Grafting as a Method to Increase the Tolerance Response of Bell Pepper to Extreme Temperatures

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Fluctuations of winter and summer and day and night temperatures strongly influence shoot and root growth, as well as the whole plant tolerance to extreme soil temperatures. We compared the response of a commercial pepper (Capsicum annuum L.) hybrid (Romance, Rijk Zwaan) to a range of soil temperatures when grafted to a new rootstock hybrid (S101, Syngenta), self-grafted, or ungrafted. The new rootstock hybrid was bred for enhancing abiotic stress tolerance. Plants were grown during winter and summer seasons in a plastic greenhouse with natural ventilation. Minirhizotron cameras and in-growth cores were used to investigate grafted bell pepper root dynamics and root and shoot interactions in response to extreme (low and high air and soil) temperatures. Soil and air temperatures were measured throughout the experiment. The variations of the grafted peppers and the ungrafted aboveground biomass exposed to low and high temperatures during winter and summer were higher in the Romance grafted on the S101 rootstock than in the self-grafted and ungrafted Romance. The plot of rootstock S101 accumulated Cl, and the rootstock efficiently allocated C into the leaves, stems, and roots and N into the leaves, stems, and fruits. These traits of rootstock S101 can be used to improve the tolerance of other pepper cultivars to low and high soil temperatures, which could lengthen the pepper growing season, as well as provide highly interesting information to plant breeders.

Abbreviations: DAT, days after transplanting; EC, electrical conductivity; ROM Romance hybrid.

Bell pepper yield is severely reduced by abiotic stresses, including low soil water content and low and high root zone temperatures. These stresses affect root and shoot interactions by reducing plant growth and development, causing wilt and necrosis, and retarding the rate of branching and fruit ripening (Ahn et al., 1999). The long bell pepper production season in Mediterranean-like climates usually includes exposure to high and low temperatures during the summer and winter seasons, respectively, making it impossible to take advantage of the plants’ full potential. A simple option to ensure continuous production is to breed new cultivars that are better adapted to high and low temperatures. However, due to the lack of practical selection methods, such as genetic markers, it is still a slow and inefficient process (Schwarz et al., 2010). Currently, grafting is regarded as an alternative to the relatively slow breeding methods and is aimed at increasing the environmental-stress tolerance of fruit vegetables (Flores et al., 2010) and enabling the use of the extensive genetic diversity of the Capsicum species scion and rootstock accessions.

The use of rootstocks to improve vegetable production is a common practice; grafting has already been used for more than 50 yr in many parts of the world (Schwarz et al., 2010). Currently, grafting is used to reduce infections by soil-borne pathogens and to enhance tolerance against abiotic stresses, including saline soils (Colla et al., 2010), soil-pH (alkalinity) stress, nutrient deficiency, toxicity of heavy metals (Savvas et al., 2010), and drought (López-Marin et al., 2017). Temperature stress is another abiotic condition necessitating rootstock application. Nevertheless, most studies on the response of grafted bell pepper rootstocks to temperature stress have focused on photosynthesis (Zheng et al., 2009), shading (López-Marin et al., 2013), and fruit quality (Savvas et al., 2010). The complexity of the root zone and its monitoring
A commercial pepper hybrid (Rewald et al., 2012a; Shelef et al., 2010) is a major obstacle in providing effective solutions for root zone stress. Therefore, field evaluations of root dynamics and C and N metabolism variations in bell pepper rootstock response to extreme air and root zone temperatures are scarce.

Populations of land plants are characterized by extensive genetic diversity, even among accessions of the same species; this intraspecific variation can often be reflected in wide phenotypic diversity (Koornneef et al., 2004; Keurentjes and Sulpice, 2009). Additionally, natural variation across a large set of genotypes can be used to detect correlations between phenotypic parameters, providing insights into the regulation underlying physiological (Driever et al., 2014) and biochemical networks (Poorter et al. 2005; Cross et al., 2006). These natural variations can be used to improve plants through the careful selection of scions and rootstocks for grafting.

Extreme-temperature-tolerant genotypes may offer significant advantages for vegetable production under low, suboptimal, and high temperatures in the field or in the greenhouse (Foolad and Lin, 2000). For example, cold-tolerant plants may grow more rapidly at early stages and thus become established faster than cold-sensitive plants. These tolerant genotypes may result in improved earliness, adaptability, and yield of high-quality fruits when grown under low and suboptimal temperatures. In addition, the crop could be grown in the field for longer periods of time (i.e., extended growing season), and thus the final fruit yield may be higher. Production may also be improved because early plantings can develop tolerance to high temperatures in summer, which can reduce fruit set during midsummer (Foolad and Lin, 2000). The tolerance traits to extreme temperature may result in higher water use efficiency because early plant growth makes better use of early season rains and available water in the root zone under field conditions (Foolad and Lin, 2001). The goal of this study was to investigate and identify the variations in bell pepper rootstock dynamics and C and N metabolism in response to extreme canopy and root zone temperatures. We therefore hypothesized that tolerant bell pepper rootstocks will adapt to extreme canopy and root zone temperatures by the use of physiological, C and N metabolism, and phenological mechanisms associated with the stress.

Materials and Methods

Plant Materials

A commercial pepper hybrid (Romance, Rijk Zwaan [ROM]) and a new rootstock hybrid (Syngenta S101) bred for enhancing abiotic stress tolerances were evaluated in this study. Romance scions were grafted on the pepper hybrid S101, self-grafted, and ungrafted. Seedlings were obtained from Hishtil.

Treatments and Experimental Design

The experiment was conducted in a greenhouse with natural ventilation at Mivtahim, in the western Negev Desert, southern Israel (31.220460 N, 34.391936 E, 130 m asl). A randomized complete block design was used with three treatments (two graft combinations and one ungrafted) replicated six times. Each plot consisted of one row measuring 5 m in length, with 25 plants at a planting distance of 0.4 m within the row and 1-m distance between plot rows. The experimental period lasted for 7 mo (December–July), and the plants were monitored on a monthly basis. Type T thermocouple sensors were embedded in the soil profile at 0.05-m intervals to a depth of 0.4 m in three replications. The air temperature was measured in the middle of the greenhouse at a height of 1.5 m using Type T thermocouple sensors located in a solar radiation shield. The plants were drip irrigated with a discharge rate of 4 L/h. The dripper spacing was 0.2 m placed at the base of the plants. A commercial fertilizer (5:1:9 N–P–K) was applied through the irrigation water with a NO₃ concentration of 150 mg/L. The first flowers of the bell peppers were removed 3 mo after transplanting to induce vegetative growth.

Minirhizotron and In-Growth Core Measurement

The total root volume and the number of roots were monitored once a month using minirhizotron cameras (BTC-2, Bartz Technology Corporation). Measurements started 2 mo after transplanting and continued until 7 mo, when the experiment ended. The minirhizotron system included tubes measuring 60 mm in outside diameter, 2 mm in thickness, and 750 mm in length. The minirhizotron tubes were installed 20 cm from each plant in a row with six replicates (Shilo et al., 2013). Additionally, in-growth cores of a length of 35 cm, a diameter of 10 cm, and a volume of about 2750 cm³ were installed at a distance of 20 cm perpendicular to the plant rows and the drip line with six replications (Van Do et al., 2016). These in-growth cores have a metal net that allows root growth; they were sampled 90 and 118 d after transplanting (DAT), and the roots were washed and scanned using WinRHIZO Pro 2005 software (Regent Instruments) to investigate the number of root tips. The roots were then dried, and the dry mass values of the different treatments were compared.

Fruit, Shoot, and Root Phenology and Elemental Analyzer

Plants and soil were sampled 150 and 249 DAT. Carbon and N contents were determined in the plant tissues sampled at the end of winter, in April at 150 DAT. The shoots were immediately separated into leaf, stem, and fruit samples and then dried, along with the roots, in an oven at 65°C for 72 h for the shoot and root samples and 120 h for the fruit samples. The dried samples were ground in a TissueLyzer (RetschGmbH and Co.), and then C and N contents of the leaves, stems, roots, and fruits were analyzed using a FlashEA1112 CHNSO analyzer (Thermo Fisher Scientific) with 2.5-mg samples of dried and ground tissues (leaves, stems, roots, and fruits) after recording the dry mass. The analysis for Cl concentration in the soil was performed by a Chloride Analyser 926 (Sherwood Scientific), and soil electrical conductivity (EC) was determined by an EC meter (Crison MM 40).
These measurements (EC and Cl) were done at a 1:1 ratio of double-distilled water and soil (Hochberg et al., 2013; Sperling et al., 2014).

**Data Analysis**

Seven separate data and statistical analyses were performed for four sets of response variables: shoot phenology, soil EC and Cl sampled at 150 and 249 DAT, C and N sampled at 150 DAT, and root morphology monitored with minirhizotron cameras and in-growth cores. One-way ANOVA was performed on the effect of extreme temperatures on each trait variation using JMP 2007 (SAS Institute). The means were separated by a Tukey–Kramer and Student’s *t*-test at $P \leq 0.05$ to identify statistically significant differences between the means.

**Results**

**Air and Soil Temperatures, Soil Electrical Conductivity, and Chloride**

The air temperature of the greenhouse varied between a minimum of 1.5°C and a maximum of 21.7°C in January and increased to a minimum of 14.7°C and a maximum of 43.2°C in June. The soil profile temperature was as low as 7.2°C in January at the 5-cm depth and rose to a maximum of 39.4°C in June (Fig. 1). The decline in temperature during July resulted from the provisioning of extra shading on the greenhouse roof to reduce rising temperatures inside the greenhouse (Fig. 1). The EC and Cl contents determined in the soil sampled at 150 and 249 DAT had higher concentrations at a depth of 10 cm in the soil profile, which reduced significantly with increasing soil depth until 40 cm (Fig. 2). Significant changes in these parameters between

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**Fig. 1.** (a) Maximum, (b) minimum, and (c) average air and soil profile temperatures to the 45-cm depth in the plastic greenhouse during the experiment from the period of January to July.

**Fig. 2.** Soil (a,c) electrical conductivity (EC) and (b,d) Cl content measured (a,b) 150 d after transplanting (DAT) in April and (c,d) 249 DAT in July after transplantation of Romance bell pepper seedlings grafted onto S101 rootstock (ROM/S101), self-grafted (ROM/ROM), or ungrafted (ROM NON) into the plastic greenhouse. Values are the means of four replicates with standard error bars. Bars not connected by the same letter are significantly different ($P \leq 0.05$; Tukey–Kramer; $n = 6$).
Fig. 3. (a) Leaf fresh mass, (b) leaf dry mass, (c) stem fresh mass, (d) stem dry mass, (e) total fresh biomass, and (f) total dry biomass of Romance bell pepper seedlings grafted onto S101 rootstock (ROM/S101), self-grafted (ROM/ROM), or ungrafted (ROM NON) and (g) fruit mass and (h) number of fruits sampled 150 and 249 d after transplanting. Values are the means of six replicates with standard error bars. Dark bars represent sampling in April and lighter bars represent sampling in July. Bars not connected by the same letter are significantly different ($P \leq 0.05$; Tukey–Kramer; $n = 6$).
plots of the same depth were observed only at the 10-cm depth (Fig. 2). The EC values of the soil samples from this depth were higher in the plot of ROM grafted on rootstock S101 than in the soil of the self-grafted ROM and ungrafted ROM sampled at 150 DAT (Fig. 2a). Similar observations were made for the Cl content in soil sampled at the same depth at 150 and 249 DAT, with greater concentrations in the plot of ROM grafted on rootstock S101 (Fig. 2b and 2d).

**Fruit, Shoot, and Root Phenological Response to Extreme Air and Soil Temperatures**

At the end of the winter growing season in April (150 DAT), it was observed that fresh and dry biomass levels were significantly greater in the ROM grafted on rootstock S101 than in the ungrafted ROM or self-grafted ROM (Fig. 3). The fruit mass and number of fruits also showed significant differences among the treatments, with ROM grafted on rootstock S101 having higher levels than other treatments at the end of the winter growing season (Fig. 3). Stem fresh and dry mass levels in the ROM grafted on rootstock S101 were significantly higher than other treatments at the end of the summer growing season in July (249 DAT) (Fig. 3).

The results of the growth and development of the bell pepper root systems as monitored by in-growth cores and minirhizotron cameras are shown in Fig. 4, 5, and 6. Root dry mass per volume of the in-growth core increased in the ungrafted ROM by 24% and by 40% in the ROM grafted on rootstock S101 between 90 and 118 DAT (Fig. 4a). The root growth rate revealed an increase of 43% in ROM grafted on rootstock S101, a faster rate than in ungrafted ROM or self-grafted ROM (Fig. 4b). The ROM grafted on rootstock S101 was the only treatment that showed a significant increase (22%) in the number of root tips between 90 and 118 DAT (Fig. 4c).

The total root volume for ROM grafted on rootstock S101, evaluated in the winter season from February to April, was greater than for the ungrafted ROM in March and April (Fig. 6). In the summer (May–July), when the air and soil temperatures increased, the root volume and number were significantly greater for the ROM grafted on rootstock S101 than the ungrafted or self-grafted ROM, especially during June and July (Fig. 5 and 6). The highly significant root numbers, as a function of the growth and development of the ROM grafted on rootstock S101, were particularly observed from soil profile depths of 0 to 30 cm in June and July (Fig. 5d, 5e, and 5f).

**Carbon and Nitrogen Allocation in Response to Extreme Air and Soil Temperatures**

The leaf, stem, root, and fruit C and N contents at 150 DAT are presented in Fig. 7. Carbon contents detected in the roots of ROM grafted on rootstock S101 were higher than in the ungrafted or self-grafted ROM (Fig. 7a). The N contents in the leaves of ROM grafted on rootstock S101 were significantly different from those of the self-grafted and ungrafted ROM. In the fruits, high N content was observed in ROM grafted on rootstock S101 relative to the self-grafted ROM (Fig. 7b). The C/N ratio in ROM grafted on rootstock S101 increased in the root by 21% and was reduced in the leaves by 18% compared with the ungrafted ROM (Fig. 7c).
Discussion

The limited understanding of belowground processes is a major limitation in predicting better mechanisms for plant growth and productivity (Rewald et al., 2012b) during extreme temperatures. The higher EC and Cl contents detected in the soil sampled at the 10-cm depth in the rootstock S101 plot at 150 DAT may have been due to the high uptake of water and nutrients and the reduced uptake of Cl. These traits of rootstock S101, coupled with its fast and deep root growth and development (Fig. 4, 5, and 6), can be used to breed and improve pepper tolerance to extreme temperatures. The high Cl concentrations found in bell peppers cultivated in saline soil have been reported to reduce photosynthetic capacity due to chlorophyll degradation, leading to yield losses (Ityel et al., 2014). However, insignificant differences in EC among the treatments at 249 DAT may be explained by the increase in water absorption due to the rise in air and soil temperatures during July.

![Fig. 5. Number of roots of Romance bell pepper seedlings grafted onto S101 rootstock (ROM/S101), self-grafted (ROM/ROM), or ungrafted (ROM NON) monitored monthly using minirhizotron cameras: (a) 81 d after transplanting (DAT) in February, (b) 112 DAT in March, (c) 142 DAT in April, (d) 173 DAT in May, (e) 203 DAT in June, and (f) 234 DAT in July. Values are the means of six replicates with standard error bars. Graphs not connected by the same letter are significantly different; n denotes not significant ($P \leq 0.05$; Tukey–Kramer; n = 6).]()}
Extreme temperatures reduce plant performance, resulting in yield limits caused by exposure to temperatures above and below the threshold for optimal biochemical and physiological activity or morphological development (Greaves, 1996; Ntatsi et al., 2014). During the generative growth phase of vegetables under extreme temperatures, fruit set is reduced as a result of poorer pollen quality and the increased period between anthesis and fruit maturity and a decreased truss appearance rate (van der Ploeg and Heuvelink, 2005). The slower fruit development and truss initiation rate at low and high temperatures mainly reduces early and late yield, respectively, in the seasons when the prices for vegetables are relatively high (Schwarz et al., 2010). The variations between the rootstocks and the ungrafted bell pepper plants’ aboveground biomass exposed to low temperatures during winter (December–April) were higher in ROM grafted on rootstock S101 than in self-grafted or ungrafted ROM. Similar trends were observed after the summer growing season in July. However, only stem fresh and dry mass values were significantly higher. This means that under stress conditions, rootstock S101 served as an important sink for improving scion growth and development, tolerance, and high yield or productivity during extreme temperatures (Fig. 3).

The reduction in root morphology of self-grafted and ungrafted ROM, compared with rootstock S101, could be due to a reduced rate of water and nutrient uptake and a reduction in root system enzymatic activity and metabolism (McMichael and Burke, 1994; Aidoo et al., 2016). However, the consistently lower effect of extreme temperatures on the root morphological traits or the possible growth of rootstock S101 indicates a tolerance mechanism resulting from effective ion uptake and transportation, allocation of C and N to the root, and reduction of root maintenance respiration (Rachmilevitch et al., 2008).

The allocation of C and N to various plant organs in this study might have enhanced protein catabolism (Kempa et al., 2008) and caused a shift in the proteome expressed as the production of greater numbers of stress-associated proteins (Cramer et al., 2013; Muneer et al., 2016), which may have contributed to the high performance of rootstock S101 during extreme temperature stress. Under low root zone temperatures, a greater portion of assimilated N has been found to be used for carbohydrate accumulation.
and root and rhizome development instead of being partitioned into shoot growth, as has been shown to occur when temperatures are optimal for shoot growth (Bowman, 2003; Aidoo et al., 2017). The allocation of more assimilated C to the root prevents C starvation and root death and increases root viability. Effective utilization of C has been shown to contribute to root survival at extreme temperatures (Rachmilevitch et al., 2006). Rootstock S101 showed tolerance by contributing to faster shoot growth (scion) and the survival of the whole plant during extreme temperature stress. These traits can be enhanced to improve the tolerance of other pepper cultivars’ response to low and high canopy and soil temperatures, which can lengthen the pepper growing season.

This study provides monitoring results of root zone biological, physical, and chemical parameters, which could be essential to calibrate and validate soil–plant–atmosphere models with root growth (Hartmann and Simunek, 2015) or with C–N allocation (Seidel et al., 2016) to evaluate the effects of abiotic stresses on crop productivity.

Acknowledgments
We acknowledge the valuable contributions of Yuval Shani for his skillful assistance with the installation of the temperature sensors and of Liron Summerfield, Kaining Zhou, and Asal Kardel for their help with the measurements. This research was partially supported by Zeranim Gedera, Syngenta, Israel and the Israeli Ministry of Agriculture and Rural Development (Eugene Kandel Knowledge Centers) as part of the program The Root of the Matter: The root zone knowledge center for advancing modern agriculture.

References