, 21.89 and 17.52 % for copper, 16.26 and 13.74 % for manganese and 13.64 and 21.71 for zinc in both seasons, respectively. These results were in agreement with Amer *et al*. (2010). They reported that the leaf iron of Indian ber were 347.33-439 ppm, leaf copper were 106 and 375 ppm for Balahy variety and 110-355.67 for Tofahy, leaf manganese were 95.67 and 106.67 ppm for Balahy and 100 and 103 ppm for Tofahy and leaf zinc were 51.33 and

86.67 ppm for Balahy variety and 52.67 and 84.67 ppm for Tofahy variety in both seasons, respectively.

B- Fruit mineral content

1- Macro elements

The averages of fruit nitrogen, phosphorous, magnesium and sodium were significantly higher in Tofahy variety than Balahy in both seasons (Table 3). Tofahy variety was more than Balahy by 4.26 and 3.64% for nitrogen, 5.94 and 4.10 % for phosphorous, 11.97 and 8.85 % for magnesium and 4.67 and 7.53 % for sodium in both seasons, respectively. Also, fruit potassium content of Tofahy variety was higher than Balahy by 22.59% in the second season and fruit calcium content of Tofahy variety was higher than Balahy by 19.91% in the first season. No other significant differences were recorded. These results were partially in line with Youssef (2005) who reported that ber fruit potassium content was ranged from 122.75 and 145.39 mg/100g and fruit sodium content in ber was 132.72 to 152.14 mg/100g.

2- Micro elements

The averages of fruit iron, copper, manganese and zinc (Table3) were significantly higher in Tofahy variety than Balahy in the first season. Tofahy variety was more than Balahy by 15.52 and 20.12 % for iron, 8.9 % in the second season for copper, 8.94 and 21.9 % for manganese and 8.82 and 8.79 % for zinc in both seasons, respectively. These results were in agreement with Amer *et. al.* (2010). They reported that fruit iron contents of ber were 116.1 to 122.73 and 108.33 to 116.67 ppm for Tofahy and Balahy varieties in two seasons of study, respectively. Also, fruit copper contents were 5.55 to 6.66 and 4.67 to 5.33 ppm for Tofahy and Balahy varieties in the two years of study, respectively. As for fruit manganese, they reported that, Tofahy variety had 10.00 to 20.44 ppm and 14.44 to 18.33 ppm for Balahy variety in both seasons, respectively and fruit zinc values were 44.43 to 66.65 ppm for Tofahy variety while they were 44.43 to 61.1 ppm for Balahy variety in two years of study, respectively.

7- Electrophoretic analysis

There is a complete lack of information on the extent of genetic diversity in ber. This is the first study to investigate the extent of genetic diversity of cultivated ber genotypes in Egypt.

Identification of the studied varieties

SDS-PAGE of leaves proteins, isozymes (peroxidase and estrase), randomly amplified polymorphic DNA using PCR (RAPD-PCR) were used to assess the genetic diversity of the two varieties under investigation.

A- Identification based on biochemical analysis

Leaf proteins provide valuable evidence for taxonomic and evolutionary relationships of plant species (Yates *et. al.*, 1990). It is worthy to note that, leaf protein profiles are often species–specific, highly stable and unlikely not to be influenced by environmental conditions and seasonal fluctuations.

SDS-protein electrophoresis in leaves

The electrophoretic banding patterns of proteins extracted from leaves of the two Indian jujube varieties were shown in Figure (11). Their densitometric analyses are illustrated in Table (4). The presence and absence of bands were assessed with (1) and (0), respectively. The results of leaves SDS-PAGE revealed a total number of seventeen bands with molecular weights (MW) ranging from about 120.0 to 11.0 kDa. The analysis of data showed 12 common bands (monomorphic), while the remaining five bands were polymorphic with 29.4 % polymorphisms. In which two of them were positive variety-specific markers at 58.0 and 46.0 KDa, respectively for Tofahy variety and the other three bands were positive variety-specific markers at 38.0, 36.0 and 25.0 KDa, respectively for Balahy variety.

B- Identification based on Isozymes banding patterns

Peroxidase banding patterns

Table (5) and Figure (12) represent peroxidase electrophoretic banding patterns among examined fresh leaf samples of selected Indian jujube varieties. A total of one band was characterized for the studied cultivars, which was present in the two cultivars at relative mobility 0.85 with high density. These results were in agreement with Hassan *et. al.* (2002) who used isozymes to identify prunus cultivars. They indicated that most of cultivars could be differentiated and some cultivars gave identical patterns. They reported the importance of electrophoretic techniques separation of proteins and isozymes in elucidating biochemical genetic markers.

C- Identification based on molecular analysis

Identification based on RAPD

In the present study, RAPD-PCR was used to analyze the genetic polymorphisms of the two studied Indian jujube varieties, and to assess their genetic relationships using similarity index and dendrogram tree.

Five arbitrary random primers were used to determine RAPD polymorphism of the two Indian jujube varieties. The resulted amplified fragments are shown in Table (11) and their densitometric analyses are illustrated in Figure (11). Banding patterns were scored as present (1) or absent (0). A total number of 72 fragments were visualized across the two investigated genotypes, Table (11). Primers produced band numbers ranging from 11 (primer OP-A03) to 20 (primer OP-B03) across species,

Figures (13 and 16). Primer OP-A03 resulted in eleven bands with molecular sizes from 160 to 752 bp, Figure (13) and Table (6). One band was polymorphic (9.0 %), in which was variety-specific marker at 710 bp which considered as positive marker for Balahy variety. Primer OP-A05 indicated the amplification of fifteen bands with molecular size range from 80 -1220 bp Figure (14) and Table (7), one band was polymorphic (9.0%) in which was variety-specific marker at 420 bp which considered as positive marker for Tofahy variety. Primer OP-B01 indicated the amplification of fourteen bands with size range from 135-1140 bp, Figure(16) and Table (8), three bands were polymorphic (21.4 %), in which were variety-specific marker at 812, 727 and 600 bp, respectively. two of them were positive markers for Tofahy variety and the third band was positive for Balahy variety. Primer OP-B03 resulted in twenty DNA fragments ranging in 105-1055 bp, Figure (17) and Table (9), three bands were polymorphic (15.0 %), in which there was variety-specific marker at 700, 380 and 230 bp, respectively which were positive specific markers for Tofahy variety. Primer OP-B05 resulted in twelve DNA fragments ranging in 130-785 bp, Figure (15) and Table (10), one band was polymorphic (8.3 %), in which was positive variety-specific marker at 380 bp for Balahy variety.

Table 1. Vegetative growth, fruit set, fruit drop and physical character of Balahy and Tofahy ber varieties in 2007 and 2008 seasons.

Characters	Tofahy		Balahy	
	2007	**2008**	**2007**	**2008**
Tree hight (cm)	408.6 a	399.2 a	375.4 b	361.0 b
Leaf area (cm^2)	37.56 a	38.48 a	29.92 b	30.40 b
Leaf length (L) (cm)	8.67 a	8.55 a	7.68 b	7.61 b
Leaf width (w) (cm)	5.30 a	5.78 a	4.73 b	4.86 b
Leaf L/W ratio	0.99 b	0.92 b	1.07 a	1.17 a
Length of primary shoots (cm)	243.40 a	231.30 a	210.50 b	207.80 b
Length of secondary shoots (cm)	90.80 a	90.80 a	82.60 b	86.00 b
No. of flowers	4768	4726	4159	4312
Fruit set %	81.00 a	82.40 a	80.80 a	79.80 b
Fruit drop %	14.60 a	13.00 a	15.80 a	16.20 a
No. of fruit/tree	3382 a	3129 a	2857 b	3078 a
Fruit weight (gm)	31.40 a	31.50 a	27.70 b	25.60 b
Fruit length (L)	4.20 b	3.30 b	4.50 a	3.90 a
Fruit width (w)	4.20 a	3.60 a	4.20 a	3.30 b
Fruit L/W ratio	0.99 a	0.92 b	1.07 a	1.17 a
Fruit volume (cm^3)	37.60 a	36.20 a	35.20 a	34.40 a
Fruit firmness	13.22 b	13.01 b	15.75 a	14.89 a
Fruit pulp thickness (cm)	1.50 a	1.40 a	1.30 b	1.20 b
Seed weight (gm)	1.27 b	1.28 b	1.45 a	1.48 a
Seed/fruit weight ratio	4.24 b	4.07 b	5.24 a	5.77 a

* Similar letters for each character in the same season are not significantly different

Table 2. Yield, leaf and fruit pigments and chemical properties of Tofahy and Balahy varieties in 2007 and 2008 seasons.

Characters	Tofahy		Balahy	
	2007	2008	2007	2008
Yield (kg)	105.00 a	97.00 a	78.80 b	77.30 b
Acidity (%)	0.23 a	0.27 a	0.15 b	0.18 a
TSS (%)	14.12 a	14.29 a	14.09 a	14.02 a
TSS/ Acidity ratio	64.26 b	56.39 a	97.12 a	87.38 a
Ascorbic acid (mg/100g)	79.20 a	80.60 a	66.40 b	64.50 b
pH	4.39 a	4.56 a	4.72 a	4.57 a
Total sugars (%)	11.74 a	12.25 a	12.86 a	12.52 a
Reducing sugars (%)	7.27 a	7.99 a	8.14 a	7.37 a
Non-reducing sugars (%)	4.47 a	4.52 a	4.72 a	4.90 a
Leaf total chlorophyll content (SPAD unit)	41.80 b	43.31 a	44.47 a	44.71 a
Fruit chlorophyllA content (mg/100g)	6.57 a	6.60 a	6.43 a	6.51 a
Fruit chlorophyll B content (mg/100g)	3.29 a	3.72 a	2.29 a	2.84 a
Fruit total chlorophyll content (mg/100g)	41.80 b	43.31 a	44.47 a	44.71 a
Fruit carotene content (mg/100g)	3.45 a	4.42 a	3.27 a	4.40 a

* Similar letters for each character in the same season are not significantly different

Table 3. Leaf and fruit mineral content of Balahy and Tofahy varieties in 2007 and 2008 seasons.

Characters	Tofahy		Balahy	
	2007	2008	2007	2008
Leaf N (%)	1.99 a	1.96 a	1.80 b	1.81 b
Leaf P (%)	0.33 a	0.29 a	0.27 b	0.23 b
Leaf K (%)	1.27 a	1.18 a	1.18 b	1.04 b
Leaf Ca (%)	3.54 a	3.53 a	2.44 b	2.36 b
Leaf Mg (%)	0.69 a	0.65 a	0.61 b	0.57 b
Leaf Na (%)	0.04 a	0.04 a	0.03 a	0.03 a
Leaf Fe (ppm)	451 a	424 a	412 b	374 b
Leaf Cu (ppm)	338 a	314 a	264 b	259 b
Leaf Mn (ppm)	109.41 a	113.82 a	91.62 b	98.18 b
Leaf Zn (ppm)	79.20 a	79.00 a	68.40 b	54.80 b
Fruit N (%)	1.08 a	1.10 a	1.04 b	1.06 b
Fruit Ca (%)	2.48 a	2.26 a	1.82 b	2.09 b
Fruit Mg (%)	2.34 a	2.26 a	2.06 b	2.06 b
Fruit Na (%)	0.96 a	0.93 a	0.92 b	0.86 b
Fruit Fe (ppm)	114.70 a	108.28 a	96.90 b	88.16 b
Fruit Cu (ppm)	4.35 a	5.10 a	4.32 a	3.94 b
Fruit Mn (ppm)	16.78 a	16.21 a	15.28 b	12.66 b
Fruit Zn (ppm)	51.15 a	49.95 a	46.64 b	45.56 b

* Similar letters for each character in the same season are not significantly different

Table 4. Densitometric analysis for SDS leaf proteins of the two Indian jujube varieties.

Band No.	MW KDa	Jujube varieties	
		Balahy	Tofahy
1	120.0	1	1
2	95.0	1	1
3	72.0	1	1
4	63.0	1	1
5	58.0	1	0
6	52.0	1	1
7	46.0	1	0
8	38.0	0	1
9	36.0	0	1
10	34.0	1	1
11	32.0	1	1
12	30.0	1	1
13	25.0	0	1
14	18.0	1	1
15	16.0	1	1
16	13.0	1	1
17	11.0	1	1
Total		15	15

Table 5. Peroxidase isozyme banding patterns for the two Indian jujube varieties.

Peroxidase groups	Relative mobility	Indian jujube varieties	
		Balahy	Tofahy
Px1	0.85	++	++

Table 6. DNA polymorphism using randomly amplified polymorphic DNA (RAPD) for the two Indian jujube varieties with primers OP-A03.

Band No.	M.W (bp)	Indian jujube cultivars	
		Balahy	Tofahy
1	750	1	1
2	710	0	1
3	490	1	1
4	375	1	1
5	300	1	1
6	275	1	1
7	236	1	1
8	220	1	1
9	190	1	1
10	175	1	1
11	160	1	1
Total		10	11

Table 7. DNA polymorphism using randomly amplified polymorphic DNA (RAPD) for the two Indian jujube varieties with primers OP-A05

Band No.	M.W (bp)	Indian jujube varieties	
		Balahy	Tofahy
1	1220	1	1
2	1080	1	1
3	960	1	1
4	825	1	1
5	725	1	1
6	550	1	1
7	420	1	0
8	390	1	1
9	330	1	1
10	300	1	1
11	260	1	1
12	210	1	1
13	170	1	1
14	120	1	1
15	95	1	1
Total		15	14

Table 8. DNA polymorphism using randomly amplified polymorphic DNA (RAPD) for the two Indian jujube varieties with primers OP-B01.

Band No.	M.W (bp)	Indian jujube varieties	
		Balahy	Tofahy
1	1135	1	1
2	1005	1	1
3	890	1	1
4	812	1	0
5	727	1	0
6	600	0	1
7	575	1	1
8	450	1	1
9	405	1	1
10	365	1	1
11	320	1	1
12	220	1	1
13	180	1	1
14	135	1	1
Total		13	12

Table 9. DNA polymorphism using randomly amplified polymorphic DNA (RAPD) for the two Indian jujube varieties with primers OP-B03.

Band No.	M.W (bp)	Indian jujube varieties	
		Balahy	Tofahy
1	1055	1	1
2	965	1	1
3	815	1	1
4	765	1	1
5	700	1	0
6	605	1	1
7	515	1	1
8	490	1	1
9	430	1	1
10	380	1	0
11	320	1	1
12	260	1	1
13	240	1	1
14	230	1	0
15	200	1	1
16	160	1	1
17	140	1	1
18	128	1	1
19	120	1	1
20	105	1	1
Total		20	17

Table 10. DNA polymorphism using randomly amplified polymorphic DNA (RAPD) for the two Indian jujube varieties with primers OP-B05.

Band No.	M.W (bp)	Indian jujube varieties	
		Balahy	Tofahy
1	785	1	1
2	700	1	1
3	510	1	1
4	470	1	1
5	415	1	1
6	380	0	1
7	280	1	1
8	265	1	1
9	210	1	1
10	155	1	1
11	135	1	1
12	130	1	1
Total		11	12

Table 11. Polymorphism detected for all systems used in Indian jujube varieties descrimination.

System	Polymorphic	Monomorphic	Unique	Total	Polymorphism %
Protein	5	12	5	17	29.4 %
Isozymes	0	1	0	1	0 %
RAPD	9	63	9	72	12.5 %
Total	14	76	14	90	15.5 %

(1) (2)

Fig.1. The leaves of Balahy variety

1: upper surface of Balahy leaf variety

2: lower surface of Balahy leaf variety

(3) (4)

Fig. 2. The leaves of Tofahy variety

3: upper surface of Tofahy leaf variety

4: lower surface of Tofahy leaf variety

Fig. 3. The flowers of Balahy variety

Fig. 4. The flowers of Tofahy variety

Fig. 5. The fruit shape of Balahy variety

Fig. 6. The fruit shape of Tofahy variety

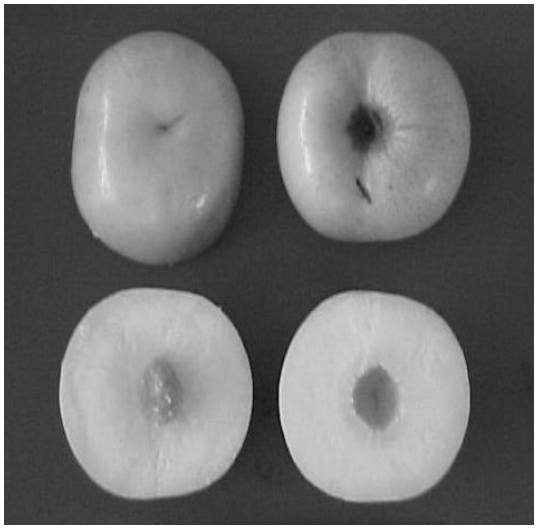

Fig. 7. Cross section in Tofahy fruit

Fig. 8. Cross section in Balahy fruit

Fig. 9. The seeds of Tofahy variety

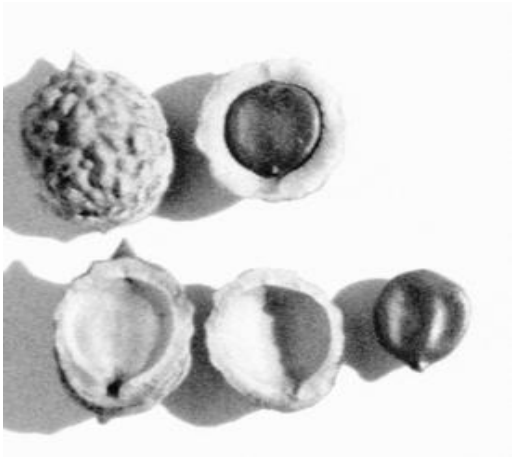

Fig. 10. The seeds of Balahy variety

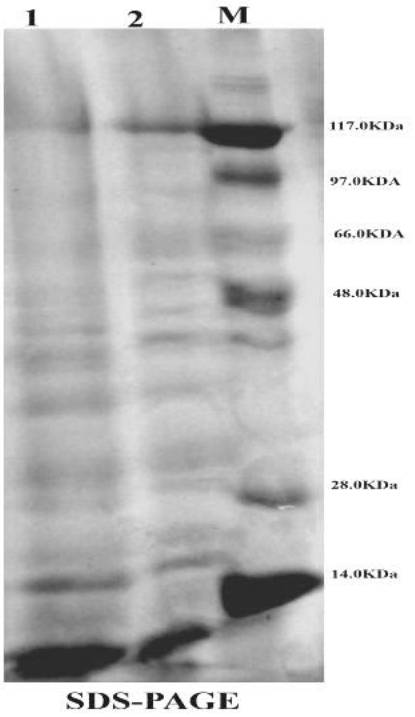

Fig. 11. Protein banding patterns for the two Indian jujube varieties

1- Balahy variety
2- Tofahy variety

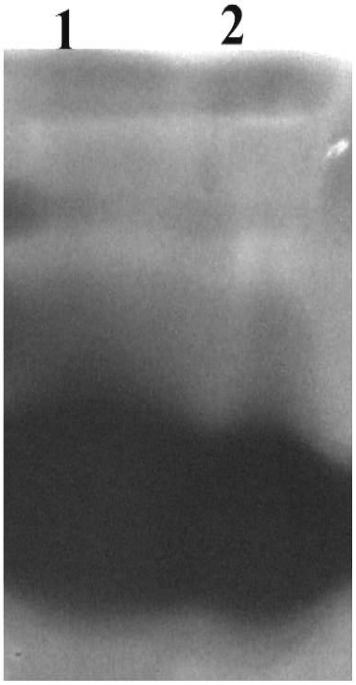

Fig. 12. Peroxidase isozyme banding patterns for the two Indian jujube varieties

1- Balahy variety
2- Tofahy variety

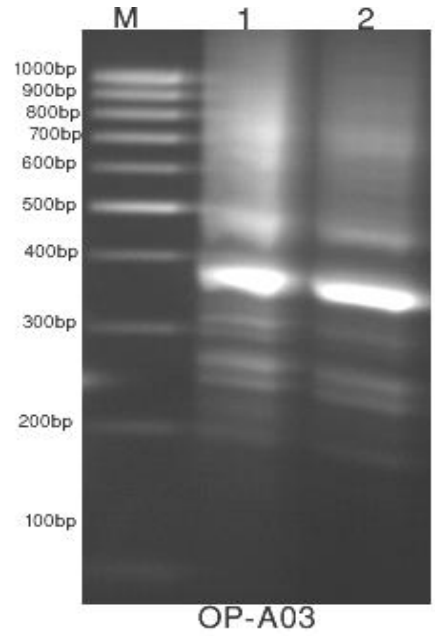

Fig. 13. DNA polymorphism of the two Indian jujube varieties amplified with primers OP-A03

1- Balahy variety
2- Tofahy variety

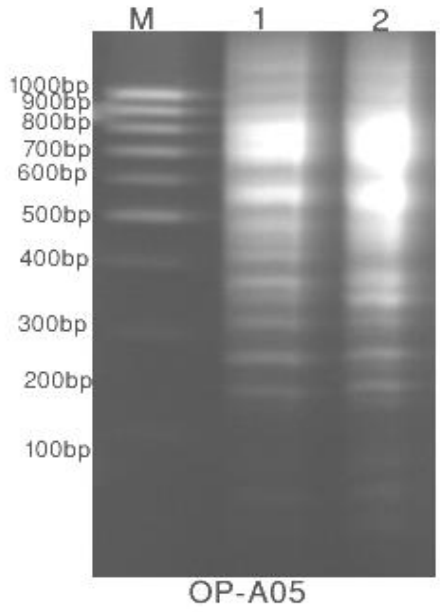

Fig. 14. DNA polymorphism of the two Indian jujube varieties amplified with primers OP-A05

1- Balahy variety
2- Tofahy variety

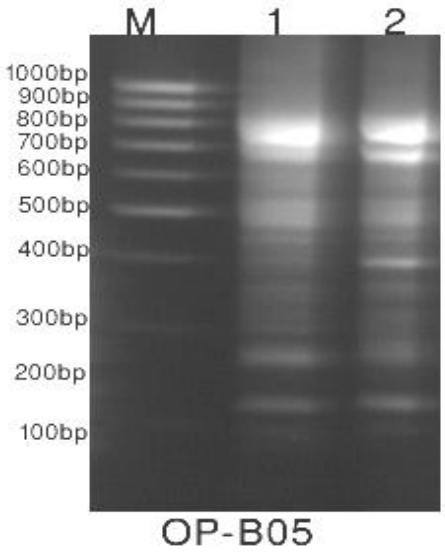

Fig 15. DNA polymorphism of the two Indian jujube varieties amplified with primers OP-B05

1- Balahy variety
2- Tofahy variety

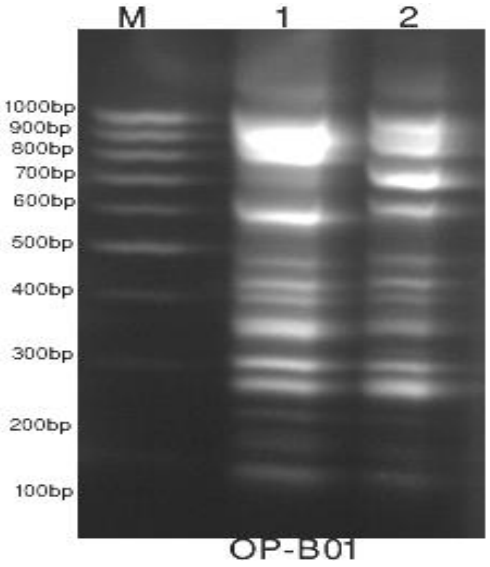

Fig. 16. DNA polymorphism of the two Indian jujube varieties amplified with primers OP-B01

1- Balahy variety
2- Tofahy variety

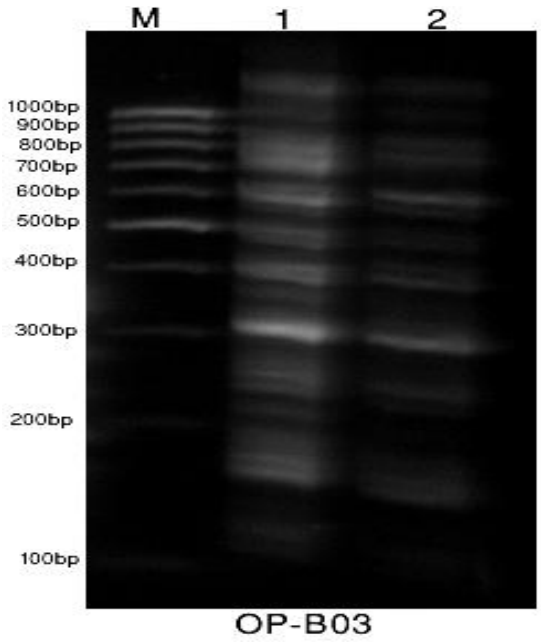

Fig. 17. DNA polymorphism of the two Indian jujube varieties amplified with primer OP-B03.

1- Balahy variety 2- Tofahy variety

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دراسات مورفولوجية وفسيولوجية وكيمو حيوية وراثية على بعض اصناف العناب الهندى الناميه فى النوباريه

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اجريت هذه الدراسه بمزرعه خاصه خلال عامى 2007 و 2008 لدراسة الصفات المورفولوجيه والفسيولوجيه والتحليل الوراثى لكل من صنفى العناب الهندى التفاحى والبلحى. واظهرت النتائج ان الصنف التفاحى سجل اعلى قوة نمو ومتوسط مساحه وطول وعرض الورقة وطول الأفرع الأوليه والثانويه بينما سجل الصنف البلحى أعلى نسبه طول الورقه الى عرضها. وسجل الصنف التفاحى اعلى نسبه عقد للثمار وعدد الثمار بالشجره ووزن الثمره والمحصول وعرض الثمره وسمك لب الثمره بينما سجل الصنف البلحى أعلى طول للثمره ونسبه طول الثمره الى عرضها و صلابه الثمار ووزن البذره ونسبه وزن البذره الى الثمره. وقد سجل صنف التفاحى أعلى نسبه حموضه وحمض الاسكوربيك بالثمار، بينما سجل صنف البلحى اعلى نسبه الكلوروفيل الكلى بالاوراق والثمار ونسبه المواد الصلبه الذائبه الكليه الى الحموضه. وسجل صنف التفاحى اعلى نسبه نيتروجين وفوسفور وكالسيوم وماغنسيوم وحديد ومنجنيز وزنك بالاوراق والثمار، ولم تسجل اختلافات معنويه بين صنفى العناب الهندى فى نسبه تساقط الثمار وحجم الثمره والمواد الصلبه الذائبه الكليه والسكريات الكليه والمختزله والغير مختزله ورقم الاس الهيدروجينى للعصير وكلوروفيل أ و ب والكاروتين بالثمار ومحتوى الاوراق من الصوديوم. وبينت الدراسات البيوكيمائية وجود تقارب وراثى بين صنفى العناب، البلحى والتفاحى حيث وجدت اختلافات طفيفه فى تتابع الاحماض الامينيه فى البروتينات والقواعد النيتروجينيه.