CHARACTERIZATION OF TUNISIAN GENETIC RESOURCES OF WATERMELON (*CITRULLUS LANATUS*)

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ABSTRACT

In Tunisia, watermelon [Citrullus lanatus (Thunb.) Matsum. & Nakai] is largely consumed in summer as a fresh fruit. This cucurbit is cultivated in various areas in the country. The landraces are a very important source of genetic diversity, constituting an important genetic resource for plant breeders. The aim of the present study was to collect and characterize fruit diversity of watermelon landraces collected from local farmers from Monastir and Sousse areas of Tunisia. The landraces were categorized for 20 variables of fruit features according to UPOV descriptors for watermelon. Also, these landraces were screened by artificial inoculation for their resistance to Fusarium oxysporun f.sp niveum (FON) races 0 and 2 to identify sources of resistance to Fusarium wilt. Significant differences were found among accessions for the quantitative traits recorded, revealing a large diversity. The evaluated watermelon landraces expressed a wide range of phenotypes including fruit size, flesh color, rind pattern, FON disease resistance and sweetness.

***Keywords**: Citrullus, disease resistance, diversity, Fusarium wilt, genetic resources, landraces, phenotypes, watermelon.*

INTRODUCTION

The annual *Citrullus lanatus* (Thunb.) Matsum. & Nakai, the dessert watermelon, is the best known among all *Citrullus* species. It is a warm-season annual vegetable crop that is grown on 3.5 million hectares worldwide (Faostat, 2015). Native to Sudan and Egypt, it includes wild and cultivated forms (Paris, 2015). It is a member of the *Cucurbitaceae* family along with cucumber, squash, and melon. Watermelon originated in western Africa (Chomicki and Renner, 2015) then spread to the Middle East and then China. The fruit is grown for its edible endocarp, rind, and seed oil. The colored flesh, though 93% water, contains significant amounts of carbohydrates, vitamin A, and lycopene (Wehner, 2008).

The sweet flesh of *C. lanatus* has resulted in its spread throughout the world and it has become one of the most extensively consumed vegetable fruit crops. The genus *Citrullus* includes six additional diploid species, three of which are also of importance (Jarret et al. 1997). These are the egusi watermelon (*C. mucosospermus* (Fursa) Fursa), the citron watermelon (*C. amarus* Schrad.) and the colocynth (*C. colocynthis* (L.) Schrad.).

Cultivars express a wide range of phenotypes including fruit size, flesh color, rind pattern, disease resistance and sweetness. Each growing region has a set of cultivars that are widely grown and are suited for local environmental conditions (Wehner, 2008). Despite geographic and phenotypic diversity, the genetic variation of cultivated watermelon is limited (Levi et al., 2001). Analysis of genome-wide diversity revealed that cultivars from Asia, Europe and America are derived from one of three subsets of sweet watermelon accessions from Africa (Nimmakayala et al., 2014). As such, estimates of genotypic variation among cultivars have been low.

The narrow genetic base of dessert watermelon (*Citrullus lanatus*) cultivars creates a continuous challenge for researchers and breeders aiming to improve the crop for disease resistance. High yield, high fruit quality and early maturity are the principle objectives for watermelon breeding (Gusmini and Wehner, 2005).

Fusarium wilt is one of the most economically important diseases of watermelon. The disease is present worldwide, and it can result in yield losses nearing 100% when severe (Egel and Martyn, 2013). The pathogen that causes this disease is the fungus *Fusarium oxysporum* f.sp *niveum* (FON). There are four known races of FON (0, 1, 2 and 3), distinguished by their ability to infect watermelon varieties with different resistance genes (Netzer,

1976; Davis et al. 2012). The Evaluation of the resistance of local cultivars to this pathogen is promising for the identification of resistance genes but also to ensure the valorization of this germplasm.

In Tunisia, watermelon genetic resources are poorly characterized and additional studies are needed to properly collect, classify and evaluate them. The aim of the present study was to evaluate the phenotypic diversity between watermelon landraces and to screen them for Fusarium wilt resistance in order to select the best accessions for further breeding programs.

MATERIAL AND METHODS

Plant material and experimental design

The watermelon material used in this study consisted of 15 watermelon landraces collected from local farmers from Monastir and Sousse areas of Tunisia. Seeds were initially sown in peat and seedlings at the three-leaf stage were transplanted to the greenhouse at the Sahline experimental station of the Regional Research Centre on Horticulture and Organic Agriculture (CRRHAB) during 2018-2019.

Three replications containing 10 plants of each accession were arranged in a randomized complete block design (RCBD). Seedlings were transplanted 1.2 m apart in-row and 1.8 m apart between rows. Fertilizer was applied with synthetic chemical fertilizers (145 kg N ha^{-1}, 140 kg P ha^{-1}, 210 kg K ha^{-1}) to plots and soluble fertilizer was applied weekly via drip irrigation. Plants were irrigated 3 times per week as needed. All plots were weeded manually to maintain proper weed control. After fruits ripening, they were harvested and moved to laboratory for investigating their characteristics.

Morphological traits evaluated

Watermelon landraces were evaluated for 20 morphological traits and productivity. Watermelons were selected randomly from the different blocks. Four ripe fruits were harvested per block per accession. Ripe watermelons were harvested, in June, at their horticultural maturity which was determined according to tendril browning, yellowing of the ground spot, and loss of surface gloss and to a thumping sound which changes from a metallic ringing when unripe to a soft hallow sound when ripe.

The following traits were recorded: (1) days to first male and female flower, (2) number of days from sowing to first mature fruit, (3) Marketable fruit from each plot were harvested, weighted, and counted, (4) average fruit weight, (5) number of fruit per plant, (6) fruit shape, (7) type of stripes, (8) width of stripes, (9) size of insertion of peduncle, (10) depression at base, (11) fruit length, (12) fruit width, (13) thickness of pericarp, (14) main flesh color, (15) flesh firmness using a hand-held penetrometer with 10 mm solid probe, (16) total soluble solids (TSS) expressed as Brix in fruit juice were determined using a digital refractometer, (17) seed color, (18) seeds number and (19) 100-seed weight.

Screening for resistance to FON

Watermelon plants (at the 1st true leaf stage) were inoculated by FON races 0 and 2. Inoculation was performed by immersing the roots in the conidial suspension (10^6 conidia/ml) while stirring for 2 min. For each accession, 12 plants were tested per FON race. The inoculated seedlings were transplanted into 0.5 l pots with sterile substrate. The pots were placed in a growth chamber at 26°C / 24°C (16 h day) for 30 days. Plants were examined every 7 days and disease severity was assessed based on a 1 to 4 scale 1 = Healthy plant; 2 = Delayed growth or atrophy of up to 50% compared to the resistant control; 3 = Presence of yellowing leaf lesions, wilting and poor root development and 4 = Death plant (Odibert et al., 2016). The line PI296341 was used as resistant control to races 0 and 2 and the line Black Diamond as susceptible control to both races. The lines Charleston Gray and Calhoun Grey were used as resistant to race 0 and susceptible to race2.

Statistical analysis

The data were subjected to basic statistics and an ANOVA was executed to provide the significant differences of characters between accessions. Duncan's multiple range tests were used to compare means for each trait. The principal component analysis (PCA) and hierarchical clustering analysis (HCA) with the squared Euclidean distance and the Ward's method were performed using the SPSS statistics version 20.

RESULTS AND DISCUSSION

Most local watermelon cultivars have disappeared from fields because they have been replaced by hybrids and modern cultivars. The assembly and the conservation of genetically and morphologically diverse watermelon germplasm are essential activities to increase genetic variability and to ensure the current and future success of watermelon breeding programs. For this reason, on-going efforts are needed to collect, maintain and evaluate *Citrullus* germplasm.

In this study, several watermelon landraces were collected from local farmers and the evaluated traits from observations of the fruits and seeds were used according to the UPOV standards. The evaluation of these watermelon genetic resources and their exploitation for sustainable agriculture is needed.

The evaluated watermelon landraces expressed a wide range of phenotypes including fruit size and shape, flesh color, rind pattern, disease resistance and sweetness. the ANOVA revealed significant differences among the watermelon landraces for all the quantitative parameters evaluated (data not shown) and a high variation was obtained for some traits which displayed a high value for coefficients of variation; fruit weight (37%), seeds number (43%) and 100-seed weight (32%) revealing a large diversity. Wehner (2008) stated that each growing region has a set of cultivars that are widely grown and are suited for local environmental conditions. The same author showed that the phenotypic diversity within populations of watermelon is high and includes variation in shape, size and color of fruits; number and size of seeds; quality, color and thickness of fruit flesh and precocity in fruit production among other traits. In contrast, Levi et al. (2001) showed that despite geographic and phenotypic diversity, the genetic variation of cultivated watermelon is limited.

Enhancing genetic diversity and host plant resistance in watermelon cultivars is a priority that will enable the maintenance and improvement of the current levels of production. In this study, the screening of watermelon landraces for resistance to FON races 0 and 2 revealed interesting genotypes which showed partial resistance to these races. The screening will be done once again in order to confirm the results.

The PCA and the Hierarchical cluster analysis divided our local watermelon landraces into 4 clusters mainly on fruit sweetness (data not shown).

Results obtained herein could be used in breeding programs and promotion of local varieties. Further analysis using molecular markers should be conducted to deepen the assessment of the genetic variation. Biochemical analysis should be conducted too, in order to select the promising genotypes.

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