Morphological and Physiological Responses of Grafted Melon Subjected to Fusarium Wilt and Water Stress

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Abstract: This study aimed to determine the effects of different rootstocks on crop water stress index (CWSI), some morphological and physiological parameters of grafted melon under soil-borne pathogen (Fusarium oxysporum f.sp.melonis) and water stress conditions. The study was carried out in Çumra District of Konya Province in Türkiye for two years. The main plots consisted of three different irrigation levels and the sub plots consisted of subjects with melon plants with different rootstocks. The experiment was carried out in a split-plot design with three replications. In the study, four different rootstocks; 1-Ungrafted, 2-Ferro, 3-Maximus and TZ 148 were used. Edali F1 variety (Cucumis melo var. Edali F1) was used as a scion in the experiment. Irrigation was done in the four vegetation periods (early vegetative, late vegetative, flowering-fruit setting and ripening) of plant. Irrigation consisted of issues to increase soil depth from 0-90 cm to field capacity (I100), 50% (I50) of soil available water field capacity and rainfed (I0). As a result of the experiment, while leaf water potential (LWP) and leaf relative water content (RWC) increased with the increase of irrigation water in both years, the chlorophyll content was higher in I100, stomatal conductance was the highest in I50 of irrigation water, while the increase in irrigation water decreased stomatal conductance. In terms of physiological parameters measured in the plant, chlorophyll content and RWC values in the first year, Ferro, TZ-148 and Maximus rootstock, respectively, in the second year, Maximus rootstock reached the best value stem diameter in both years. CWSI was also affected by the applications. 

Keywords: Melon, Fusarium wilt, Rootstocks, Chlorophyll content, Stomatal conductance, CWSI.

INTRODUCTION

In the global horticultural industry, melon yield plays a major role. Drought is the most devastating environmental stress for agriculture which results in a significant reduction in crop productivity worldwide. The impact of climate change is going to aggravate the situation in particular in the semi-arid regions, the most drought-prone areas [1, 2], unless urgent measures are taken. The productivity of melon decreases significantly due to the stress factors such as soil-borne pathogens, drought and extreme temperatures [3]. The excessive use of fertilizers and pesticides in the production of this vegetable to achieve the desired yield causes many problems both economically and in terms of human and environmental health. On the other hand, the decrease in precipitation and consequently the available water resources is a serious problem in Türkiye, especially in the Central Anatolia Region, as in many regions of the world.

According to 2021 data, approximately 1.7 million tons of melon yields were produced in Türkiye. The country ranks 2nd rank after China in world melon production [4].

High soil temperatures, low moisture and drought improve the symptoms of the wilt pathogen by providing a suitable environment [5, 6]. For these reasons, the melon yield has decreased in the Çumra region, which leads the melon production in Konya.

Some methods for pathogen control have been developed. Vegetable grafting is one of these methods. This was used to manage a soil borne disease (Verticillium, Fusarium, and Meloidogyne spp). Grafting has become widespread; not only to control biotic stress such as soil borne pathogens but also to provide tolerance to abiotic stress such as drought, cold, salinity and heavy metal toxicity [7]. Thereafter, grafting was soon applied to melons. The use of grafted plants in production has been increased with the ban of the broad-spectrum soil fumigant methyl bromide [8, 9].

Studies have shown that the strong root system of the rootstock is more effective in the intake of water and plant nutrients in grafted plants. As a result, it has determined that the growth performance of the rootstocks has a positive effect on product increase.

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and disease control. Grafts were used to enhance nutrient uptake [10].

Morphological and physiological changes during fruit growth, maturation, and ripening of melon have not been studied extensively. In this respect, this study produced important data.

This research aims to investigate the effect of different irrigation levels and rootstocks on the some morphological (stem diameter, plant habitus, covering the status of the fruits of the leaves, fibrillation in fruit, an internal rupture in fruit) and physiological (leaf relative water content, leaf water potential, chlorophyll content, stomatal conductance) parameters of melon under the soil-borne pathogen (Fusarium oxysporum f.sp.melonis).

MATERIALS AND METHODS

Material

The Experimental Site, Soil and Irrigation Water Descriptions

The experiment was conducted at Çumra plain in Konya province, Türkiye, between 37° north longitude and 32° east latitude. The pH content of the soils of the trial area is slightly alkaline, more calcareous, organic matter and phosphorus content are low, and potassium level is sufficient. The physical properties of the soils, on the other hand, have a clay texture in the 0-90 cm soil layer and their water holding capacity is high. The infiltration rate of the soils of the experimental area was determined as 2.88 mm h⁻¹.

The canal water from Apa Dam was used in the experiment. Irrigation water is classified as C₂S₁ class in the both years and has characteristics suitable for aquaculture. EC, pH and SAR contents of irrigation water are changed between 0.31-0.026 dS m⁻¹, 7.89-8.74 and 0.26-0.34, respectively.

The climate in Çumra and its surroundings is cold and snowy in winters and hot and dry in summers. The autumn and spring months are periods of precipitation. While the temperature increases in the summer months, the humidity decreases. Total precipitation during the vegetation period was 42.4 mm in 2019 and 16.8 mm in 2020 (Table 1).

The Rootstocks and Melon Variety Used in the Experiment

Three rootstocks and one non-grafted variety as a scion were used in the study. The rootstocks were Ferro, Maximus and TZ 148. The scion was Edali F₁ (Cucumis melo var. Edali F₁). All the rootstocks are Cucurbita maxima x Cucurbita moschata hybrids. Grafting was performed through the cotyledon splice grafting method [11].

Ferro rootstock: It is resistant to Fusarium oxysporum. It does not cause fibrillation in the fruit flesh. It provides a stronger development.

Maximus rootstock: Maximus rootstock, which is known to be resistant to wilting (Fusarium oxysporum f.sp. niveum) especially in watermelons was used. Rootstock is a medium - strong rootstock, it provides a high yield in the variety it is grafted to, and it has no negative effect on the taste, shape and other quality elements of the fruit.

Table 1: Some Climatic Data of the Experimental Site during Vegetation Period

<table>
<thead>
<tr>
<th>Months of Vegetation period</th>
<th>Evaporation (mm)</th>
<th>Rain (mm)</th>
<th>Mean Temperature (°C)</th>
<th>Mean Relative Humidity (%)</th>
<th>Minimum Temperature (°C)</th>
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<tr>
<td>May (3 days)</td>
<td>170.3</td>
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<td>June</td>
<td>181.9</td>
<td>36</td>
<td>21.5</td>
<td>54.5</td>
<td>10.8</td>
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<td>July</td>
<td>237.6</td>
<td>6.4</td>
<td>22.3</td>
<td>47.7</td>
<td>10.5</td>
<td>36.7</td>
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<td>August (16 days)</td>
<td>208.3</td>
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<td>22.8</td>
<td>48.9</td>
<td>12.4</td>
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</table>

| 2020                         |                   |           |                       |                           |                          |                          |
| June (28 days)              | 193.7             | 6.8       | 20.7                  | 47.4                      | 8.1                      | 33.9                     |
| July                        | 281.3             | 10        | 24.6                  | 43.3                      | 14.5                     | 35.3                     |
| August (24 days)            | 257.1             | -         | 23.1                  | 39.2                      | 11.6                     | 35.9                     |
TZ 148 rootstock: This rootstock is resistant to Fusarium spp, Verticillium spp, Phomopsis spp and Meloidogyne spp.

Scion-Variety (Edalı F$_1$): Edalı F$_1$ (Cucumis melo var. Edalı F$_1$), a registered melony variety widely used in melon production in the region, was used as the scion. The variety is susceptible to soil borne pathogens. Its plant is big and strong. Its fruit is ‘hıdır’ type, oval and green freckled on a yellow background. The flesh is thick and firm. The ripening period is medium late. Fruit weight is 2 - 2.5 kg.

METHODS
The Establishment of the Experiment
The experiment was conducted for 2 years in 2019-2020 in a field naturally infested as in a split-plot design in random blocks with three replications. The experiment was conducted in a farmer’s land. The trial area is located at Cumra plain in Konya province. The main plots were constituted of three irrigation levels (I100, I50 and I0) while the subplots were with different rootstocks (Non-grafted, Ferro, Maximus, TZ 148). The soil samples taken from the experimental area were analysed by the soil-borne Fusarium oxysporum f.sp.melonis pathogen causing the disease. It was detected as 32.2x10$^{-1}$ spores g$^{-1}$.

Irrigation Treatments
The main parts of the irrigation system have been placed on the land before planting. Irrigation was not applied to the issues based on rainfall after the subject applications started, only enough water was given to apply fertilizer at the time of fertilizer applications. During the vegetative period, irrigation was carried out depending on the growth periods of the plants (early vegetative, late vegetative, flowering-fruit setting and ripening).

Irrigation follow-up was made with neutron-meter readings as a result of neutron calibration and the available moisture was determined as a percentage of volume. Measurements were made at every 30 cm layer with a depth of 0-120 cm. Thus, it was observed that there was no deep infiltration during the irrigation and the plants did not suffer from the stress. The water deficiency in the soil determined before irrigation was completed to the field capacity on full water (100%) (I100) and the amount of irrigation water for I50 were determined by reducing the amount to be applied by 50%. The other issue has been applied based on rainfall (I0), with no irrigation.

The amount of irrigation water was determined using equation 1 below [12];

\[ Dw = (Pwfc - Pwc) \times As \times Drz \times P \times 1 / 10 \]  

Where, Dw is the irrigation water amount (mm), Pwfc is field capacity (g g$^{-1}$), Pwc is the water amount before irrigation in plant root profile (g g$^{-1}$), As is the bulk density (g cm$^{-3}$), Drz is the effective root zone (0-90 cm), and P is the canopy cover percentage.

Evapotranspiration (ET) from the treatments was determined using the Eq. 2 [13];

\[ ET = P + I - Rf - Dp \pm \Delta S \]  

Where, ET is crop water consumption (mm), I is irrigation water amount (mm), P is the rainfall (mm), Dp is the deep percolation (mm), Roff is the runoff (mm) and \( \Delta S \) is the change of moisture content in the root depth (mm).

Cultural Practices
Cultivation has been done with seedlings, and the seedlings have been obtained from the Antalya Seedling Company (Antalya-Türkiye). They were planted at a 2.0 m inter-row width with 1.0 m spacing on May 27, 2019, and June 02, 2020, respectively. The dimensions of the plot at planting and harvesting were 10×8 m (80 m$^2$). A total of 150 kg N ha$^{-1}$, 100 kg P$_2$O$_5$ ha$^{-1}$, and 100 kg K$_2$O ha$^{-1}$ fertilizers were applied during the vegetation period. Half of the nitrogen and the whole of the phosphorus and potassium were applied to the soil before the seedlings were planted. The remaining nitrogen fertilizer was applied with a drip irrigation system. Harvests were made on Aug. 16, 2019 and Aug. 24, 2020, respectively.

Measurements and Observations

Morphological Parameters
Stem diameter (SD)
Plant stem diameter (mm) was determined with a compass below the branching point of the plant in 6 plants in each per plot.

Plant habitus (PH)
It was evaluated as compact, semi-open and open depend on a 1-5 scale.
Covering Status of The Fruits of The Leaves (CFL)

Covering status of the fruits of the leaves status, which are important in terms of sunburn, were evaluated as good, moderate and weak.

Fibrillation in Fruit (FF)

Fibrillation in fruits was evaluated as present or absent by cutting the fruits in half after harvest.

Internal Rupture in Fruit (IRF)

Internal rupture of the fruit, which is an important indicator of firm fruit flesh, depended on a 1-5 scale by cutting the fruits in half after harvest.

Physiological Parameters

Relative Water Content (RWC)

At the end of the stress, the RWC determination was made by measuring the fresh weights of the leaf samples taken from the plants then the leaf was kept in pure water for 4 hours and at the end of this period, the turgor weights were determined. Leaf samples whose weights were determined were dried in an oven at 65°C until they reached a constant dry weight. The fresh and dry weights obtained were proportioned with the help of the following formula and the RWC (%) of the leaves was calculated.

\[
RWC(\%) = \frac{(FW-DW)}{(TuW-DW)} \times 100
\]  (3)

Where, RWC: Relative water content (%), FW: Fresh weight (g), DW: Dry weight (g), TuW: Turgor weight (g).

Leaf Water Potential (LWP-Ψ)

LWP was made with a portable pressure chamber (pressure chamber, PMS Instrument Company Model 615) device in the middle of the day (12:30-14:30) before irrigation, once a week, in two replications. For this purpose, measurements were made on two leaves (fully developed, facing the sun) from six plants in each plot and their average was taken as the mid-day leaf water potential value. In the leaf water potential measurement, the leaf blade was placed in the pressure chamber with the stem out, and pressure was applied to the leaf blade from the pressure source of the device and the pressure was increased until a water drop appeared on the outside end of the stem, and the value when a water bubble appeared on the petiole tip was taken as the leaf water potential value.

Chlorophyll Content (SPAD)

Measurements made with the help of a chlorophyll meter as an indicator of the chlorophyll content in the leaf were made between 10:00 and 14:00 from on the adaxial surface of six random leaves (third–fourth leaflets from the top) in each plot when the sun’s angle of incidence on the earth changes at least on days when the weather is not cloudy. Three consecutive readings were taken on the chlorophyll meter so that no shadows were cast on the leaf during measurement.

Stomatal Conductance (mmol m⁻² S⁻¹)

A porometer device was used to determine stomatal conductivity in melon leaves. Measurements were made once a week between 12:00 and 14:00 in open air conditions on six plants selected in the middle of each plot, under and over the top two sun-exposed fully developed leaves. The instrument was calibrated before each porometer reading.

Crop Water Stress Index (CWSI)

Crown temperature (Tc) measurements were made with an infrared thermometer between 12:00 and 14:00 in conditions where the weather was completely clear or the clouds did not block the sun. Crown temperature observations were made during plant growth stages (early vegetation, late vegetation, flowering-fruit setting and ripening) before and after irrigations, on days that did not limit the measurements. Measuring in the direction of the diagonals of the plots, the average crown temperature was found for that plot by taking the average of a total of 12 values at 4 corners and with 3 replications in each. At the beginning and end of the plant crown temperature measurements, the psychrometer and wet and dry thermometer values were read. Measurements taken one or two days after irrigation in full irrigation subjects without water stress (I100) were used to develop the lower bound (LL) equation. The upper bound equation (UL), representing the full stress, was determined from the measurements made in a plot created next to the trial plots and in which no water was applied. The plant water stress index (CWSI) was calculated from the following the Eq. 4 [15].

\[
CWSI = \frac{(Tc-Ta)-UL}{(Tc-Ta)UL-(Tc-Ta)UL}
\]  (4)

Where, LL represents the non-water-stressed baseline (lower base-line) and UL represents the non-transpiring upper baseline; Tc = canopy temperature (°C); Ta = air temperature (°C). LL for the canopy–air temperature difference (Tc–Ta) versus the vapor pressure deficit.
(VPD) relationship was determined using data collected only from the unstressed treatments (FI). UL was computed according to the procedures explained by Idso et al. (1981). To verify the upper baseline, canopy temperatures of the fully stressed plants were determined several times during the growing season of melon.

**Pathogen isolation**

The isolation of the pathogen from the soil was performed by dilution plate count method using Komada's selective medium for *Fusarium oxysporum* [16].

**RESULTS**

**Applied Irrigation Water and ET Amounts**

ET values according to irrigation treatments (I100, I50 and I0) were calculated as 205, 143 and 47 mm for 2019 and 231, 177 and 80 mm for 2020, respectively.

**Morphological Parameters Measurement**

Data plant stem diameter values measured for morphological parameters are shown in Figure 1. Plant stem diameter increased due to the increased amount of irrigation water and the progress of the vegetation period. It showed a steady increase towards the end of the vegetation period.

According to the plant habitus1-5 scale, the subject of I0 is open and semi-open in both years. The I50 is generally in the C3-C4 range, and the I100 has developed as Compact-5. Leaf closure status is weak to moderate, generally weak on I0. The I50 subject was generally moderate and the I100 subject was generally good. The fibrillation did not occur in all subjects in both years. While internal rupture is not generally seen in all subjects, its incidence is generally seen at the level of 2 (less than 10 fruits) in I0-I50 subjects and more than I100 subjects. On the other hand, the internal rupture was more common in the second year than in the first year (Table 2). This may be due to the fact that the experiment was carried out in a fixed experiment area. For this reason, fruit quality may have decreased.

**Physiological Parameters Measurement**

Data on leaf proportional water content (RWC-%), leaf relative water potential (LWP-bar), chlorophyll content and stomatal conductance (porometer-mmol/m²S) values measured for physiological parameters are shown in Figures 2, 3, 4 and 5. Accordingly, in 2019, while stomatal conductance and RWC reached the highest point on the 53rd day after planting, while the chlorophyll and LWP contents were at the highest point on the 32nd day after planting, the values decreased as the vegetation period passed. In 2020, stomatal conductance reached the highest point on the 66th day after planting and the RWC on the 73rd day, while the chlorophyll and LWP contents were at the highest point on the 50th day after planting, and the values decreased as the vegetation period passed.

In both years, the plant stem diameter has increased continuously during the vegetation period. While the plant stem diameter increased with the increase in the irrigation water of LWP and RWC, the chlorophyll content was higher in the subjects where the deficient moisture field capacity was completed (I100), while the stomatal conductance was the highest in the I50 of the irrigation water, the increase in the irrigation water decreased the stomatal conductance. In terms of rootstocks, TZ-148 and Ferro rootstocks reached the best values in terms of chlorophyll content and RWC values in the first year, respectively, and Maximus rootstock in terms of plant stem diameter in both years. In terms of stoma conductance, Maximus

![Figure 1: Variations of plant stem diameter values during the vegetation period, 2019-2020.](image-url)
Table 2: The Effect of Subjects on the Morphological Parameters of Melon

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IL: Irrigation Levels, R: Rootstocks
A0-Non-grafted, F-Ferro rootstock, M-Maximus rootstock, T-TZ 148 rootstock
According to the 1-5 scale of plant habitus; C-Compact, SO-Semi Open, O-Open
According to the 1-5 scale IRF-the internal rupture; [1-healthy, 2-mild (less than 10%), 3-moderate (11-25%), 4-severe (25-50%), and 5-very severe (more than 50%).]

Figure 2: Leaf relative water content (RWC) change during the vegetation period, 2019-2020.

Figure 3: Leaf water potential (LWP) change during the vegetation period, 2019-2020.

rootstock in the first year gave the best results in the second year, while TZ 148 rootstock reached the highest value in LWP values in both years.
Relationships between Yield and Measured Parameters

The relations between yield values of each measured parameter were examined (Figure 6, 7, 8, 9 and 10). The relationship between yield and plant stem diameter was found to be $R^2=0.58-0.73$, $R^2=0.64-0.71$ for chlorophyll content, $R^2=0.65-0.54$ for stomatal conductance, $R^2=0.69-0.89$ for LWP and $R^2=0.51-0.56$ for RWC by years, respectively.

Crop Water Stress Index (CWSI)

The first and second year CWSI values for I100, I50, and I0 irrigation treatments were in the range of 0.56-0.47, 0.86-0.71 and 0.97-0.93, respectively. CWSI

Figure 4: Change of leaf chlorophyll content during the vegetation period, 2019-2020.

Figure 5: Variation of stomatal conductance values during the vegetation period, 2019-2020.

Figure 6: The relationship between plant stem diameter and yield, 2019-2020.
value approached 1 under water deficit conditions and, 0 with grafting and increasing irrigation water amount. (Figure 11).
DISCUSSION

Increased irrigation levels and rootstock use had significant positive effects on most of the studied morphological and physiological parameters. The implementation of new management techniques is essentially a necessary practice to alleviate the adverse effect of climate change undesirably impacting the precipitation frequency and global warming contributing to the spread of the drought-prone areas and to significant decline in crop yielding and in freshwater resources around the world [17]. The chlorophyll content in the leaf indicates the physiological state of a plant. Chlorophyll is among the obligatory pigments in the conversion of light energy into chemical energy. The amount of radiation absorbed from the sun also depends on the amount of photosynthetic chlorophyll in the leaf. Therefore, the amount of chlorophyll content is related to photosynthetic activity [18]. The amount of chlorophyll gives an estimate of the relationship between the amount of nitrogen from plant nutrients and the pigment ratio [19]. Leaf chlorophyll level is directly related to plant stress and aging. Thanks to the leaves containing high chlorophyll, higher quality and more fruit can be obtained [20]. Leaf water potential was -2.02 bar in grafted plants and -1.62 bar in ungrafted plants, and YSP increased significantly in grafted plants [21]. Rootstocks indicate less damage to chlorophyll. Bikdeloo et al. 2021 [22] and Kumar et al. 2015 [23] also reached similar results in different vegetables.

In terms of physiological parameters measured in the plant, in 2019; while stomatal conductance, RWC, chlorophyll and LWP contents reached the highest value due to the increase in the days after planting, the values decreased as the vegetation period passed. In both years, the plant stem diameter has increased continuously during the vegetation period. While the plant stem diameter increased with the increase in the irrigation water of LWP and RWC, the chlorophyll content was higher in the subjects where the deficient moisture field capacity was completed (I100), while the stomatal conductance was the highest in the I50 of the irrigation water, the increase in the irrigation water decreased the stomatal conductance. The stomatal conductance value generally increased in applications based on precipitation with the subject of limited irrigation (I50). This increase in stomatal conductance indicates that the stomata are significantly closed. In this case, plants in I50 and 10 subjects also show that the transpiration rate is slow [24]. The drought reduces stomatal aperture through the production of root chemical signals. The regulation of the stomatal closure was appropriate for the maintenance of an adequate water status insuring the plant growth without detrimental effects on canopy water relations [25-27].

In terms of rootstocks, in terms of chlorophyll content and RWC values in the first year, Ferro rootstock TZ-148 and Ferro rootstock, respectively, in the second year, Maximus rootstock reached the best value in terms of plant stem diameter in both years. In terms of stomatal conductance, Maximus rootstock in the first year gave the best results in the second year, while TZ 148 rootstock reached the highest value in RWC values in both years. In addition, RWC was higher in irrigation water increase and grafted plants compared to control plants. On the contrary, LWP has decreased. However, the research findings of a previous experiment carried out by Bikdeloo et al. (2021) [22] and Bigdelo et al. (2017) [28], increases in plant vigor rather than tolerance to stresses. Furthermore, this rootstock has also been shown to increase water and nutrient uptake in grafted watermelon which was likely associated with greater rootstock’s vigorous root system [29-31].

The CWSI values increased with increasing water stres [32] and decreased with grafting. Melon should be irrigated at CWSI values between 0.56-0.71.
The pathogens fungi, *Fusarium oxysporum* f. sp. *melonis*, generally increased with decreasing soil moisture content and non-grafted applications. As with most other Fusarium wilts, low soil moisture favor the pathogen and accentuates the wilting symptom. Maximum disease incidence was observed in the I50 and I0 treatments. The results obtained are consistent with the results of previous studies [29, 30, 33, 34]. As was determined in the current study, soil-borne pathogens occur in high temperatures and low moisture are closely related to irrigation. It has been reported that the grafting approach against Fusarium wilt in melon is effective in disease control and other parameters [35, 29, 36].

**CONCLUSION**

Grafted plants developed a more compact habitus than the control in both years. Leaf development and stem thickness were also higher. In both years, the plant stem diameter has increased continuously during the vegetation period. While the plant stem diameter increased with the increase in the irrigation water of LSP and RWC, the chlorophyll content was higher in the subjects where the deficient moisture field capacity was completed (I100), while the stomatal conductance was the highest in the I50 of the irrigation water, the increase in the irrigation water decreased the stomatal conductance.

In terms of rootstocks, Ferro rootstock gave the best results in terms of chlorophyll content and RWS values in the first year, while TZ-148 and Ferro rootstock gave the best results, respectively, in the second year. Maximus rootstock reached the best value in terms of plant stem diameter in both years. In terms of stomata conductivity, Maximus rootstock in the first year gave the best results on the second year TZ-148 rootstock, while the highest LWP value was measured in TZ 148 rootstock in both years.

In addition, strong correlations were found between yield and investigated parameters. The results revealed that CWSI can be used for irrigation scheduling for melons.

In the study carried out, grafting and rootstock-scion combinations increased disease control. Low soil moisture and lack of vaccination for Fusarium wilt disease promoted the growth of the pathogen and the appearance of wilt symptoms. Ferro, TZ 148 and Maximus rootstocks were effective in controlling *Fusarium oxysporum* f.sp.*melonis*, respectively.

On the other hand, rootstock and scion combinations should be carefully selected according to the variety, specific soil, climate and regional conditions. The relationship between rootstock and scion can help determine the tolerance of the root system. As it is known, healthy seedlings are the guarantee of abundant and quality products. In addition, the increase in the investigated parameters may also be due to the increased water and nutrient intake due to the strong root system of the rootstock.

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