MOLECULAR AND MORPHOLOGICAL EVALUATION OF POTATO GENOTYPES CULTIVATED IN SANDY SOIL

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Potato (*Solanum tuberosum* L.) is considered as an important crop worldwide and it comes after the cereal crops in the economical importance (Mahgoub *et al.*, 2015). Over the years, potato has become an important crop for both farmers and consumers worldwide. In Egypt, potato was introduced on the small scale during the nineteenth century, it is nowadays the second most important vegetable crop after tomato; and Egypt is one of the largest producers and exporters of potatoes in Africa (Ramadan, 1981). Potatoes are grown in Egypt for local consumption, export and processing industries (Abd-Elgawad and Youssef, 2008). Furthermore, beside carbohydrates it is a good source of vitamins, mainly B and C complexes and mineral salts.

Production of potato (*Solanum tuberosum* L.) takes a very important

place in the world agriculture, with a potential production of about 270 million tons which harvested from 12.2 million hectares of planted area. The cultivated potato area was increased in Egypt especially in the new lands under new irrigation systems by adding the organic fertilizers and pesticides to the irrigation water. Moreover, Potato is one of the main crops in Egypt where the production is about 4.80 million tons which harvested from 0.178 million hectare (FAOSTAT, 2013). The success of potato breeding programs is depending on identification of the amount and distribution of genetic diversity in the gene pool, to identify the gaps in germplasm collections and to develop effective conservation and management strategies (Esfahani *et al.*, 2009). The correct identification, characterization and evaluation of conserved genotypes are fundamentally important for genetic improvement programs and for detecting duplicates in germplasm banks (Goncalves *et al*., 2008; Sudre *et al*., 2010; Abdellatif *et al.*, 2012). The genetic deviation can be evaluated based on agronomic, morphological, biochemical, physiological and molecular characteristics (Goncalves *et al.*, 2008). Studies with molecular markers have made significant contributions for understanding the genetic diversity. When compared with other types of markers, they present a greater number of polymorphic loci, which allows distinguishing between accessions that may have similar to the morphological and agronomical traits (Abouzied *et al.*, 2013).

Several methods were recommended for potato cultivar identification such as RAPD (McGregor *et al*., 2000), AFLP (Van Treuren *et al.*, 2004), SSR (Ghislain *et al.*, 2004) and ISSRs (Miz *et al.*, 2008; Aguilera *et al.*, 2011; Hardiganl *et al*., 2014). ISSR marker is often chosen to perform these studies considering the advantages regarding high speed and polymorphism (Abdellatif and Soliman, 2013), therefore, it clearly shows genetic variations among studied potato cultivars (Mahfouz *et al.*, 2012). The superiority of ISSR marker over other techniques has been identified in several studies. Prevost and Wilkinson (1999) found that five ISSR primers were adequate to distinguish among 35 varieties of potatoes. Aversano *et al.* (2009) and Mumtaz *et al.* (2010) identified thirteen ISSR primers to investigate the broad variability in cytoplasmic and nuclear DNA of *Solanum* genotype regenerated plantlets and emphasized that ISSR markers, due to its fast, high reproducibility and low cost, it offered useful information, and suitable for the analysis of genetic variations in this method of proliferation.

This study was conducted to investigate the genetic relationships among 26 potato (*Solanum tuberosum* L.) genotypes grown in Egyptian sandy soil using both morphological characteristics and molecular markers. The potato growth, yield

and tuber quality were evaluated in this study.

MATERIAL AND METHODS

Plant material

Twenty-six potato genotypes including five cultivated genotypes (Spunta, Desiree, Sophie, Red Sun and Safari) have been kindly obtained from HZPC and DE NIJS potato companies (Table 1). These potato genotypes were used for both morphological and molecular experiments.

Morphological experiment

Two field experiments were carried out during the two growing seasons of 2014 and 2015 at the farm of Environmental Studies and Research Institute, University of Sadat City, Minoufiya, Egypt. The morphological experiments were carried out in order to study the characteristics of yield and its components and quality of tubers under sandy soil conditions to evaluate these twenty-six potato genotypes. The physical and chemical analyses of the soil are presented in Table (2). The twenty-six potato genotypes were cultivated in a randomized complete block design (RCBD) with three replications. Each genotype was planted in three rows of 3 m long and 0.9 m wide. Tubers from each genotype were sown at the end of January in the two growing seasons (2014 and 2015) and spaced at 25 cm apart. The normal agri-culture practices for growing potato plants were applied whenever required.

Total yield and tuber quality

After 120 days of planting, tubers from each plot were harvested, weighted, counted and graded for recording the following data; number of tubers/plant, average weight of tuber (g), average yield of tubers/plant (g), total yield/plot and then calculated as ton/ Feddan. The potato tubers were graded into four sizes: grade 1 (more than 70 mm in diameter); grade 2 (55 to <70 mm in diameter); grade 3 (35 to >55 mm in diameter); and grade 4 (less than 35 mm in diameter). Tubers of each grade were weighted and its percentage from the total marketable yield was calculated.

Morphological Statistical analysis

The recorded morphological data were subjected to statistical analysis of variance as described by Snedecor and Cochran (1967) to identify significant treatments and/or interaction effects using 'F test' by the SAS program. The means of the treatments were compared by the Student's Least Significant Difference (LSD) value at a 5% of probability level. The averages of the morphological traits were calculated for each genotype (the averages of the two seasons and the three replications). The averages of the morphological data were used for constructing the two-way hierarchical analysis using JMP IN 7 software (Lehman et al., 2005; SAS, 2003).

Molecular marker analysis

1. DNA extraction

About a 100 mg of the tubers of each genotype was grounded in liquid nitrogen using a pestle and mortar to the fine powder. The grounded samples were used for DNA isolation using the DNA extraction kit (iNtRON Biotech., Inc.) according to their manufacturer instruction and the concentration was adjusted at 25 ng/µl.

2. PCR amplification and electrophoresis

Ten random 10-*mer* primers were used for RAPD analysis (Table 3). The PCR reaction was contained a 50 ng DNA template, 7.5 µl of PCR master mix (iNtRON Biotech., Inc.) and 0.25 µm of the primer. The volume was adjusted up to 15 µl using ddH$_2$O. The PCR program consisted of an initial denaturation step at 94°C for 5 min., followed by 35 cycles of a template denaturation step at 94°C for 40 seconds, primer annealing step at 32°C for 35 seconds and primer extension step at 72°C for 40 seconds, followed by storage at 10°C. The products were separated on a 1.5% agarose gel electrophoresis and then gels were photographed for analysis.

Ten ISSR primers were used to perform ISSR analysis (Table 3). PCR reaction was performed in a 15 µl of reaction volume containing 7.5 µl of master mix, 50 ng (2 µl) of DNA template and 0.25 µm of the primer. PCR program was carried out for 36 cycles at 94°C for 45 seconds, 49°C for 50 seconds and 72°C

for 45 seconds. A primary denaturation step at 94°C for 5 min. and a final extension step at 72°C for 7 min were applied. PCR products were separated on a 2% agarose gel electrophoresis, and then gels were photographed for analysis.

3. Data handling and cluster analysis

Both RAPD and ISSR gels were scored for presence or absence of the amplified fragments for each primer as 1 or 0, respectively. The scored data of both ISSR and RAPD markers were used to generate dendrograms. Similarity, matrices were calculated using Jaccard coefficient's algorithm (Sokal and Sneath, 1963) and used to construct dendrograms using UPGMA method (Rohlf, 1998). Dendrogram was used to determine the genetic relationships among the different investigated genetic material under study. The analysis was conducted using NTSYS-pc software (Rohlf, 1998).

RESULTS AND DISCUSSION

Morphological experiment of yield and its components

The analysis of variance (ANOVA) of the potato yield traits and its components showed highly significant differences among genotypes, growing seasons and the interaction between genotypes and growing seasons for all the studied traits (Table 4).

Some traits showed better performances during the growing season of 2015 such as tuber weight in grams, tuber

yield/plant in grams and the percentage of tuber size (35-55 and 55-70 mm in diameter traits); while the other parameters (e.g. total yield/Feddan and percentage of tuber size <35 and >70 mm in diameter) were better in the growing season of 2014 (Table 5).

The NAP genotype gave the highest significant values for the yield parameters (yield/plant 960 g and total yield/Feddan 16107 Kg) overall the other genotypes and the NIZ genotype gave the highest significant value (146.5 g) for the tuber weight trait. On the other hand, OH, Cleopatra and Sophie potato genotypes showed the least significant values for tuber weight (50.9 g), yield/plant (550 g) and total yield/Feddan (8663 Kg), respectively (Table 6). These results are in good harmony with those reported by Bekhit *et al.* (2005) and Danilchenko *et al.* (2005). Similar results were recorded by Kate *et al.* (2005), Levya and Tai (2013), Khan *et al.* (2013) and Habib *et al.* (2014). They found highly significant differences among potato genotypes concerning potato yield and its components.

Tuber size traits

LSD values of the tuber size traits showed that the highest percentage of the tuber size with more than 70 mm in diameter was obtained from the NAP; (60.2%); and the lowest percentage of both sizes less than 35 mm (6.6%) and 35-55 mm in diameter (12.8%) were also obtained from the same genotype (Table 6). On the other hand, the highest percentage of the small size; <35 mm in diameter (55.2%); and the least percentage of the big size; >70 mm in diameter (0.7%); were obtained from the OH genotype. The highest significant percentages of the medium tuber size were recorded for the genotypes Vr808 for the size 35-55 mm (35.2%) and Cleopatra for the size 55-70 mm in diameter (49.7%); while the lowest significant percentage of the size 55-70 mm in diameter (15.9%) was noted for the Mondia genotype (Table 6). Such differences in the total yield and its components among the tested cultivars may be related to the differences in their vegetative growth vigor and to their response to fertilizers and suitable weathering to growth characteristics. These results are in agreement with those reported by Arafa (2004), Alva *et al.* (2008) and Alva *et al.* (2012). Khan *et al.* (2013), on the contrary, reported that pyramid height for Desiree cultivar indicated that maximum tuber yield, size and weight traits among all potato cultivars which contributed to its high yield.

Morphological Two-way Hierarchical Cluster Analysis

A two-way hierarchical cluster analysis was carried out using JMP IN 7 software for the twenty-six potato genotypes among the seven morphological traits. According to this analysis, the potato genotypes were separated into three main cluster groups. The first cluster was included the ALF, Liseta, Mozart, NIZ, Red Sun, Safari, Spunta, LYS, Adora, Mondial, RAF and NAP genotypes. The second group was included the

Red Scarlett, Desiree, MEM, FIS, RAF, Fortus, Vr808, EIB, Colomba, Dynamica, Astrix and Cleopatra genotypes. The third cluster was included the OH and Sophie genotypes (Fig. 1).

In the second way of the hierarchical clustering (traits clustering), the morphological traits were separated into two clusters. The first cluster included four morphological traits (tuber weight, tuber size >70 mm in diameter, yield/plant and total yield/Feddan) while the second cluster contained three morphological traits (tuber sizes of <35 mm and from 35-55 and 55-70 mm in diameter) (Fig. 1). This result means that the yield traits and its component were clustered together along with the trait of tuber size >70 mm in diameter. Therefore, the tuber size >70 mm in diameter trait may be strongly correlated with the yield traits in potato plants.

Similar results were reported by Haydar *et al.* (2007), who studied the genetic diversity of tuber yield traits and its components of 30 potato genotypes and they reported that the genotypes were grouped into six clusters and the maximum diversity was contributed by tuber weight/plant.

Molecular analysis

Ten RAPD and ten ISSR primers were used for PCR amplification of the 26 potato genotypes. The amplification of fragments was in different sizes depending upon the genotype (Fig. 2). All RAPD and the half of the ISSR primers produced polymorphic fragments (Table 3). The total amplified fragments generated from each RAPD primer were ranged from 14 fragments for the OPW07 primer to 32 fragments for the OPB10 primer, while they were ranged from six to 13 fragments for the ISSR UBC818 and UBC811 primers, respectively. The polymorphic fragments percentage of the RAPD primers was ranged from 76.19% for the OPR02 primer to 88.46% for the OPA09 primer; and from 72.72% to 90.9% for the ISSR UBC817 and UBC810 primers, respectively (Table 3). Comparable results were reported by Abbas *et al.* (2008), who obtained amplification of 26.3 alleles per potato genotype using RAPD primers. They mentioned that the size of score able fragments were ranged from approximately 250 to >1000 bp. Similarly, Gauchan *et al.* (2012) produced 29 different marker fragments of which 69.0% were polymorphic. The same result was recorded for ISSR markers, whereas, Nováková *et al.* (2010) reported that both SSR and ISSR markers afford sufficient polymorphism for variety identification in Czech potato variety. Torabi-Giglou *et al.* (2015) studied the genetic diversity of wild and cultivated potato and they found that the average numbers of score able fragments which produced per primer using ISSRs were eight polymorphic fragments for all genotypes and the UBC826, UBC820 and UBC824 primers gave the best results for all attributes.

RAPD cluster analysis

Dendrogram was established depending upon the RAPD data (Fig. 3).

According to the cluster analysis, the Mondial genotype was separated at the uppermost of the dendrogram apart of the other genotypes and the Red Sun genotype was separated at the lowermost of the dendrogram. The other 24 potato genotypes were separated into five clusters. The first cluster was located at the uppermost part of the dendrogram and contained the NAP, Safari, Adora, RAF, Cleopatra, RAF and Mozart genotypes. The second cluster was included the EIB, ALF, Spunta, NIZ, OH and FIS genotypes. The third cluster was involved the Desiree, Dynamica, MEM, LYS, Asterix and Sophie genotypes. The fourth cluster was contained the Fortus and Vr808 genotypes while the last cluster included the Liseta, Red Scarlett and Colomba genotypes (Fig. 3). Genetic diversities among potato genotypes were identified by several researchers (Abbas *et al.*, 2008; Rocha *et al.*, 2010; Gauchan *et al.*, 2012; Hoque *et al*., 2013). All of them found a high level of genetic diversity among potato genotypes using cluster analysis.

ISSR cluster analysis

According to the ISSR analysis, the Mozart and ALF genotypes were separated apart of all other genotypes at the lower most of the dendrogram while the Mondial, Sophie, Liseta, NAP and Red Sun genotypes were distributed separately throughout the dendrogram (Fig. 4). The other genotypes were separated into four clusters. The first cluster included the Cleopatra, EIB, OH, Adora, FIS and Vr808 genotypes. The second cluster contained RAF, Spunta, Fortus, Dynamica and Safari genotypes. The third cluster contained the Bartina and Asterix genotypes. The fourth cluster included the NIZ, Desiree, LYS, MEM, Red Scarlett and Colomba genotypes (Fig. 4). Novák-ová *et al.* (2010) discriminated genetic relationships among twenty potato varieties using cluster analysis and they reported that the similarity values among these varieties were ranged between 65-80%. On the other hand, Torabi-Giglou*et al.* (2015) mentioned that pairwise species matrix of Neigenetic distance were varied from 0.058 to 0.645 among some potato genotypes based on ISSR marker.

Thus, it can be concluded that both morphological and molecular markers could be efficiently used to study genetic diversity in potato genotypes. Although the morphological characteristics are affected with the environment, their results could be supported by the molecular markers results.

SUMMARY

Twenty-six potato genotypes including seventeen cultivars were used to investigate the genetic diversity of potato plants (*Solanum tuberosum* L.) grown in Egyptian sandy soil using both morphological characteristics and molecular markers. The analysis of variance (ANOVA) of the potato yield traits and its components showed highly significant differences among the genotypes, the growing seasons and the interaction between genotypes and the growing seasons for all the studied traits. The NAP geno-

type gave the highest significant values for the yield traits and the highest percentage of the tuber size more than 70 mm in diameter trait. The NIZ genotype gave the highest significant value (146.5 g) for the tuber weight trait. On the other hand, OH, Cleopatra and Sophie potato genotypes showed the least significant values for tuber weight and tuber size traits. According to the two-way hierarchical cluster analysis, the potato genotypes were separated into three main cluster groups, while in the second way of the hierarchical clustering (traits clustering), the morphological traits were separated into two clusters. According to RAPD cluster analysis, the Mondial genotype was separated at the uppermost of the dendrogram apart of the other genotypes and the Red Sun genotype was separated at the lowermost of the dendrogram. The other 24 potato genotypes were separated into five clusters. Depending upon ISSR cluster analysis; the Mozart and ALF genotypes were separated apart of all other genotypes at the lowermost of the dendrogram, while the Mondial, Sophie, Liseta, NAP and Red Sun genotypes were distributed separately throughout the dendrogram. The other genotypes were separated into four clusters. Thus, it can be concluded that both morphological and molecular markers could be efficiently used to study the genetic diversity among potato genotypes. Although the morphological characteristics are affected with the environment, their results could be supported by the molecular markers results.

REFERENCES

Abbas, S. J., G. Rasool, S. R. U. Shah and A. Iqbal (2008). Analysis of genetic diversity in Pakistani potato cultivars by using Randomly Amplified Polymorphic DNA (RAPD) primers. American-Eurasian Journal of Sustainable Agriculture, 2: 50-53.

Abd-Elgawad, M. M. and M. Youssef (2008). Programs of research development in Egypt. First International Workshop on Ecology and management of plant-parasitic nematode communities in South-Mediterranean Ecosystems, 17-19 March.

Abdellatif, K. F. and Y. A. Soliman (2013). Genetic relationships of cotton (*Gossypium barbadense* L.) genotypes as studied by morphological and molecular markers. African J. Biotech., 12: 4736-4746.

Abdellatif, K. F., Y. A. Khidr, Y. M. El-Mansy, M. M. El-Lawendy and Y. A. Soliman (2012). Molecular diversity of Egyptian cotton (*Gossypium barbadense* L.) and its relation to varietal development. Journal of Crop Science and Biotechnology, 15: 93-99.

Abouzied, H. M., S. M. M. Eldemery and K. F. Abdellatif (2013). SSR-based genetic diversity assessment in tetraploid and hexaploid wheat.

British Biotechnology J., 3: 2231-2927.

Aguilera, J. G., L. A. Pessoni, G. B. Rodrigues, A. Y. Elsayed, D. J. H. Silva and E. G. de Barros (2011). Genetic variability by ISSR markers in tomato (*Solanum lycopersicon* Mill.). Revista Brasileira de Ciências Agrarias, 6: 243-252.

Alva, A. K., H. P. Collins, R. A. Boydston and A. D. Moore (2008). Managing deficit irrigation for potatoes. http:// www.intlcss. org/ files/icss/congress-proceedings/ 2008-papers /cs1-s5/cs1-s5-o5-ashok-k-alva.pdf.

Alva, A. K., A. D. Moore and H. P. Collins (2012). Impact of deficit irrigation on tuber yield and quality of potato cultivars. Journal of Crop Improvement, 26: 211-227.

Arafa, M. M. (2004). Effect of different NPK treatments on growth, yield, quality and chemical components of two potato cultivars. Annals of Agric. Sci. Moshtohor, 42: 753-766.

Aversano, R., S. Savarese, J. Maria, D. Nova, L. Frusciante, M. Punzo and D. Carputo (2009). Genetic stability at nuclear and plastid DNA level in regenerated plants of (*Solanum* spp.) and hybrids. Euphytica, 165: 353-361.

Bekhit, S. R., A. H. Hassan, M. H. Ramadan and A. M. A. El-Anany (2005). Effect of different levels and sources of nitrogen on growth, yield and quality of potatoes grown under sandy soil condition. Annals of Agric. Sci. Moshtohor, 43: 381-394.

Danilchenko, V., R. Dris and R. Niskanen (2005). Influence of organic manure and mineral fertilization on yield, composition and processing quality of potatoes. J. Food Agric., 3: 143-144.

Esfahani, S. T., B. Shiran and G. Balali (2009). AFLP markers for the assessment of genetic diversity in European and North American potato varieties cultivated in Iran. Crop Breed. and Appl. Biotech., 9: 75-86.

FAOSTAT (2013). Production quantities by country, Statistics Division. Food and Agriculture Organization of The United Nation. Available at http:// faostat3.fao.org/browse /Q/QC/E.

Gauchan, D. P., C. Brinegar, A. Hada and D. Singh (2012). Molecular characterization of Nepali potato cultivars using Randomly Amplified Polymorphic DNA (RAPD) markers. Journal of Advanced Laboratory Research in Biology, 3: 72-75.

Ghislain, M., D. M. Spooner, F. Rodrıguez, F. Villamon, J. Nunez

and C. Vasquez (2004). Selection of highly informative and user-friendly microsatellites (SSRs) for genotyping of cultivated potato. Theor. Appl. Genet., 108: 881-890.

Goncalves, L. S., R. Rodrigues, A. T. Amaral and M. Karasawa (2008). Comparison of multivariate statistical algorithms to cluster tomato heirloom accessions. Genet. Mol. Res., 7: 1289-1297.

Habib, M., S. Nasrollahzadeh, Y. Raiyi and D. H. Panah (2014). Responses of potato genotypes to limited irrigation. International Journal of Agronomy and Agricultural Research (IJAAR), 5: 13-19.

Hardiganl, A. M., J. Bamberg, R. C. Buell1 and S. D. Douches (2014). Taxonomy and genetic differentiation among wild and cultivated 1 germplasm of *Solanum* sect. potatoes. The Plant Genome doi:10.3835/plant genome 2014.06.0025.

Haydar, A., M. B. Ahmed, M. M. Hannan, M. A. Razvy, M. A. Mandal, M. Salahin, R. Karim and M. Hossain (2007). Analysis of genetic diversity in some potato varieties grown in Bangladesh. Middle-East Journal of Scientific Research, 2: 143-145.

Hoque, M. E., H. Huq and N. J. Moon (2013). Molecular diversity analysis in potato (*Solanum tuberosum*

L.) through RAPD markers. SAARC J. Agri., 11: 95-102.

Kate, D. M., A. V. Solanke, T. K. Tiwaride and S. M. Nemade (2005). Growth and yield of potato cultivars as affected by integrated nutrient management system. J. Maharashtra Agric. Univ., 30: 236-237.

Khan, M. F., N. Tabassum, A. Latif, A. Khaliq and M. Malik. (2013). Morphological characterization of potato (*Solanum tuberosum* L.) germplasm under rain fed environment. African Journal of Biotechnology, 12: 3214-3223.

Lehman, A., L. Creighton, J. Sall, B. Jones, E. Vang, M. Blackwelder, A. D. Zangi and B. Corcoran (2005). JMP IN 7 software, Statistical Analysis System SAS Users Guide: Statistics. SAS Institute Inc. Editors, Cary, NC.

Levya, D. and G. C. C. Tai (2013). Differential response of potatoes (*Solanum tuberosum* L.) to salinity in an arid environment and field performance of the seed tubers grown with fresh water in the following season. Agricultural Water Management, 116: 122-127.

Mahfouze, S. A., K. A. F. El-Dougdoug, O. E. El-Sayed, M. A. Gomaa and E. K. Allam (2012). Genetic diversity of cultivated and wild-type potatoes under potato spindle tuber

viroid infection. New York Science Journal, 5: 9-18.

Mahgoub, H. A. M., G. S. A. Eisab and M. A. H. Youssef (2015). Molecular, biochemical and anatomical analysis of some potato (*Solanum tuberosum* L.) cultivars growing in Egypt. Journal of Genetic Engineering and Biotechnology, 13: 39-49

McGregor, C. E., C. A. Lambert, M. M. Greyling, J. H. Louw and L. Warnich (2000). A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum* L.) germplasm. Euphytica, 113: 135-144

Miz, R. B., L. A. Mentz and T. T. Souza-Chies (2008). Overview of the phylogenetic relationships of some southern Brazilian species from section Torva and related sections of spiny *Solanum* (*Solanum subgenus* and *Leptostemonum, Solanaceae*). Genetica, 132: 143-158.

Mumtaz, A. S., M. Naveed and Z. K. Shinwari (2010). Assessment of genetic diversity and germination pattern in selected cotton genotypes of Pakistan. Pak. J. Bot., 42: 3949-3956.

Nováková, A., K. Šimáčková, J. Bárta and V. Čurn (2010). Utilization of DNA markers based on microsatellite polymorphism for identification of potato varieties cultivated in the Czech Republic. Journal of Central European Agriculture, 11: 415-422.

Prevost, A. and M. J. Wilkinson (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor. Appl. Genet., 98: 107-112.

Ramadan, M. S. (1981). Report on potato production in Egypt. International Potato Course: production, storage, and seed technology. Report of Participants. International Agricultural Center, Wageningen, The Netherlands, 1981.

Rocha, E. A., L. V. Paiva, de Carvalho, H. H. and C. T. Guimarães (2010). Molecular characterization and genetic diversity of potato cultivars using SSR and RAPD markers. Crop Breeding and Applied Biotechnology, 10: 204-210.

Rohlf, F. J. (1998). NTSYSpc. Numerical taxonomy and multivariate analysis system, version 2.01c. Exeter Software, New York.

SAS (2003). Statistical Analysis System SAS Users Guide: Statistics SAS Institute.

Snedecor, G. W. and W. G. Cochran (1967). Statistical methods 6th edition, Oxford and IBH Publishing Co. New Delhi.

Sokal, R. and P. Sneath (1963). Principles of Numerical Taxonomy, San Francisco: W. H. Freeman.

Sudre, C. P., L. S. A. Goncalves, R. Rodrigues, A. T. do Amaral, E. M. Riva-Souza and C. D. Bento (2010). Genetic variability in domesticated *Capsicum* spp. as assessed by morphological and agronomic data in mixed statistical analysis. Genet., Mol., Res., 9: 283-294.

Torabi-Giglou, M., P. Jaber, S. A. Mohammadi, F. Z. Nahandi, A. M. Azar and J. Śliwka (2015). DNA and morphological diversity and relationship analysis of selected cultivated, wild potatoes and some promising hybrids. Journal of Biodiversity and Environmental Sciences (JBES), 6: 175-186.

Van Treuren, R., A. Magda, R. Hoekstra and J. L. Van Hintum (2004). Genetic Resources and Crop Evolution 51: 277.

Table (1): Sources and some morphological characteristics of potato genotypes that used in this study.

No.	Genotypes	Skin Color	Source	Cross breeding	Maturity	Flesh color	Shape
1	Mondial	Y	HZPC	SPUNTA x VE 66-295	L	LY	LO
2	NAP	Y	HZPC	Unknown	L	LY	O
3	Cleopatra	R	HZPC	ZPC 50 35 x DESIREE	E	LY	ROO
4	Mozart	R	HZPC	REDSTAR x CAESAR	ME	Y	O
5	RAF	Y	HZPC	Unknown	ME	LY	OLO
6	EIB	Y	HZPC	Unknown	ME	LY	OLO
7	ALF	DR	HZPC	Unknown	ME	W	RLO
8	NIZ	DR	HZPC	Unknown	ME	Y	RLO
9	Spunta	Y	DE NIJS	Bea x USDA 96-56	Medium E	Light Y	Long O/long
10	OH	DR	HZPC	Unknown	ME	LY	ROO
11	FIS	Y	HZPC	Unknown	L	CREM	RO
12	Adora	Y	HZPC	PRIMURA x ALCMARIA	VE	LY	O
13	Bartina	R	HZPC	SATURNA x ZPC 62- 75	L	Y	ROO
14	VR 808	DY	HZPC	LADY CLAIRE x ATLANTIC	E	Y	RO
15	Fortus	Y	HZPC	Unknown	ME	Y	O
16	Liseta	Y	HZPC	SPUNTA x VE 66-295	E	LY	LO
17	Colomba	Y	HZPC	CARRERA x AGATA	E	Y	ROO
18	Red Scarlett	R	HZPC	ZPC 80 O239 x MANS.MGB78-286	ME	Y	OLO
19	Desiree	R	DE NIJS	Urgenta x Depesche	Medium L	Light Y	OLO
20	Dynamica	R	HZPC	CORNADO x RZ- 86-2918	M	LY	OLO
21	MEM	R	HZPC	Unknown	ME	LY	OLO
22	LYS	Y	HZPC	Unknown	ME	Y	ROO
23	Asterix	R	HZPC	CARDINAL x VE 70- 9	L	Y	LY
24	Sophie	Y	DE NIJS	TE 93-26-02 x Lady Claire	Medium L	Deep Y	Round
25	Red Sun	R	DE NIJS	Inova x Amadeus	Medium E	Y	O
26	Safari	Y	Greenvale AP	Obelix x Amadeus	Medium E	Y	RO

Y: Yellow, R: Red, DR: Dark red, E: Early, L: Late, O: Oval, VE: very early, LY: Late Yellow, ROO: Round Oval/ Oval, OLO: Oval Long/Oval and LO: Long Oval

Table (2): Some physical and chemical characteristics of the soil and nutrients of Bentoniet in Sadat City.

PH (KCl)	EC Mg/l	% OM	% CaCO$_3$	CECcmol. K g^{-1}	% Sand	% Silt	*% Clay*	Texture
7.72	8.23	0.116	1.45	13.90	69.90	20.50	7.66	Sandy loam

PH	% Total C	N ppm	P ppm	K ppm	Fe ppm	Mn ppm	Zn ppm	Cu ppm
7.39	0.37	13.10	7.85	300	14.98	3.01	1.82	1.01

Table (3): RAPD and ISSR primer sequences and their polymorphisms percentage.

	RAPD markers				ISSR markers			
Primer	Sequence	# Bands	% Polymor-phism	Primer	Sequence	# Bands	% Polymor-phism
OPA07	GAAACGGGTG	25	88.00	UBC807	(AG)$_7$T	Not detected	--
OPA09	GGGTAACGCC	26	88.46	UBC808	(AG)$_8$C	10	80.00
OPB10	CTGCTGGGAC	32	84.38	UBC810	(GA)$_8$T	11	90.90
OPB12	CCTTGACGCA	20	85.00	UBC811	(GA)$_8$C	13	76.92
OPC05	GATGACCGCC	17	76.47	UBC812	(GA)$_8$A	Not detected	--
OPC09	CTCACCGTCC	16	81.25	UBC814	(CT)$_8$T	Not detected	--
OPR02	CACAGCTGCC	21	76.19	UBC815	(CT)$_8$G	Not detected	--
OPR10	CCATTCCCCA	21	90.48	UBC816	(CA)$_8$T	Not detected	--
OPR20	ACGGCAAGGA	23	78.26	UBC817	(CA)$_8$A	11	72.72
OPW07	CTGGACGTCA	14	85.71	UBC818	(CT)$_8$G	6	83.33

Table (4): Analysis of variance of seven morphological traits calculated from two growing seasons of the 26 potato genotypes.

Source	DF	Mean of squares						
		Tuber weight (g)	Yield/ plant (g)	Total yield/Fed. (Kg)	%tuber size < 35	% tuber size (35-55)	% tuber size (55-70)	% tuber size > 70
Genotypes	25	51127.6 **	869666.6 **	289771682 **	10379.6 **	3871.1 **	6563.8 **	18799.3 **
Years	1	2070.2 **	74311.5 **	8806824 **	4.3 **	9.6e-5 **	10.5 **	0.9 **
Genotypes x Years	25	17903.9 **	852106.5 **	248965446 **	161.7 **	239.9 **	160.0 **	204.1 **

Table (5): LSD differences between the two growing seasons (2014 and 2015) of seven morphological traits. α = 0.050, t = 2.00758.

Years	Tuber weight (g)	Yield/ plant (g)	Total yield /Fed. (Kg)	% tuber size <35	% tuber size (35-55)	% tuber size (55-70)	% tuber size >70
2015	109.4 A	734.1 A	11533.2 B	17.3 B	23.863 A	28.2 A	30.6 B
2014	100.5 B	680.7 B	12115.2 A	17.7 A	23.861 B	27.6 B	30.8 A

Levels not connected by same letter are significantly different.

Table (6): LSD differences among the 26 potato genotypes between of seven morphological traits recorded during the two growing seasons (2014 and 2015). α = 0.050, t = 2.00758.

Genotypes	Tuber weight (g)	Yield/plant (g)	Total yield/ Fed. (Kg)	% of tuber size (mm) <35	35-55	55-70	>70
Mondial	100.5 Q	718.5 M	11993.5 M	18.8 J	14.4 X	*15.9 Z*	51.2 B
NAP	133.1 C	*950.0 A*	*16107.0 A*	*6.6 Y*	*12.8 Z*	20.1 V	*60.2 A*
Cleopatra	91.7 T	*550.0 Z*	8999.5 Y	19.1 H	28.1 H	*49.7 A*	3.1 Y
Mozart	121.6 G	775.5 E	13016.0 F	7.9 X	15.6 W	34.0 F	42.5 C
RAF	108.8 L	767.0 G	12857.0 H	30.9 C	16.0 V	20.8 T	32.3 M
EIB	102.9 O	814.5 C	13697.5 C	16.8 K	25.3 N	24.7 Q	33.2 K
ALF	109.3 J	708.0 O	11816.0 N	13.8 O	14.0 Y	30.0 H	42.2 D
NIZ	*146.5 A*	805.5 D	13546.0 D	15.3 M	21.6 R	22.2 S	41.0 G
Spunta	128.7 D	640.5 U	11110.5 R	10.9 S	19.0 T	27.8 N	42.1 E
OH	*50.9 Z*	558.5 Y	9155.0 X	*55.2 A*	26.1 I	18.0 Y	*0.7 Z*
FIS	88.7 W	715.0 N	11435.5 P	12.5 Q	25.5 M	34.8 E	27.3 Q
Adora	121.9 F	734.0 K	12274.5 K	20.2 E	22.0 P	18.5 X	38.9 I
Bartina	105.4 N	729.0 L	12188.0 L	10.8 T	25.9 K	28.2 M	35.3 J
Vr 808	89.8 U	648.0 S	10744.0 T	21.9 D	*35.2 A*	31.7 G	11.2 X
Fortus	97.7 R	578.5 W	9510.5 W	15.1 N	25.8 L	29.1 K	29.9 N
Liseta	115.2 H	820.0 B	13799.0 B	10.0 U	18.6 U	29.9 I	41.6 F
Colomba	93.9 S	743.0 I	12435.0 J	19.7 F	31.8 B	24.0 R	24.5 T
Red Scarlett	107.9 M	646.0 T	10705.0 U	12.3 R	31.5 C	27.0 O	29.2 O
Desiree	102.6 P	698.0 P	11638.0 O	13.6 P	31.5 D	26.3 P	28.7 P
Dynamica	89.3 V	770.0 F	12918.5 G	19.3 G	26.0 J	29.5 J	25.3 S
MEM	109.2 K	680.0 Q	11316.0 Q	15.1 N	30.1 F	28.8 L	26.3 R
LYS	114.5 I	656.5 R	10898.0 S	19.0 I	20.9 S	20.4 U	39.6 H
Asterix	74.9 X	759.0 H	12718.5 I	15.5 L	28.5 G	35.1 D	23.9 U
Sophie	57.0 Y	559.0 X	*8663.0 Z*	36.4 B	30.6 E	18.6 W	11.8 W
Red Sun	138.8 B	736.5 J	13321.5 E	9.5 V	21.8 Q	45.0 B	23.6 V
Safari	127.4 E	631.5 V	10566.0 V	8.8 W	22.4 O	36.0 C	32.6 L

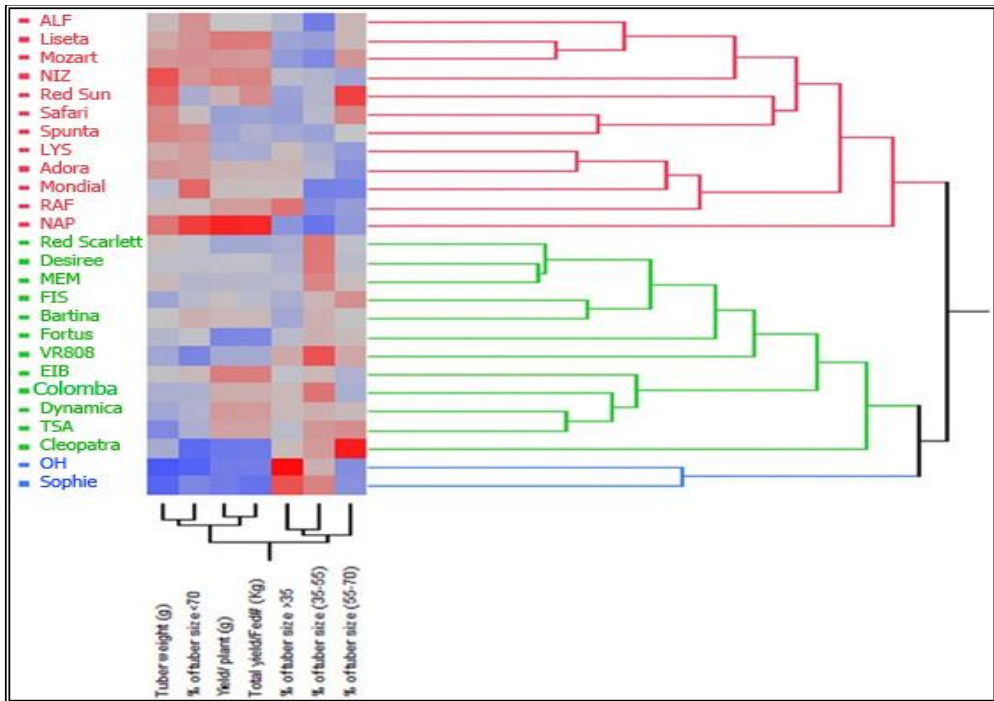Levels not connected by same letter are significantly different.

Fig. (1): Two-way hierarchical cluster analysis of the 26 potato genotypes and the seven
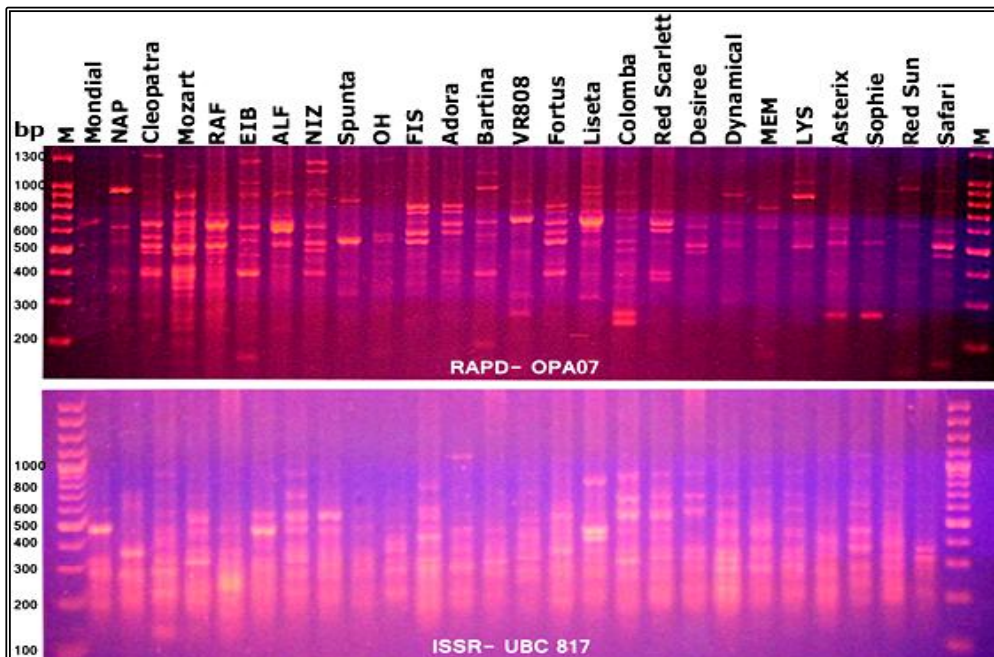morphological traits collected from the two growing seasons.

Fig. (2): DNA polymorphasim of potato genotypes based on RAPD-PCR using OPA07
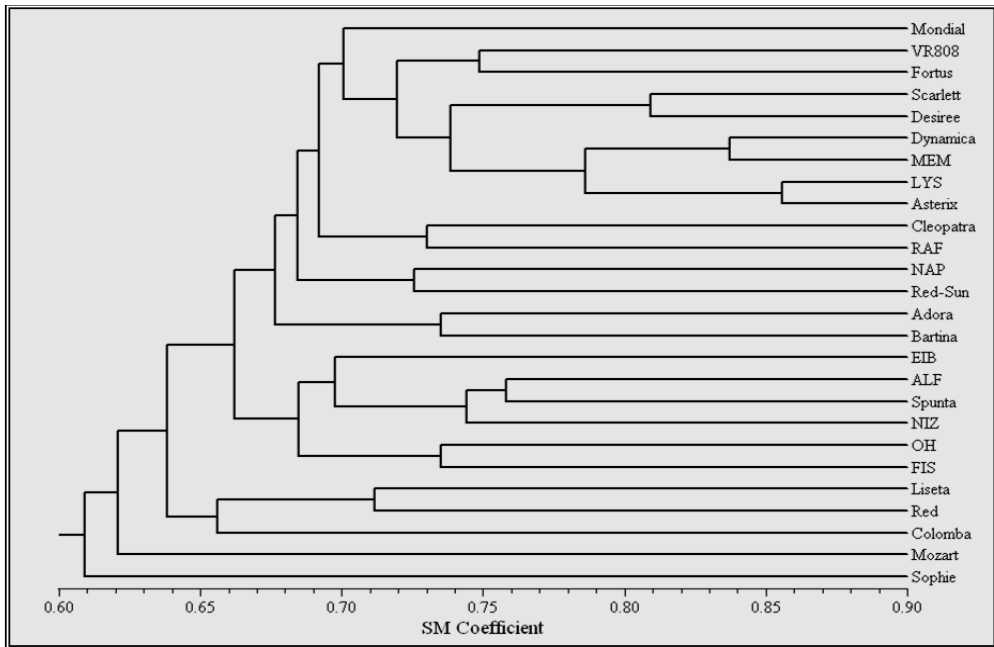primer and ISSR-PCR using UPC817 primer.

Fig. (3): Cluster analysis using the UPGMA method for the 26 imported potato genotypes based on RAPD-PCR, according to the similarity index of Jaccard.
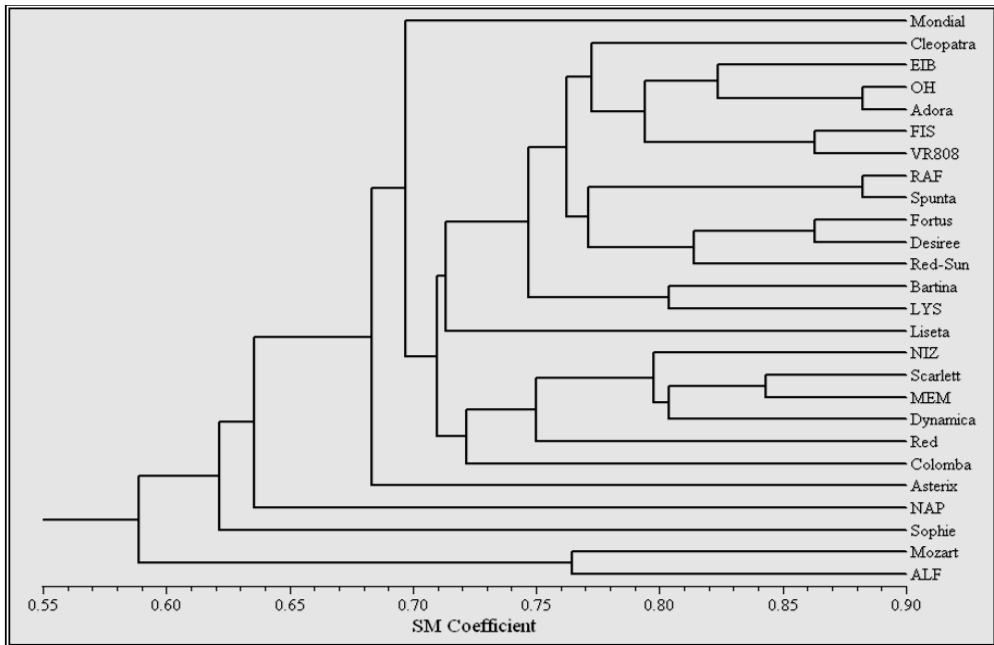

Fig. (4): Dendrogram generated using UPGMA analysis, showing relationships among potato genotypes, using ISSR data.