IMPROVEMENT OF SOME NUTRITIONAL VALUES OF TOMATOES VIA SALINITY

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Tomato (Solanum lycopersicon) is commonly used in the Mediterranean diet and is one of the most widely cultivated economically important vegetable in the world. From the nutritional and health points of view, tomato is characterized by its high content of antioxidants such as vitamin C and vitamin E (Sgherri et al., 2007). The consumption of fresh tomatoes and tomato products has been found to be inversely related to the incidence of some types of cancer and cardiovascular diseases (La Vecchia, 1997; Giovannucci, 1999).

Viruses form major threat to tomato plants. The most important virus affecting tomato is Tomato yellow leaf curl virus (TYLCV). Goodman (1977) reported that TYLCV is a member of family Geminiviridae which is transmitted by whiteflies. Morris et al. (2002) stated that the detection of TYLCV in tomato plants was achieved two weeks after whitefly fumigation with an improved frequency of detection at four weeks. They also found out that the PCR method is more sensitive than triple-antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) for the detection of TYLCV isolates in all known hosts. Abou-Jawdah et al. (2006) used the PCR reaction to confirm the presence of TYLCV in tomato plants in Lebanon. El-Gaied and El-Sherif (2012) stated that some antioxidant activity increased in the TYLCV infected tomato plants.

Soil salinity constitutes a major abiotic stress in plants worldwide. This has led to research involving salt tolerance and isolation of salt induced proteins with the aim of improving crop plants (Hoyos and Zhang, 2000; Majoul et al., 2000). Changes in the environmental conditions, such as salt concentration, affect the concentration of vitamins and soluble sugars of cherry tomato (Cristina et al., 2008). Tomato, a moderate-salinity-tolerant species, has been shown to respond positively to irrigation with diluted seawater, increasing its nutritional value (Giovannucci, 1999; Cristina et al., 2008).

Antioxidants play a fundamental role in the protection of plant cells from oxidative damage. In fact, antioxidants like vitamin C, vitamin E and β-carotene, with their antagonistic functions against free radicals, are very useful to protect humans against various diseases (De Pascale et al., 2001). The antioxidant ac-
tivity status is important to determine the nutritional value of fruits and vegetables (Rice-Evans et al., 1996). Increased antioxidant levels not only have high benefits in preventing widespread human diseases, including cancer and cardiovascular pathogens but enhance shelf life as well (Sgherri et al., 2007). Nutritional value of food products is of great importance for modern consumer society and can be affected by various substances; some of them are antioxidants (Smidova and Izzo, 2009).

Vitamin C (ascorbic acid) is a water soluble organic compound; mammals are not able to synthesize it. Vitamin C reduces hydrogen peroxide (H$_2$O$_2$), which preserves cells against reactive oxygen species (Zbynek et al., 2008). Vitamin C plays an important role in resistance to oxidative stresses such as heavy metals, salts, ultraviolet radiation, etc. (Taqi et al., 2011). Vitamin C is important in forming collagen, muscle and blood vessels. It also has rich contents of phytonutrients (Hsu et al., 2008).

Vitamin E applies to a family of eight related compounds, each with its own biological activity, potency and functional use in the body; α-tocopherol is considered as the most powerful antioxidant where it appears to provide better protection than other types of tocopherols (Sgherri et al., 2007).

Vitamin E (tocopherols) synthesized exclusively by photosynthetic organisms is a major antioxidant in biomembranes (Ouyang, 2011). Previous studies have shown that stress activated the expression of genes responsible for the synthesis of tocopherols in higher plants (Shao, 2007). Vitamin E and vitamin C are essential components of the human diet and perform numerous critical functions including scavenging and quenching of free radicals and various reactive oxygen species (ROS) (Szymańska and Kruk, 2007).

The present study focused on the changes in some antioxidants, as well as some types of carbohydrates in TYLCV-free tomato plants stressed by using 100 mM NaCl under greenhouse conditions. In particular, vitamin C, vitamin E, glucose and fructose which play a significant role in determining the nutritional value of tomatoes, were analyzed.

**MATERIALS AND METHODS**

**Plant material**

Seeds of tomato (Castle Rock) from Agriculture Research Center (ARC) were germinated in greenhouse at AGERI, ARC with 16 hr illumination per day. Plants were kept in cages to avoid infection with TYLCV by whiteflies.

**Salt treatment**

Sodium chloride was added in concentrations of 100 mM to plants when they were 30 days old. The plants were then grown for the next 45 days during which the salinized solutions were changed every 3 days under greenhouse conditions. Five plants were grown for
salinity treatment and the control plants were irrigated with water. After 45 days of salt treatment, at age of 75 days old, plants were tested for the presence of TYLCV using PCR to confirm that it is free virus, followed by determination of some antioxidants levels as vitamin C, vitamin E using HPLC and glucose and fructose levels using UPLC.

**DNA extraction**

Leaves of stressed plants, at the age of 75 days old, were used to extract DNA with DNeasy Plant Mini Kit supplied from Qiagen Hilden Germany catalog number 69104 (Riha et al., 1998). Extracted DNA was subjected to PCR using whitefly transmitting geminivirus (WTGs) specific primers HD1 & HD2 and TYLCV-CP specific primers to confirm that the plants are virus-free.

**Polymerase chain reaction (PCR)**

Primers used in this work were designed from the nucleotide sequence for Tomato yellow leaf curl virus Egyptian isolate (TYLCV-Eg) (Abdallah et al., 1993). The oligonucleotide primers were synthesized at Agriculture Genetic Engineering Research Institute, Agriculture Research Center (AGERI, ARC, Giza, Egypt) on an ABI 392 DNA/RNA synthesizer (Applied BioSystem, Lincoln Center Drive, Foster City, CA, USA). The PCR was carried out as described by Essam et al. (2004). The primers used to amplify TYLCV-Eg viral genome and position of the PCR products are shown in Table (1).

**Vitamins determination**

**Sample preparation**

Five gram of stressed tomato leaves were dried at 105°C till the weight was constant for moisture percent determination. Levels of vitamin C and vitamin E were determined in 75 days old stressed and virus free tomato leaves by a reverse-phase HPLC technique.

Vitamin C level was determined from 1 gm fresh weight stressed tomato leaves as a block. The method was carried out according to Zbynek et al. (2008). Twenty µl of supernatant were injected in HPLC (Hewlett Packards series 1050, detector UV 1050 and column ODS 5x4.6x25 ultra sphere).

α-tocopherols were determined in lipid extracts of one gm fresh weight stressed tomato leaves as a block and a control sample. Extraction was performed in the dark according to Nielsen and Hansen (2008) using HPLC as previously indicated.

**Glucose and fructose**

0.1 gm of dry weight from stressed tomato leaves as a block was analyzed using Acquity ultra performance liquid chromatography (UPLC) H-class waters USA. The detector used was MS/MS detector XEVO-TQD waters USA. The column used was Acquity UPLC. BEH amide 1.7 µm 1.0x100 mm column waters USA. The way of determination was used according to the application note of the instrument.
RESULTS AND DISCUSSION

Seeds of tomato were germinated in greenhouse at AGERI in cages with 16 hr illumination per day. One month old plants were subjected to 100mM NaCl salt stress for 45 days.

Salt level (100 mM NaCl) was studied on tomato plants where caution in practice of over irrigation with salty water should be held to avoid deleterious impact on the soil. Some researchers studied the effect of this concentration on tomato plants (Amir Nawaz et al., 2012). Also, El-Gaied and El-Sherief (2012) found that the concentration of 100 mM NaCl gave the best concentration of antioxidants. This concentration also is in conformity with Syed et al. (2011) who studied the effect of 75 mM NaCl salinity on tomato to determine Na⁺/K⁺ ratio and proline content and with Shah et al. (1994) who studied the effect of 30, 60 and 100 mM NaCl on the contents of glucose, fructose, ascorbic acid and citric acid in tomato plants and found that the concentration of 100 mM NaCl gave the best results of glucose and fructose contents.

DNA extraction and PCR

DNA extracted from stressed tomato plants, 75 days old, was used for PCR analysis with WTG specific primers (Cp-F, Cp-R and HD1, HD2). The cloned TYLCV genome was used as positive control in PCR experiments lanes 3 and 6. The DNA extracted from salt-treated plants gave negative results compared with DNA extracted from positive control where a 787 bp band was amplified in case of Cp-F, Cp-R and 674 bp bands was amplified in case of HD1 & HD2 (Fig. 1).

This experiment was carried out to confirm that the plants are virus-free since the TYLCV virus was shown to affect the quantity of antioxidants in tomato plants (El-Gaied and El-Sherif, 2012). This result indicates that the plants are not infected with TYLCV virus as compared with the positive control. These results were in agreement with the results of Abou-Jawdah et al. (2006) and Morris et al. (2002), as they used the PCR reaction to confirm the presence of TYLCV in tomato plants.

Vitamin quantification

Vitamin quantification was calculated from the curve generated by plotting the peak area of each authentic standard versus concentration (Fig. 2).

Treatment of virus-free tomato plants with 100 mM NaCl increased the contents of vitamin C and Vitamin E (α-tocopherol) by considerable amounts, as compared with the control plants (treated with water). These results represent the average ratio between the plants. These results agreed with Shah et al. (1994) who observed considerable enhancement of vitamin C in tomato plants treated with 100 mM NaCl as compared with the control.

Results obtained in this work are also consistent with previous studies of Sgherri et al. (2007) and Cristina et al.
(2008), who showed the benefit of irrigation with salt solution or diluted sea water on food quality of tomato berries, as salinity increased the amounts of vitamin C and α-tocopherol. They reported that vitamin C could have been involved in the regeneration of α-tocopherol. Similar observations were reported by some investigators (Flavia and Riccardo, 2008). Abbasi et al. (2007) who observed that tobacco plants treated with 400 mM NaCl displayed 6 fold increases in total tocoopherol content. The total vitamin E content is closely related to concentration of NaCl in the growing medium. Also, Smidova and Izzo (2009) found that elevating salinity level corresponded to electric conductivity of 8 mS/cm of tomato berries cultivar Gimar increased the content of vitamin C and of α-tocopherol. On the other hand, Gossett et al. (1996) observed increased amount of α-tocopherol in salt tolerant cotton plants.

**Glucose and fructose content**

Sugar analysis was calculated from the curve generated by plotting the peak area of each authentic standard versus concentration in Fig. (3).

The contents of glucose in virus-free plants were clearly increased by using 100 mM NaCl salt stress as compared with the control plants while there was a slight increase in the amount of fructose in salt stressed plants compared with the control plants. These results agreed with the results obtained by Amini and Ehsanpour (2005) who found that in response to increasing salt concentration of the medium, the average amount of total carbohydrate in stem-leaf of tomato plants cv. Shirazy increased. Whereas, Adams (1988) reported that increasing the levels of NaCl always improved tomato fruit quality; increased dry matter, sugar contents and acidity of the fruit juices.

The results also agreed with Smidova and Izzo (2009) who reported increasing contents of reducing sugar by 54% in tomato plants cultivar Jama under elevated salinity level.

Change in soluble sugars content under salt stress has already been reported for a number of plant species. Ashraf and Tufai (1995) determined the total soluble sugars content in five sunflower accessions differing in salt stress. They found that sugar content increased in all five lines with increasing salt in the growth medium.

**SUMMARY**

The antioxidant activity status is important to determine the nutritional value of fruits and vegetables. Increased antioxidant levels not only have high benefits in preventing widespread human diseases, including cancer and cardiovascular pathogens but also it enhances shelf life as well.

This study was carried out to test the effects of 100 mM NaCl on some antioxidants (vitamins C and E) and some monosaccharides (glucose and fructose) of TYLCV virus-free tomato plants (*Solanum lycopersicon*) cultivar Castle Rock.
The plants were treated at 30 days old with 100 mM NaCl for the next 45 days.

At 75 days old, mature leaves were harvested and tested for TYLCV infection, as this virus affects the amount of antioxidants in tomato plants. Plants were kept in cages to avoid infection with TYLCV. The plants gave negative results, confirming that they are virus-free.

Levels of vitamin C and vitamin E were determined by a reverse-phase HPLC technique while levels of glucose and fructose were determined using UPLC-MS in 75 days treated tomato leaves. Plants showed an increase in vitamin C, α-tocopherol, glucose and a slight increase in fructose contents versus the control plants.

These results are of great importance from the nutritional and health points of view where salt stress improved the plant quality by increasing the concentrations of important antioxidants (vitamin C and vitamin E). In conclusion, the use of controlled salinity level can be an effective method to produce tomatoes of good nutritional quality and with higher market price.

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Table (1): Nucleotide sequences of the whitefly transmitting geminiviruses (WTG) specific primers used to amplify the TYLCV-Eg viral genome and position of PCR product.

| Primer name | Nucleotides Sequence | Added restriction sites | Position of amplified fragment | Length bp |
|-------------|----------------------|-------------------------|--------------------------------|-----------|
| Cp-F        | 5’-CGGAATTCATGTCGAAGCGACCAGG-3’ | EcoRI | 467-1253 | 787 |
| Cp-R1       | 5’-CGGGATCTTAAATTTGATAATGAATC-3’ | BamHI | 1855-2528 | 674 |
| HD-1        | 5’-CGGAATTCGCCACCAATAAACTGTAGC-3’ | EcoRI | | |
| HD-2        | 5’-CGGGATCCGAGTCCGGTATTGAGAAACTTAC-3’ | BamHI | | |

Fig. (1): Amplified PCR fragments produced from using WTGs specific primers (Cp-F & Cp-R and HD1 & HD2). Stressed tomato plants with 100 mM NaCl (lanes 1, 2) showed negative results in case of first couple of primers and lanes (4, 5) showed negative results for second couple of primers compared with the cloned TYLCV genome which was used as positive control (+) lanes (3, 6) and gave the expected molecular weight. Lane M represents the DNA marker (1 KB Ladder from Fermentas).
Fig. (2): Effect of 100 mM treatment on average concentration of vitamin C and vitamin E in stressed tomato plants. (A): Concentration of Vitamin C (µg/1000µl) and (B): Concentration of Vitamin E (µg/1000µl).

Fig. (3): Effect of 100 mM NaCl treatment on average concentration of (A) fructose% and (B) glucose% in dried leaves of five stressed tomato plants.
GENETIC DIVERSITY IN OLD AND MODERN EGYPTIAN BREAD WHEAT (Triticum aestivum L.) VARIETIES REVEALED BY SIMPLE SEQUENCE REPEATS

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Wheat (Triticum aestivum L.) is the first and strategic important cereal crop in Egypt. National wheat breeding programs cultivated wheat represented essentially spring-type. Since the initiation of wheat breeding program in Egypt, new cultivars were developed by (1) selection from local populations, (2) introduction of new varieties and (3) crossing and selection for yield and its components. Several high-yielding bread wheat cultivars were produced. Current breeding objectives are aimed at improving productivity of wheat through increased resistance to biotic and abiotic stresses and to develop varieties with good milling and bread-making properties and a high nutritional value (Morgounov et al., 2001). The development of such varieties requires a continuous supply of a source of desirable genes and/or gene complexes. The sources of such genes could be (i) bread wheat varieties which have not been used very intensively but have a higher general adaptation, (ii) landraces, (iii) wild relatives, and (iv) weedy species. A prerequisite for efficient utilization of the plant material is knowledge about the genetic diversity, within the Egyptian bread wheat germplasm.

Molecular markers that reveal polymorphism at the DNA level have been shown to be a very powerful tool for genotype characterization and estimation of genetic diversity. In this regard, microsatellites or simple sequence repeats (SSRs), due to their multiallelic nature, have been extensively used in several crops (Gupta and Varshney, 2000).

In recent years, due to the availability of SSR marker sequences for oligonucleotide synthesis, involvement of PCR amplification, the simplicity of protocol that produces reliable and highly detectable amplification products, their codominance and single localization constitutes their advantages over AFLP, RFLP and RAPD markers (Varshney et al., 2005). Molecular markers developed from SSR resources for crop plants have been popularly called as genic molecular markers (Varshney et al., 2007).

Wheat microsatellite markers (WMS) (Röder et al., 1998), are known to
be abundant, highly polymorphic, reliable and relatively easy in application, have already been used in several studies to estimate the genetic diversity in wheat (Plaschke et al., 1995; Ben Amer et al., 2001; Chebotar et al., 2002; Huang et al., 2002; Alamerew et al., 2004; Colomba and Gregorini, 2011; Sardouie-Nasab et al., 2013; Akfirat and Uncuoglu, 2013).

The objectives of this study were to (i) assess the genetic diversity within old and modern bread wheat (Triticum aestivum L.) varieties cultivated in Egypt by using SSR markers and (ii) assess whether old Egyptian varieties could be a potential source for improving genetic diversity in modern wheat breeding in Egypt.

**MATERIALS AND METHODS**

**Plant material**

Thirty-three diverse bread wheat varieties (Triticum aestivum L) released from 1947 to 2004 and with Egyptian origin were used in this study. Grains of all Egyptian varieties were obtained from the Agricultural Research Center (ARC), Giza, Egypt. A List of the wheat varieties, year of release, pedigree and released group is presented in Table (1).

**DNA extraction**

Total genomic DNA was extracted from young leaves for five seedlings from 8-weeks-old seedlings of each genotype. Only one replication was sampled for DNA extraction. DNA extraction was performed according to Plaschke et al. (1995).

**PCR amplification**

PCR reaction contained 50-100 ng template DNA, 250 nM forward primer, 250 nM reverse primer, 0.2 mM dNTPs, 2.5 μl PCR buffer (10 X), 1.5 mM MgCl, 1 U Taq DNA polymerase in a total volume of 25 μl. Amplifications were carried out using the following programs: 5 min at 94°C followed by 35 cycles of 1 min 94°C, 1 min 50°C or 55°C or 60°C according to primer annealing temperature and 2 min at 72°C, with a final extension of 5 min at 72°C as described by Röder et al. (1998). The amplification products were resolved on 10% polyacrylamide denaturing gels (PAGE) (Röder et al., 1998).

**SSR analysis**

Seventeen gatersleben wheat microsatellite (Xgwm) markers (Table 2) were selected from Röder et al. (1998). The microsatellite primers used were described by Röder et al. (1998). Fragment detection for SSR markers was carried out as given in Röder et al. (1998).

**Data collection and diversity**

Gels were scored as binary data matrix. The presence (1) and absence (0) of alleles for each microsatellites marker were recorded for each variety. Gene diversity was calculated according to the formula of Nei (1973) using the equation.
PIC = 1 – \sum_{i=1}^{k} P_i^2$, where $k$ is the total number of alleles detected for a locus of a marker and $P_i$ is the frequency of the $i^{th}$ allele in the set of thirty-three Egyptian wheat varieties investigated. Anderson et al. (1993) indicated that gene diversity is essentially the same as the polymorphism information content (PIC) as used by Botstein et al. (1980).

**Genetic similarity estimation and cluster analysis**

The data were analyzed using the SIMQUAL (Similarity for Qualitative Data) routine to generate Dice similarity coefficient (Dice, 1945). The similarity coefficient were used to construct dendrogram using Unweighted Pair Group Method with Arithmetic Average (UPGMA) algorithm using the Numerical Taxonomy and Multivariate Analysis System (NTSYS), version 2.1 (Rohlf, 2002).

**RESULTS AND DISCUSSION**

**Characteristics of SSR markers**

All microsatellite markers used in this study yielded polymorphic fragments among the varieties tested. In total, 66 and 82 alleles were detected with an average of 3.88 and 4.82 alleles in both old and modern varieties, respectively (Table 3). The number of alleles per locus ranged from 3 to 6 with an average of 3.88 for old wheat varieties, while the number of alleles per locus ranged from 3 to 9 with an average of 4.82 for modern wheat varieties (Table 3). Huang et al. (2002) reported an average allele number of 18.1 in 998 gene bank accessions of hexaploid wheat originated from 68 countries of five continents. Khlestkina et al. (2004) found an average allele number of 6.6 in 54 Siberian old and modern common spring wheat varieties. Roussel et al. (2005) reported an average allele number of 16.4 in 480 wheat varieties originating from 15 European geographical areas and released from 1840 to 2000. Salem et al. (2008) detected an average of 3.2 alleles in seven wheat varieties. The data for the average number of alleles obtained in the present study of Egyptian bread wheat was lower than most previous studies, but it was comparable with Satchel's results, which detected 4.8 alleles per locus in wheat varieties (Satchel et al., 2000) and 3.2 alleles per locus in seven wheat varieties detected by Salem et al. (2008).

The correlation coefficient between gene diversity and the number of alleles for SSRs markers was high, $r = 0.603$ and $r = 0.503$, ($P<0.01$), for old and modern varieties, respectively. The linear relationship between them is shown in Fig. (1). However, the correlation coefficient between gene diversity and the number of alleles for wheat genomes was $r = 0.842$ and $r = 0.373$, ($P<0.01$), for old and modern varieties, respectively. The linear relationship between them is shown in Fig. (2). While, the correlation coefficient between gene diversity and the number of alleles for homologous groups was $r = 0.710$ and $r = 0.029$, ($P<0.01$), for old and modern varieties, respectively. The linear
relationship between them is shown in Fig. (3). The value of gene diversity increased with the number of alleles at a given locus. There was significant correlation between gene diversity and the number of alleles. Therefore, the number of alleles can be used for the evaluation of genetic diversity. The data obtained in the present investigation agreed with those of Huang et al. (2002) who reported that the PIC value was correlated with the number of alleles.

**Genetic diversity of A, B and D genomes**

The 17 loci were distributed basically on A, B & D genomes (6, 6 and 5, respectively) Table (4). Compared to old varieties, the modern groups showed the highest number of alleles of the three wheat genomes 30, 28 and 24 (B, A & D), respectively. Regarding to average genetic richness, the modern varieties showed higher number of alleles/loci 5, 4.8 and 4.67 (B, A & D), respectively than that in the old varieties (Table 4). As for genetic diversity, the modern varieties showed higher genetic diversity 0.703, 0.635 and 0.617 (B, A & D), respectively than that in the old varieties (Table 4). Indeed, the B genome showed the highest diversity. The number of alleles was different for individual genomes, (27 and 30) for B genome, (21 and 28) for A genome and (18 and 24) for D genome in old and modern wheat varieties, respectively. This might suggest that D genome is the most conserved. This may be due to the pattern of evolution of wheat genomes, as D genome was incorporated into hexaploid wheat much later than A and B genomes, so it may be less diverse. On the other hand, the number of SSR alleles located on B genome may reflect its greater variability sustained during evolution (Feldman, 2001). Those results were consistent with data achieved by Röder et al. (1998), Fahima et al. (1998), Huang et al. (2002), Alamerew et al. (2004), Colomba and Gregorini (2011), Li et al. (2012), Sardouie-Nasab et al. (2013) and Akfirat and Uncuoglu (2013) for SSR markers.

**Genetic diversity of the 7 homologous groups**

Homologous group 7 possessed the highest average of allelic numbers, while group 2 was the lowest values for both modern and old varieties. The order from the highest to the lowest for modern varieties was 19 for group 7, (17) for group 4, (15) for group 1, (12) for group 5, (11) for group 3, (8) for group 2, while the order for old group was 14 (group 7), 13 (group 4), 12 (group 1), 10 (group 3=5) and 7 (group 2). Regarding to the average of genetic richness, the modern varieties had the highest values than the old varieties. In addition, the average of genetic richness from 1st to 7th homoeologous group for the modern varieties was 5, 4, 3.67, 5.67, 4.00 and 6.33, respectively. So, group 7 still hold the highest genetic richness and group 3 was the lowest in both modern and old varieties (Table 4). With regards to PIC, the highest value was 0.753 and 0.718 for group 4 in both modern and old varieties, respectively. Whereas, the lowest PIC values was 0.595 (group 5) and
0.528 (group 2) for both modern and old varieties, respectively. There were not large differences between A and D genomes in the average genetic richness for both old and modern wheat varieties, but average genetic richness for A genome was obviously lower. While, B genome had the highest average genetic richness. This indicated that there were more key genes/QTLs controlling important agronomic characteristics, and domestication and modern breeding provided much higher selective pressures to A genome (Peng et al., 2003). Among the 7 homologous groups, the genetic diversity of group 5 was much lower for both old and modern Egyptian wheat varieties. Therefore, it was estimated that breeding might have brought much higher selection pressure on genes conveyed by this group. This was consistent with the opinions of Börner et al. (2002) and Peng et al. (2003).

**Cluster analysis**

Similarity index and consensus tree were developed on the bases of the scorable banding patterns of the 6 released wheat groups which resulted from 33 wheat varieties using the 17 SSR markers as shown in Table (4 and 5). The similarity index showed that the two most closely related groups were C and B with the highest similarity index 0.588. On the other hand, the two most distantly related groups were (E and B) and (E and C) with the low similarity index 0.228.

Cluster analysis was conducted based on SSR data to group the wheat varieties and to construct a dendrogram as presented in Fig. (4). All 33 varieties were divided into six groups according to year of release. Two major clusters corresponded to the old and modern groups. Two groups can be distinguished by truncating the dendrogram at GS value of 0.25. With genetic distance (GD) < 0.588 as the standard of sub-cluster.

The consensus dendrogram showed that the Egyptian bread wheat varieties were divided into two main clusters (I and II). The first included two groups from four of the old wheat varieties (group A and C). Group (A) consisted of two varieties (Giza 139 and Giza 144), while group (C) consisted of two varieties (Giza 157 and Sakha 8). The second main cluster was divided into two sub-clusters (IIa and IIb). The first subcluster (IIa) included the other two old groups (group B and D). Group (B) contained two varieties (Giza 150 and Giza 155). However group (D) contained seven varieties (Sakha 61, Sakha 69, Giza 160, Sakha 92, Giza 162, Giza 163 and Giza 164). The second sub-clusters (IIb) included the modern Egyptian wheat varieties (group E and F). Group E had twenty varieties (Gemmiza 1, Sahel 1, Giza 167, Sids 1, Sids 2, Sids 3, Sids 4, Sids 5, Sids 6, Sids 7, Sids 8, Sids 9, Gemmiza 3, Gemmiza 5, Gemmiza 7, Gemmiza 9, Giza 168 and Sakha 93). However, group F contained only the two wheat varieties (Sakha 94 and Gemmiza 10).

The above discussion amply demonstrates the utility of microsatellites, which can be profitably utilized in wheat
not only for detecting polymorphism and tagging genes (Prasad et al., 1999; Roy et al., 1999) but also for genotype identification and for estimation of genetic diversity. We conclude, therefore, that on the basis of microsatellite markers, diverse parents can be selected. In addition to provide new information about the relationships between the old and modern Egyptian bread wheat varieties analyzed. Also, the obtained data may be suggested that old Egyptian bread wheat varieties are a potential basis for genetic diversity in modern wheat breeding in Egypt.

**SUMMARY**

The objective of the present study was to assess genetic diversity within old and modern bread wheat varieties cultivated in Egypt and to find out whether old Egyptian varieties could be a potential source for genetic diversity in modern wheat breeding in Egypt. A set of 33 varieties was analyzed using 17 SSR markers, determining 17 loci located on 15 different chromosomes. A total of 66 and 82 alleles were detected with an average of 3.88 and 4.82 alleles in both old and modern wheat varieties, respectively. The average genetic diversity value was 0.617 in old varieties while in modern varieties it was 0.652. Compared to old varieties, the modern varieties showed the highest number of alleles for the three wheat genomes 30, 28 and 24 (genome B, A & D), respectively. Regarding the average genetic richness, the modern varieties showed higher number of alleles/locus 5, 4.8 and 4.67 (genome B, A & D), respectively than that in the old varieties. As for genetic diversity, the modern varieties showed higher genetic diversity 0.703, 0.635 and 0.617 (genome B, A & D), respectively. Indeed, the B genome showed the highest diversity. In generally, homologous group 7 possessed the highest average of allelic numbers, while group 2 was the lowest for both modern and old varieties. Cluster analysis was conducted based on SSRs data to group the bread wheat varieties and to construct a dendrogram. Two groups can be distinguished by truncating the dendrogram at GS value of 0.25.

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Table (1): List of bread wheat cultivars released by the Wheat Research Department, ARC, Giza, Egypt during the last 50 years*.

| No | Varieties | Year of release | Pedigree | Released Group |
|----|-----------|-----------------|----------|----------------|
| 1  | Giza 139  | 1947            | Hindi 90/ Kenya B256 | Group A |
| 2  | Giza 144  | 1958            | Regent/2* Giza 139 | Group A |
| 3  | Giza 150  | 1960            | Mida-Cadet/2* Giza 139 | Group B |
| 4  | Giza 155  | 1968            | Regent/2* Giza 139/Mida-Cadet/2* Hindi 62 | Group B |
| 5  | Giza 157  | 1977            | Giza 155//Plt 62 /LR 64/3/Tzpp/Knott | Group C |
| 6  | Sakha 8   | 1977            | Indus/Norteno “s” | Group C |
| 7  | Sakha 61  | 1980            | Inia/RL 4220//7C/Yr “s” | Group D |
| 8  | Sakha 69  | 1980            | Inia/RL 4220//7C/Yr “s” | Group D |
| 9  | Giza 160  | 1982            | Chenab70/Giza 155 | Group D |
| 10 | Sakha 92  | 1987            | Napo 63/Inia 66//Wern “s” | Group D |
| 11 | Giza 162  | 1987            | Vcm/Cno 67//7C/CM33009-F-15M-4Y-1M-1Y-1M-0M | Group D |
| 12 | Giza 163  | 1987            | T.aestivum/BN/Cno/7C CM33009-F-15M-4Y-1M-1Y-1M-0M | Group D |
| 13 | Giza 164  | 1987            | Kyz/Buha “s”//Kal/Bb CM33027-F-15M-500y-0M | Group D |
| 14 | Gemmiza 1 | 1991            | Maya 74/On//1160,147//3/Bb//Gall/4/Chat’s” CM58924-1GM-OGM | Group E |
| 15 | Sahel 1   | 1994            | N.S.732/Pim//Vee’s” | Group E |
| 16 | Giza 167  | 1995            | Au/Ukp301//Gl/Sx/Pea”s”//4/Mai’s”//May’s”//Pw”s”/CM67245-1M-2Y-1M-1Y-1M-0M | Group E |
| 17 | Sids 1    | 1996            | HD 2172/Pavon’s”//1158.57/Maya 74 “s” SD46-4SD46-4SD-2SD-1SD-0SD | Group E |
| 18 | Sids 2    | 1996            | HD 2206/Hork’s”//3/Napo63//Inia66//Wern “s” SD63-4SD-1SD-1SD-0SD | Group E |
| 19 | Sids 3    | 1996            | Sakha 69//Giza155 SD723-7SD-1SD-0SD | Group E |
| 20 | Sids 4    | 1994            | Maya “s”//Mon “S”//CM H74.A592//3/Giza 157*2 | Group E |
| 21 | Sids 5    | 1994            | Maya “s”//Mon “S”//CM H74.A592//3/Giza 157*2 SD10001-7SD-4SD-2SD-0SD | Group E |
| 22 | Sids 6    | 1994            | Maya “s”//Mon “S”//CM H74.A592//3/Sakha 8*2 SD10002-4SD-3SD-1SD-0SD | Group E |
| 23 | Sids 7    | 1994            | Maya “s”//Mon “S”//CM H74.A592//3/Sakha 8*2 SD10002-3SD-1SD-0SD | Group E |
| 24 | Sids 8    | 1994            | Maya “s”//Mon “S”//CM H74.A592//3/Sakha 8*2 SD10002-1SD-3SD-1SD-0SD | Group E |
| 25 | Sids 9    | 1994            | Maya “s”//Mon “5SD”//CM H74.A592//3/Giza 157*2SD10003 | Group E |
| 26 | Gemmiza 3 | 1997            | Bb//C42//Y50//Kai//Sakha84//Prv//Ww//5//3/Bg’s”//OnCGM.40 | Group E |
| 27 | Gemmiza 5 | 1998            | Vee’s”//SWM 6525 CMG.4017-1GM-6 GM-3 GM-0GM | Group E |
| 28 | Gemmiza 7 | 2000            | CMH74 4.630//5x//Seri 82//3/Agen CGM.4611-2GM-3GM-1GM-0GM | Group E |
| 29 | Gemmiza 9 | 2000            | Ald”s”//Hua’w”//CMH74A.630//5x//Seri 82//3/Agen CGM.4583-5GM-1GM-0GM | Group E |
| 30 | Giza 168  | 1999            | Mil/Buc//Seri | Group E |
| 31 | Sakha 93  | 1999            | Sakha 92//TR 81032 | Group E |
| 32 | Sakha 94  | 2004            | Opata/Rayon//Kauz | Group F |
| 33 | Gemmiza 10| 2004            | Maya 74 “s”//On//1160,147//3/Bb//4/Chat’s”//S/5/Ctohad | Group F |

*Thanks are due to ARC, Ministry of Agric., Egypt.
Table (2): Characteristics of 17 wheat SSR markers, their chromosomal location, primer sequence, motif, annealing temperature and fragment size.

| No. | SSR markers And their chromosomal location | Primer sequence (L) Left (R) right | Motif | Annealing Tm (°C) | Fragment size in CS (bp) |
|-----|--------------------------------------------|------------------------------------|-------|------------------|------------------------|
| 1   | Xgwm3-3D                                   | GCA GCG GCA CGT GTA CAT TT AAT ATC GCA TCA CTA TCC CA | (L)   | (CA)$_{18}$      | 55                     | 79                     |
| 2   | Xgwm18-1B                                  | TGG CCG CAT GAT TGC ATT ATC TTC GGT TGC TGA AGA ACC TTA TTT AGG | (L)   | (CA)$_{17}$GA (TA)$_{4}$ | 55                     | 183                    |
| 3   | Xgwm 46-7B                                 | GCA CGT GAA TGG ATT GGA C TGA CCC AAT AGT GGT GGT CA | (L)   | (GA)$_{3}$GC (GA)$_{33}$ | 60                     | 179                    |
| 4   | Xgwm 95-2A                                 | GAT CAA ACA CAC ACC CCT CC AAT GCA AAG TGA AAA ACC CG | (L)   | (AC)$_{16}$      | 60                     | 179                    |
| 5   | Xgwm155-3A                                 | CAA TCA TTT CCC CCT CCC AAT CAT TGG AAA TCC ATA TGC C | (L)   | (CT)$_{19}$      | 60                     | 144                    |
| 6   | Xgwm160-4A                                 | TTC AAT TCA GTC TTG GCT TGG CTG CAG GAA AAA AAG TAC ACC C | (L)   | (GA)$_{21}$      | 60                     | 182                    |
| 7   | Xgwm165-4A                                 | TGC AGT GGT CAG ATG TTT CC CTT TTC TTT CAG ATT GCG CC | (L)   | (GA)$_{20}$      | 60                     | 190                    |
| 8   | Xgwm186-5A                                 | GCA GAG CCT GGT TCA AAA AG CGC CTC TAG CGA GAG CTA TG | (L)   | (GA)$_{26}$      | 60                     | 135                    |
| 9   | Xgwm190-5D                                 | GTG CTT GCT GAG CTA TGA GTC GTG CCA CGT GAG TCT TTT G | (L)   | (CT)$_{22}$      | 60                     | 209                    |
| 10  | Xgwm261-2D                                 | CTC CCT GTA CGC CTA AGG C CTC GCC CTA CTA GCC ATG AAT G | (L)   | (CT)$_{21}$      | 55                     | 189                    |
| 11  | Xgwm389-3B                                 | ATC ATG TCG ATC TCC TTG ACG TGC CAT GCA CAT TAG CAG AT | (L)   | (CT)$_{14}$ (GT)$_{16}$ | 60                     | 129                    |
| 12  | Xgwm408-5B                                 | TCG ATT TAT TGG GGC CAC TG GTA TAA TTC GTT AGC ACG C | (L)   | (CA)$_{22}$ (T) (CA)$_{9}$ (TA)$_{9}$ | 55                     | 176                    |
| 13  | Xgwm437-7D                                 | GAT CAA GAC TTT TGT ATC TCT C GAT GTC CAA CAG TTA GCT TA | (L)   | (CT)$_{24}$      | 50                     | 107                    |
| 14  | Xgwm458-1D                                 | AAT GGC AAT TGG AAG ACA TAG C TTC GCA ATG TTT ATT TGG C | (L)   | (CA)$_{13}$      | 60                     | 113                    |
| 15  | Xgwm513-4B                                 | ATC GTG AGC ACC TAC TGG TCA GGT CTG TTC ATG CCA CAT TG | (L)   | (CA)$_{12}$      | 60                     | 140                    |
| 16  | Xgwm631-7A                                 | ---                                | (GT)$_{23}$ |              | 60                     | 196                    |
| 17  | Xtaglgap-1B                                | GCA GAC CTG TGT CAT TGG TC GAT ATA GTG GCA GCA GGA TAC G | (L)   | (CAA)$_{31}$      | 60                     | 282                    |
Table (3): Characteristics of SSR markers used with the chromosomal location, marker name, allele size range, number of alleles per locus and gene diversity calculated over a set of 33 old and modern wheat varieties.

| Locus      | Position | Allele size range (bp) | Number of alleles | Gene diversity |
|------------|----------|------------------------|-------------------|----------------|
|            |          | Min allele             | Max allele        | Old | Modern | Old | Modern |
| Xgwm 3     | 3D       | 77                     | 84                | 3   | 3      | 0.615 | 0.535 |
| Xgwm 190   | 5D       | 204                    | 212               | 3   | 3      | 0.569 | 0.558 |
| Xgwm 261   | 2D       | 165                    | 192               | 3   | 4      | 0.500 | 0.575 |
| Xgwm 437   | 7D       | 91                     | 130               | 6   | 9      | 0.694 | 0.835 |
| Xgwm 458   | 1D       | 109                    | 122               | 3   | 5      | 0.569 | 0.645 |
| Xgwm 18    | 1B       | 186                    | 192               | 4   | 3      | 0.513 | 0.605 |
| Xgwm 46    | 7B       | 147                    | 187               | 5   | 7      | 0.722 | 0.780 |
| Xgwm 389   | 3B       | 119                    | 136               | 4   | 5      | 0.694 | 0.750 |
| Xgwm 408   | 5B       | 178                    | 194               | 4   | 3      | 0.583 | 0.595 |
| Xgwm 513   | 4B       | 141                    | 150               | 5   | 7      | 0.778 | 0.725 |
| Xtaglgap   | 1B       | 212                    | 280               | 5   | 7      | 0.769 | 0.765 |
| Xgwm 631   | 7A       | 190                    | 200               | 3   | 3      | 0.500 | 0.244 |
| Xgwm 95    | 2A       | 109                    | 131               | 4   | 4      | 0.556 | 0.803 |
| Xgwm 155   | 3A       | 129                    | 147               | 3   | 3      | 0.486 | 0.515 |
| Xgwm 160   | 4A       | 177                    | 189               | 3   | 5      | 0.611 | 0.740 |
| Xgwm 165   | 4A       | 187                    | 202               | 5   | 7      | 0.764 | 0.795 |
| Xgwm 186   | 5A       | 122                    | 134               | 3   | 6      | 0.569 | 0.605 |
| Total      |          |                        |                   | 66  | 82     |       |       |
| Mean       |          |                        |                   | 3.88| 4.82   | 0.617 | 0.651 |

Table (4): Genetic diversity between the old and modern varieties in different genomes and homologous chromosome groups.

| Location | Number of loci checked | Number of alleles | Average genetic richness | Gene diversity |
|----------|------------------------|-------------------|--------------------------|----------------|
|          |                        | Old wheat varieties | Modern Wheat varieties | Old wheat varieties | Modern Wheat varieties |
| Genome   |                        |                   |                          |                |
| A        | 6                      | 21                | 28                       | 3.50           | 4.67           | 0.581 | 0.617 |
| B        | 6                      | 27                | 30                       | 4.50           | 5.00           | 0.677 | 0.703 |
| D        | 5                      | 18                | 24                       | 3.60           | 4.80           | 0.589 | 0.635 |
| Homologous chromosome Group | | | | |
| 1        | 3                      | 12                | 15                       | 4.00           | 5.00           | 0.617 | 0.672 |
| 2        | 2                      | 7                 | 8                        | 3.50           | 4.00           | 0.528 | 0.689 |
| 3        | 3                      | 10                | 11                       | 3.33           | 3.67           | 0.598 | 0.600 |
| 4        | 3                      | 13                | 17                       | 4.33           | 5.67           | 0.718 | 0.753 |
| 5        | 3                      | 10                | 12                       | 3.33           | 4.00           | 0.574 | 0.595 |
| 7        | 3                      | 14                | 19                       | 4.67           | 6.33           | 0.639 | 0.620 |
Table (5): Genetic similarity matrix values for the six groups of wheat varieties based on SSR markers.

|            | Group A | Group B | Group C | Group D | Group E | Group F |
|------------|---------|---------|---------|---------|---------|---------|
| Group B    | 0.294   |         |         |         |         |         |
| Group C    | 0.352   | 0.588   |         |         |         |         |
| Group D    | 0.235   | 0.457   | 0.571   |         |         |         |
| Group E    | 0.231   | 0.228   | 0.228   | 0.319   |         |         |
| Group F    | 0.261   | 0.294   | 0.294   | 0.236   | 0.400   |         |

Fig. (1): Correlation between gene diversity and the number of alleles over 17 SSR loci in total of 33 old (left) and modern (right) bread wheat varieties.

Fig. (2): Correlation between gene diversity and the number of alleles over all wheat genomes in total of 33 old (left) and modern (right) bread wheat varieties.
Fig. (3): Correlation between gene diversity and the number of alleles over 6 homologous groups in total of 33 old (left) and modern (right) bread wheat varieties.

\[
y = 33.164x - 9.3072, \quad R^2 = 0.7109
\]

Fig. (4): Dendrogram reflecting genetic similarity between 33 Egyptian bread wheat, based on the analysis of 17 microsatellite loci.

\[
y = 11.465x + 6.1593, \quad R^2 = 0.0298
\]