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Phytopathological evaluation of exotic watermelon germplasm as a basis for rootstock breeding

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ABSTRACT

Most of the watermelons grown in the Mediterranean basin are grafted mainly on *Cucurbita* rootstocks which provide efficient protection against a wide range of soilborne pathogens. In certain cases, however, grafting may cause a reduction in fruit quality. Grafting watermelon on watermelon rootstocks may eliminate the fruit-quality issues resulting from the use of *Cucurbita* rootstocks. The response of 22 exotic watermelon accessions to Fusarium wilt caused by *Fusarium oxysporun* f. sp. *niveum*, Fusarium crown rot caused by *F. oxysporun* f. sp. *radices cucumerinum*, the nematodes *Meloidogyne javanica* and *Meloidogyne incognita* was evaluated in pot experiments. The response to *Macrophomina phaseolina* and *Monosporascus cannonballus* was evaluated under field conditions. The screened accessions exhibited various responses to the tested diseases. The findings indicate the possibility of breeding watermelon rootstocks with high levels of resistance to several diseases and no negative effect on fruit quality. The most promising accessions are PI 457916, PI 459075 and BDA. In addition, phytopathological data on such a germplasm collection can serve as a tool for studying the resistance mechanisms and the genetics of disease resistances.

1. Introduction

Grafting has been used in eastern Asia for decades; more recently, it has been adopted on a large scale in the Western world. The ban imposed on methyl bromide (Anonymous, 2010) has resulted in the massive adoption of grafting technology in both the Mediterranean basin and Europe (Echevarria and Castro, 2002; Lopez-Galarza et al., 2004; Miguel et al., 2004). It has also increased interest in grafting in the United States, to the extent that approximately 40 million grafted tomatoes are now grown annually in North American greenhouses (Kubota et al., 2008).

Grafting provides a fast and easy solution to acute pathological problems, in contrast to long and expensive breeding programs. Two types of grafting are used: intra- and interspecific (Davis et al., 2008). Intraspecific grafting, in which the rootstock and the scion belong to the same species, is common in tomato (*Solanum lycopersicum* L.) for example, for which a large collection of tomato rootstocks that vary in specific traits is available (Augstin et al., 2002; Paplomatas et al., 2002). In cucurbits, however, interspecific grafting is common. Most of the watermelons grown in the Mediterranean basin, including Israel, are grafted mainly on *Cucurbita* rootstocks (F_1 hybrids of *Cucurbita moschata* Duchesne × *C. maxima* Duchesne). Grafting watermelon [*Citrullus lanatus* (Thunb.) Matsum.&Nakai] on *Cucurbita* rootstock provides non-specific but efficient protection against a wide range of soilborne pathogens and some abiotic stresses (Yestisir et al., 2003). Growth invigoration and proliferation of certain combinations of grafted plants often allows reducing the number of transplants for a given cultivated area in melon (*Cucumis melo* L.) and watermelon without yield reduction (Cohen et al., 2007). On the other hand, in certain cases grafting may cause a reduction in fruit quality, namely, less crispy and harder fruit flesh with white fibers, changes in fruit size and fruit deformation. These changes are more pronounced in mini watermelon cultivars grafted onto *Cucurbita* rootstocks (Lopez-Galarza et al., 2004; Proietti et al., 2008).

Intraspecific grafting, that is, grafting melon on melon rootstocks (Cohen et al., 2007) or watermelon on watermelon rootstocks (Davis et al., 2008; Lee and Oda, 2003), may prevent

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the fruit-quality problems resulting from interspecific grafting. However, developing such rootstocks requires finding sets of resistances that are absent or unknown in commercial watermelon cultivars and breeding the multiresistant rootstocks.

Some important pathogens were screened for their interactions with watermelons in this study. These include Fusarium wilt of watermelon, a major disease of watermelon worldwide. Three physiological races of *Fusarium oxysporum* f. sp. *niveum* have been identified: 0, 1 and 2. Partial resistance to races 0 and 1 are available (Martyn and Vakalounakis, 2012), but resistance may break down under high inoculum levels (Martyn and Vakalounakis, 2012). No commercial race 2-resistant cultivars are available to growers. Fusarium crown rot attacks mainly greenhouse-grown cucumber and to a lesser extent melons (Vakalounakis, 1996; Vakalounakis et al., 2005). Watermelon can be damaged by this pathogen following artificial inoculation. The emerging trend of growing mini watermelons in greenhouses motivated us to evaluate our germplasm for its response to *Fusarium oxysporum* f. sp. *radices cucumerinum*.

Root-knot nematodes, especially *Meloidogyne javanica*, *M. incognita* and *M. arenaria*, are known to attack watermelon (Winstead and Riggs, 1959; Montalvo and Esnard, 1994; Sumner and Johnson, 1973), and increase susceptibility to *F. oxysporum* f. sp. *niveum* (Sumner and Johnson, 1973). Commercial rootstocks used for watermelon, such as *C. moschata* × *C. maxima* and *Lagenaria siceraria*, are very susceptible to root-knot nematodes, even more susceptible than non-grafted watermelon cultivars (Edelstein et al., 2010; Thies and Levi, 2003). Due to the susceptibility of watermelon cultivars and their rootstocks to these nematodes, several attempts have been made to find sources for resistance (Hussey and Barker, 1973; Thies et al., 2010; Thies and Levi, 2003, 2007). Some accessions from the wild-type watermelon *C. lanatus* var. *citroides* were found to be moderately resistant to *M. incognita* and *M. arenaria* (Sumner and Johnson, 1973; Thies and Levi, 2007), and could be useful rootstocks for watermelon (Thies et al., 2010).

Macrophomina phaseolina is a pathogen of a wide number of cultivated and wild plant species in warm, temperate and tropical regions of the world (Bruton et al., 1987). *M. phaseolina* causes crown or stem rot in cucurbits and it can be fairly serious in melons under hot and dry conditions (Bruton et al., 1987; Bruton and Wann, 1996). This pathogen was the leading cause of melon collapse and of the drastic reduction in melon cultivation in the northern Negev in Israel (Krikon et al., 1982; Reuveni et al., 1982). In the last few years, *M. phaseolina* has been the main fungus isolated from collapsed watermelon and melon roots in most growing areas in northern Israel (Cohen et al., 2012a).

Monosporascus root rot and vine decline (sudden wilt), caused by the soilborne fungus *Monosporascus cannonballus*, has become one of the most important diseases of melon and watermelon worldwide. To the best of our knowledge, no commercial watermelon cultivar resistant to this disease is available (Cohen et al., 2012b).

In the last 15 years, the practice of grafting watermelons and melons to reduce damage caused by soilborne pathogens has been expanding rapidly in Israel. Grafting has been effective in reducing the incidence of fusarium wilt, Monosporascus sudden wilt and vine decline caused by *M. phaseolina* (Cohen et al., 2002a,b, 2012a,b).

The objectives of this study were to evaluate the response of exotic watermelon accessions to important soilborne pathogens threatening watermelon cultivation as a first step toward breeding watermelon rootstock. The diseases and pests evaluated were Fusarium wilt, Fusarium crown rot, two nematodes, *M. javanica*, and *M. incognita*, Monosporascus sudden wilt and charcoal rot caused by *M. phaseolina*. Brief report presenting part of the results was published (Tyutyunik et al., 2012).

Table 1
Full names, abbreviations and origin of the watermelon accessions evaluated.

Accession name	Source – country of origin
Black Diamond (BDA)	USA
Congo (CON)	Congo
Cream of Saskatchewan (COS)[a]	Russia
Early Moonbeam (EMO)	USA
Hungarian (HUN)	Hungary
King and Queen (KAQ)	USA
Malali (MAL)	Israel
Nwau-858770 (NWAU)	China
Red Seeded Citron (RSC)	Africa
Wanli (WAN)	Philippines
WIS	No data
PI 270549	Ghana
PI 273481	Ethiopia
PI 296341	Cape Province, South Africa
PI 307609	Nigeria
PI 307750	Philippines
PI 326515	Ghana
PI 441722	Brazil
PI 457916	Liberia
PI 459075	Botswana
PI 482260	Zimbabwe
PI 482318	Zimbabwe
Extazy – commercial mini	"HaZera Genetics", Israel, used as scion
Leopard – commercial midi	"HaZera Genetics", Israel, used as scion

[a] Brought by Russian immigrants to Saskatchewan, Canada. http://www.heritageharvestseed.com/watermelon.html.

2. Materials and methods

2.1. Response of watermelon accessions to F. oxysporum f. sp. niveum and F. oxysporum f. sp. radices cucumerinum – pot experiments

Twenty-two watermelon accessions were evaluated in this study. Part of them are old commercial cultivars and the others are wild watermelon accessions marked as Plant Introduction (PI) numbers. These accessions will be called hereafter "exotic watermelon accessions". Their names and origin are presented in Table 1. The same root-dipping method was used for the two Fusarium pathogens evaluated. Seeds of the tested watermelon accessions (Tables 2 and 3) were germinated in sandy soil. Two days after emergence, the seedlings were removed from the soil and washed under running tap water. The roots were pruned to approximately half their length, and the seedlings were inoculated by dipping their roots in a conidial suspension (10^6 conidia/ml) of *F. oxysporum* f. sp. *niveum* race 2 (Table 2) or *F. oxysporum* f. sp. *radicis cucumerinum* (Table 3) for 2 min. The pathogens used for inoculation were grown on potato dextrose agar (Difco) at 27 °C, in the dark, for 7 days. Conidial suspensions for seedling inoculation were prepared by macerating 1-week-old cultures with 100 ml water (Cohen et al., 2002a,b). Inoculated plants were transplanted into 250-ml pots containing sandy soil. The inoculated plants were maintained in a controlled growth chamber at 27 °C under a photosynthetic flux density of 90 μM/m^2 s for 14 days. For each watermelon accession tested, 35 seedlings (7 plants per pot × 5 replicates per treatment) were evaluated. The plants were manually irrigated daily to drainage with tap water. The plants were not fertilized during the experiment.

The number of wilted plants was recorded periodically, and disease incidence was calculated. Two experiments were conducted for each pathogen. The final disease incidences are shown in Tables 2 and 3.