JOURNAL OF OASIS AGRICULTURE AND SUSTAINABLE DEVELOPMENT

www.joasdjournal.org

JOASD

p-ISSN: 2724 – 699X
e-ISSN: 2724-7007

First study of Genetic diversity in watermelon (*Citrullus lanatus*) germplasm collected from Southern Tunisia using RAPD markers

Elbekkay Mokhtar*, Hammadi Hamza, Bekri Tozer, Haddad Mansour and Ferchichi Ali

Arid and Oases Cropping Laboratory, Arid Area Institute, Medenine 4119, Tunisia.

Article info

Article history:
Received 12 July 2019
Accepted 03 September 2019

Keywords:
Watermelon, RAPD, Local cultivar, Tunisia.

https://doi.org/10.56027/JOASD.012019

*** Corresponding author**
mokhtar123ira@gmail.com

Conflict of Interest : The authors declare no conflict of interest

Abstract

The genetic diversity in local Tunisian watermelon (*Citrullus lanatus*) was studied using RAPD markers. Eight watermelon cultivars originating from south of Tunisia and belong to four populations were analysed and compared to two commercial varieties (Giza and Sugar-baby) widely produced in this area. Five of nine RAPD primers generated a total of 86 reproducible bands, 85 of which were polymorphic (98.4%). Cluster analysis of the accessions considered in this study employing RAPD data indicated that commercial varieties are significantly different of all the local cultivars. The relationships among the local cultivars (four populations) showed that Medenine population and the most genotypes of Benguerdane population were grouped together and significantly different from Kebili population. The AMOVA showed significant differentiation between populations (27%). In addition, the data showed clusters according to some fruit characteristics such as fruit shape and fruit weight. This proved that RAPD markers are useful for germplasm discrimination as well as for investigation of patterns of variation in watermelon.

1. INTRODUCTION

Watermelon (*Citrullus lanatus,* 2n=22) belong to the family of Cucurbitaceae. It has been an important vegetable in North Africa for at least 4000 years (Robinson and Decker-Walters, 1997; Paris and Janick, 2008). It is an economically important crop and largely consumed in summer as a fresh fruit (Solmaz et al., 2010). The world production in 2009 exceeds 98 million tons (FAOSTAT, 2011). The major country in the world for the watermelon production is China with 65 million tons.

In Tunisia, watermelon is an important crop which takes more and more a place in national economy and in society. This cucurbit occupies more than 20000 ha with more than 450000 tons of production (FAOSTAT, 2011). In Tunisia, which belongs to the centre of diversity area of watermelon, this crop covers all areas throughout the country (Elgazah and Chalbi, 1995; Elbekkay et al., 2009). These are located in sites with contrasting climates and soils (plain, seacoast, oases and moist areas of high altitude). However, for several decades, the local cultivars are replaced by new introduced varieties which are more uniform and highly productive.

Many autochthones Tunisian cultivars are cited and characterized morphologically by Novikof (1951). In spite of their nutritional and agronomic values, these cultivars are regressing and sometimes disappearing by the use of especially modern watermelon varieties (Elbekkay et al., 2008). In addition, few of farmers are relying on their own harvest to select seeds for the following season (Elbekkay et al., 2009).

Genetic resources are valuable reservoirs of potentially useful genes and can be beneficial in breeding studies for future needs. According to Wehner (2008), watermelon is a useful species for genetic research because of its small genome size, and the many available gene mutants and variant. Like some other cultivated cucurbits, watermelon has high genetic variability in vine, seed and fruit traits. Consequently, it is very critical to determine the germplasm diversity for effective utilization of genetic resources (Solmaz et al., 2010).

Although watermelon is a very important crop for Tunisia, few studies were conducted on the diversity of Tunisian watermelon genetic

resources. Elbekkay et al. (2009) reported that remarkable morphologic diversity exists among Tunisian watermelon genetic resources.

The molecular markers are beneficial complements to morphological and phenological characters because they are substantive of tissue or environmental factors and enable cultivar identification in the early stage of development (Wehner, 2008 and Solmaz et al., 2010). Comparisons of the performance of several types of molecular markers in measuring genetic diversity have been carried out in several plant species. According to Garcia-Mas et al. (2000), RAPD have been introduced for measuring genetic relationships in many plant species. The easiness of the method, which only requires PCR technology, has determined its replacement of RFLPs for genetic variability assessment.

Considering that there are no studies undertaken to ascertain the diversity of local watermelon in Tunisia, the objective of this study was to examine the molecular diversity among local watermelon collected from the south of Tunisia and compared with introduced varieties.

2. MATERIAL AND METHODS

2.1 Plant material

Genetic variations in eight watermelon (*Citrullus lanatus*) accessions collected from four regions (Kebili, Tataouine, Medenine and Benguerdane) in south of Tunisia were examined. Three individuals of each accession were evaluated. In addition, tow commercial variety (cv Giza and cv Sugar Baby), widely cultivated in the South of Tunisia, were included in this study (Table1, Fig 1).Plants for DNA extraction were grown in the greenhouse following standard horticultural practice.

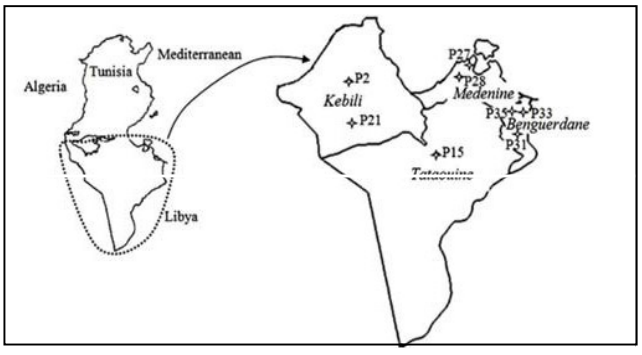

Fig 1. Origin of the 8 accessions of watermelon collected from south Tunisia

Table1. Watermelon (*Citrillus lanatus* [thumb]) germplasm used for diversity analysis. (a: according to Elbekkay et al., 2009)

Code Accessions	Provenance	Population	Decimal Degrees		Fruit Shape[a]	Fruit weight[a]
			Latitude	Longitude		
P2	Faouar	Kebili	33.3393	8.30909	Spherical	4050 g
P21	Nouail	Kebili	33.4954	8.86574	Spherical	2714 g
P15	Ferche	Tataouine	32.9591	10.3578	Spherical	3587 g
P27	Oued-sider	Medenine	33.5385	10.6717	Elongate	1689 g
P28	Bougrara	Medenine	33.4992	10.6423	Elongate	1322 g
P31	Benguerdane	Benguerdane	33.1339	11.1553	Spherical	1865 g
P33	Benguerdane	Benguerdane	33.1381	11.2173	Spherical	1414 g
P35	Benguerdane	Benguerdane	33.3627	10.6175	Spherical	1766 g
Giza	Introduced variety		-	-	Spherical	881 g
Sugar-baby	Introduced variety		-	-	spherical	913 g

2.2 Molecular analysis

DNA was extracted from watermelon leaves according to Aras et al. (2003). RAPD reactions were performed using nine primers (Table 2).

Table 2. Primers used for RAPD (Tm: melting temperature)

Primer name	Sequence (5'—3')	Tm (°C)	Total number of Bands	Number of polymorphic bands	Percentage of polymorphic Bands
OBP12	CCTTGACGCA	32	15	15	100%
OPL11	ACGATGAGCC	32	12	12	100%
OPW02	ACCCCGCCAA	34	22	22	100%
OPW04	CAGAAGCGGA	32	23	23	100%
OPY15	AGTCGCCCTT	32	14	13	92%
OPF04	GGTGATCAGG	32	-	-	-
OBP15	GGAGGGTGTT	32	-	-	-
OPC12	TGTCATCCCC	32	-	-	-
OPK14	CCCGCTACAC	34	-	-	-

The PCR was carried out in 25 µl final volume using 50 ng of genomic DNA containing 2mM MgCl2, 200 µM dNTPs, 20 pmol random primer, 1 unit of Taq DNA polymerase. The mixture was made up to 20 µl by addition of sterilized distilled water. The mixture was amplified in a thermal cycler (GeneAmp® PCR System 9700) which was programmed for one cycle of initial denaturation at 94°C for 5 min, 45 cycles of {94°C for 1 min, followed by specific annealing temperature for 1 min and ended by extension at 72°C for 2 min} and a final extension cycle that performed at 72°C for 10 min. The PCR machine was adjusted to hold the product at 4°C. The PCR products and 1

kb DNA ladder were electrophoresed on 2% agarose gel (stained with EtBr). The separated fragments were visualized with an ultraviolet (UV) transilluminator.

2.3 Data analysis

Fragments of the same molecular weight were considered as the same locus. The numbers of bands produced for each primer were scored manually for presence (1), or absence (0) and a binary matrix was generated and then used for analysis. All molecular analyses were done by the GenALEx program version 6.1 (Peakall and Smouse, 2006). The percentage of polymorphism, the mean number of observed alleles per locus were calculated. The watermelon cultivars scores were coordinated in a bi-dimensional space by principal component analysis (PCA) by computing matrix based on the Nei genetic distances (Nei, 1972). With AMOVA (Excoffier et al., 1992), pair-wise comparison between groups was tested using 999 re-sampled individuals.

3. RESULTS

A total of nine primers were screened for their ability to generate consistently amplified band patterns and to assess polymorphism in the tested accessions. Among these primers, only five have revealed unambiguously scorable polymorphic bands. These are identified as OBP12, OPL11, OPW02, OPW04 and OPY15. In fact, these mentioned primers generated multiple banding profiles with 12 to 23 polymorphic amplified DNA bands ranging in size from 160 to 1600bp. The maximum number of fragments was 23 bands produced by the primer OPW04 with 100% polymorphism. The minimum number of fragments was 12 bands produced by the primers OPL11 and PO6 with 100% polymorphism (Table 2). A total of 85 out of 86 were polymorphic (98.4%) with a mean of 17.2 bands per primer.

Genetic distances between individuals were assessed by Jacckard distance (table 3). Genetic distances ranged between 0.30 and 0.76 with a mean of 0.53. The smallest distance value of 0.30 was between P21_1 and P21_3. These individual belong to the same accession collected from Kebili. The maximum distance value of 0.76, suggesting great dissimilarity, was observed between Giza and P15_3 (individual Tataouine accession).

The genetic distances were used to analyze the variability of the studied cultivars by a principal component analysis (PCA). The first three axes of the PCA explained 66.56% of the total variability. The distribution of cultivars (Fig 2) shows the distinction of the tow introduced varieties (Sugar Baby and Giza) and the Kebili cultivars from the others. These cultivars have the highest genetic distances relative to the rest of the genotypes studied. No other geographical group was distinguished; the different cultivars are classified regardless of their provenance.

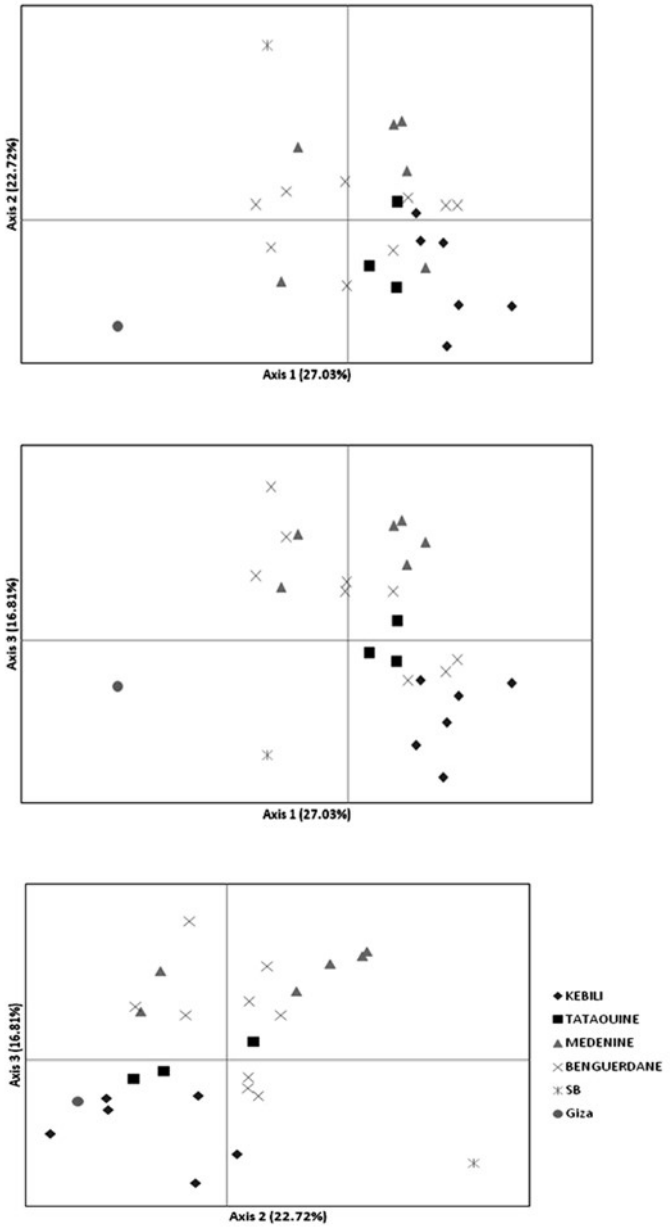

Fig 2. Principal coordinate analysis distribution using RAPD data generated from 85 bands of five primers for four population and two commercially varieties (SB: Sugar-Baby)

Cluster analysis (Neighbour Joining) employing RAPD data resulted in a dendrogram with four main branches (Fig 3). The first, containing

Medenine and Benguerdane accessions and the second containing Kebili, Tataouine accessions and three individual genotypes from Benguerdane. The two others branches separate the introduced variety (Giza and Sugar-baby).

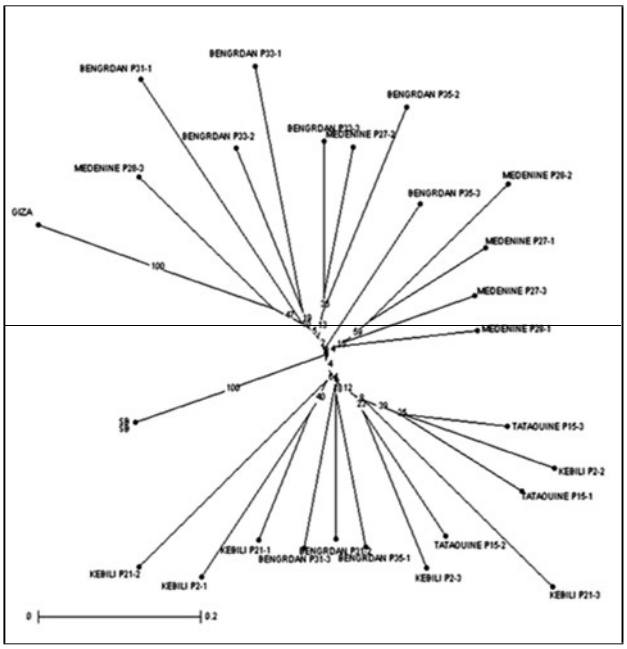

Fig 3. Dendrogram of RAPD data generated from 85 bands of 5 primers for the eight local cultivars and the two commercially varieties (SB: Sugar-Baby).

The five primers were used to evaluate the degree of polymorphism and genetic relation between studied populations. Nei genetic distances were examined for all pairwise comparisons between populations (Table 3). The distances are ranging from 0.068, between Medenine and Benguerdane populations, to 0.429 between Sugar-baby and Giza. In addition, these two introduced varieties have showed the highest genetic distances with all the Tunisian local populations.

Table 3. Pairwise Population Matrix of Nei Genetic Distance

	KEBILI	TATAOUINE	MEDENINE	BENGUERDANE	SUGAR-BABY	GIZA
KEBILI	0.000					
TATAOUINE	0.125	0.000				
MEDENINE	0.192	0.140	0.000			
BENGUERDANE	0.133	0.106	0.068	0.000		
SUGAR-BABY	0.357	0.333	0.290	0.241	0.000	
GIZA	0.389	0.333	0.358	0.277	0.429	0.000

AMOVA tests showed significant genetic differentiation (p=0.001) among populations. Indeed, there is 27% of total genetic diversity being detected among populations. For the local cultivars, pair-wise comparisons of populations (Table 4) showed that Kebili population is significantly differentiated with Medenine and Benguerdane populations. No genetic differentiation was observed between Tataouine and Kebili populations. The introduced varieties are significantly differentiated with all local population except the Tataouine one. This is in accordance with PCA and dendrogram distribution in which Kebili cultivars are jointly clustered.

Table 4. PhiPT values between different populations based on five RAPD primers.

	KEBILI	TATAOUINE	MEDENINE	BENGUERDANE	SUGAR-BABY	GIZA
KEBILI	0.000	0.248	0.007	0.002	0.011	0.015
TATAOUINE	0.038	0.000	0.218	0.437	0.103	0.101
MEDENINE	0.231	0.032	0.000	0.291	0.012	0.016
BENGUERDANE	0.144	0.000	0.010	0.000	0.006	0.006
SUGAR-BABY	0.546	0.465	0.438	0.336	0.000	0.001
GIZA	0.566	0.465	0.493	0.373	0.000	0.000

PhiPT values below diagonal. Negative *PhiPT* values converted to zero. Probability values based on 999 permutations are shown above diagonal

4. Discussion

Fingerprinting of watermelon germplasm collected from south of Tunisia was carried out using RAPD in order to obtain molecular data of local gene pool. The present study shows the reliability of RAPD analysis to detect DNA polymorphisms in this crop. According to Garcia-Mas et al. (2000), RAPD have been introduced for measuring genetic relationships in many plant species. The easiness of the method, which only requires PCR technology, has determined its replacement of RFLPs for genetic variability assessment.

Genetic diversity in watermelon was widely studied using RAPD markers. A limited number of polymorphisms was underscored in previous works (Lee et al., 1996; Levi et al. 2001a; Levi et al., 2001b). However, recent studies (Solmaz et al., 2010 and Mujaju et al., 2010) confirmed the high percentage of polymorphism obtained in the current study.

The used primers generated 86 bands with 98.83% polymorphism. Using five specifics primers tested in 26 watermelon genotypes, we registered a mean of 17 markers by primer. Thus, we assume that the studied local Tunisian watermelons are characterized, as showed by the morphological studies (Elbekkay et al., 2009), by high genetic diversity at the DNA level. This is significantly higher than reported by Solmaz et al. (2010) and Levi et al. (2001a). These authors

noted that the mean number of the total bands per primer was, respectively, 10.96 and 11.52 and the average of the percentage of polymorphism was 60.6% and 90.97%, respectively.

The cluster analysis divided the genotypes studied in this work into groups with different geographic origin. In fact, cultivars from Kebili were jointly cluster and the introduced varieties are separated from the others. The AMOVA tests showed genetic variation of 27% among the populations. In addition, Kebili landraces were significantly differentiated from all the other cultivars except Tataouine ones. This indicates that geographic proximity is an indicative of genetic similarity and can be a guide for understanding the genetic structure of this specie. In Kebili, this crop has a specific genetic characteristics resulting by the use of seeds selected and conserved by the farmers for a long time and they limit the seeds source introduced. In addition, Kebili watermelon was conducted in the oasis as an associated species to the date palm with a low commercial value. The introduced varieties were separated from the rest of the studied accessions, this could be attributed to the genetic structure of these selected genotypes which contains interested genes such us resistance to *alternaria* (Wehner and Barrett, 2010). Genetic similarity detected between Medenine and Benguerdane cultivars could be the result of exchanging vegetative material. The discrimination of this closely related accessions can be resolved by the use of others effectives markers like SSR (Jarret et al., 1997) and AFLP (Levi et al., 2004).

Furthermore, the studied accessions are also distinguished by some fruit characteristics like fruit shape and color of seeds (Elbekkay et al., 2009). Kebili and Tataouine accessions, which are jointly clustered, are characterized by spherical fruit, high fruit weight and dark seeds. However, the introduced varieties present fruits with low weight and bright seeds in comparison to the Tunisian local germplasm (Elbekkay et al., 2009). These outcomes are more consistent than those noted by Levi et al., (2001aandb) who didn't detect any association between RAPD markers and fruit shape.

Conclusion

In conclusion, our study is the first report evaluating genetic diversity in watermelon population collected from the south of Tunisia. It indicates the feasibility of RAPD markers to show the particularity of the genetic structure of the Tunisian local accession in comparison with the introduced varieties. In the future, these finding should be able to be utilized in improving and preservation strategies of this crop in Tunisia.

Acknowledgements

Wethankallthetechnicalstaff of theArid and OasesCroppingLaboratory of Arid Area Institute.

REFERENCES

Aras S, Duran A, Yenilmez G (2003). Isolation of DNA for RAPD analysis from dry leaf material of some Hesperis L. specimens. Plant Mol. Biol. Rep. 21: 461a-461f.

Elgazah M, Chalbi N (1995). Ressources génétiques et amelioration des plantes ? AUPELF-UREF (eds.). John LibbeyEurotext. Paris©, pp123-129.

Excoffier L, Smouse PE, Quattro JM (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics. 131: 479-491.

FAOSTAT (2011). http://faostat.fao.org/site/567/default.aspx# ancor

Garcia-Mas J, Olivier M, Gomez-Paniagua H, de Vicente MC (2000).Comparing AFLP, RAPD and REFLP markers for measuring genetic diversity in melon.Theor. Appl. Genet. 101 : 860-864.

Jarret RL, Merrick LC, Holms T, Evans J, Aradhya MK (1997). Simple sequence repeats in watermelon [*Citrullus lanatus* (Thunb.) Matsum.&Nakai].Genome. 40: 433–441.

Lee SJ, Shin JS, Park KW, Hong YP (1996).Detection of genetic diversity using RAPD-PCR and sugar analysis in watermelon [*Citrullus lanatus* (Thumb.) Mansf.] germplasm. Theor. Appl. Genet. 92 : 719-725.

Levi A, Thomas CE, Keinath AP, Wehner TC (2001b). Genetic diversity among watermelon (*Citrullus lanatus* and *Citrullus colocynthis*) accessions. Genetic.Resources and Crop. Evolution. 48:559-566.

Levi A, Thomas CE, Thies J, Simmons A, Xu Y, Zhang X, Reddy OUK, Tadmor Y, Katzir N, Trebitsh T, King S, Davis A, Fauve J, Wehner T (2004). Resistance to a severe strain of zucchini yellow mosaic virus in watermelon. In: A. Lebeda and H. S. Paris (Eds.) Progress in cucurbit genetics and breeding research. Proc.

Cucurbitaceae 2004, 8th EUCARPIA meeting, pp 223-230. Olomouc, Czech Republic.

Levi A, Thomas CE, Wehner TC, Zhang X (2001a). Low genetic diversity indicates the need to broaden the genetic base of cultivated watermelon. HortScience. 36: 1096-1101.

Elbekkay M, Hamza H, Haddad M, Ferchichi A, Kik C (2008). Genetic erosion in melon (Cucumismelo): a case study from Tunisia. Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae (Pitrat M, ed), INRA, Avignon (France), May 21-24th, 2008. Cucurbitaceae, pp 295-300.

Elbekkay M, Laarayedh L, Lamari R, Hamza H, Ferchichi A (2009). Characterization of Several Local Cultivars of Watermelon Collected from Arid Region in Tunisia. Proceedings of the International Conference on DESERT TECHNOLOGY IX. Journal of Arid Land Studies. 19 (1): 205-208.

Mujaju C, Sehic J, Werlemark G, Garkava-Gustavsson L, Fatih M, Nybom H (2010). Genetic diversity in watermelon (*Citrullus lanatus*) landraces from Zimbabwe revealed by RAPD and SSR markers. Hereditas. 147 : 142-153.

Navikof VA (1951). Essais d'amélioration des variétés dans les cultures de melon et de pastèque en Tunisie. Annal. Service Bota. Agronom. INRAT. 24: 65-100.

Nei M (1972). Genetic distance between populations. Amer. Naturalist. 106: 283-292.

Paris HS, Janick J (2008). Reflections on linguistics as an aid to taxonomical identification of ancient Mediterranean cucurbits: the Piqqus of the Faqqous.1 Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae (Pitrat M, ed), INRA, Avignon (France), May 21-24th, 2008. Cucurbitaceae, pp 43-51.

Peakall R, Smouse PE (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes. 6: 288-295.

Robinson RW, Decker-Walters DS (1997).Cucurbits. CAB International, New York.

Solmaz I, Sari N, Aka-Kacar Y, Yalcin-Mendi Y (2010). The genetic characterization of Turkish watermelon (*Citrullus lanatus*) accessions using RAPD markers. Genet. Resour. Crop Ev. 57: 763-771

Wehner TC, Barrett C (2010). Cucurbit Breeding, Horticultural science. Watermelon Crop Information, Disease, Insect, and Stress Resistance. http://cuke.hort.ncsu.edu/cucurbit/wmelon/wmhndbk/wmresistance.html

Wehner T C (2008). Watermelon. In: J. Prohens and F. Nuez (eds.). Handbook of Plant Breeding; Vegetables I: Asteraceae, Brassicaceae, Chenopodiaceae, and cucurbitaceae. Springer Science+Business LLC, New York, NY. 17: 381-418.

Straten van der M.J., Potting R.P.J. & Linden van der A. (2011). Introduction of the tomato leaf miner *Tuta absoluta* into Europe pages 23-30. In: Proceedings of the Netherlands Entomological Society Meeting 22, Netherlands.