

Responses of Apple Plants to Drought Stress

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Abstract

In order to screen apple rootstocks for drought tolerance, two different drought levels moderate and severe stress, and a control were applied to apple cultivar Red Chief grafted onto M9 and MM106 rootstocks. Apple plants were subjected to drought stress by withholding water for 15 and 19 days in the greenhouse conditions, while the control treatment was continued watering. Data were recorded 15 (moderate drought stress) and 19 days (severe drought stress) after application of drought stress. At the end of the experiment, both rootstocks were significantly affected under drought conditions. Severe drought stress caused decrease in SPAD value in Red Chief grafted onto M9 and MM106 by 15.7 % and 11.1 %, respectively. Severe drought stress declined anthocyanin content in M9 and MM106 by 7.8 % and 28.4 %, respectively. Stomatal conductance was remarkably affected by drought stress. Effects of drought stress on plants depended on rootstocks, severity and duration of drought stress. As a result, the more invigorating rootstock MM106 was found more drought-tolerant when compared to M9 that is needed to be evaluated with more parameters.

Keywords: apple, drought stress, malus, response, rootstock

1. Introduction

Apple (*Malus domestica* Borkh) from the family Rosaceae, which includes one-third of all flowering plants, is the main fruit crop of temperate regions of Turkey and in the world. In Turkey, it is grown on an area of 175.357 hectares with annual production of 3.032.164 tons

(FAOSTAT, 2017). However, the apple cultivation in several regions can be restricted due to drought stress since apple is a drought stress sensitive crop (Zhang *et al.*, 2013). Drought stress is commonly the challenging factor in intensive apple growing areas due to high temperatures and water deficit in the summer. Furthermore, dwarfing rootstocks are used in intensive orchards that causes decrease in water uptake from soil due to its shallow roots under water deficit conditions. Nutrient utilization by plants decreases under water deficit conditions that results in decline in photosynthesis (Nemeskéri *et al.*, 2009).

Apple cultivation under drought conditions causes several deleterious effects which includes increases in reactive oxygen species (ROS), reduction in nutrient uptake, and damages in cell membranes (Šircelj *et al.*, 2007; Bhusal *et al.*, 2019). ROS cause oxidative stress that damages chlorophyll membranes (Fita *et al.*, 2015). Plants exhibit many physiological and biochemical responses in order to cope with the malignant effects of stresses and the responses are related with rootstock ability in grafted plants. To deal with drought damage, drought tolerant rootstocks can be used. Rootstocks improve tree size, fruit yield and quality. It's been demonstrated that rootstocks play a pivotal role in plant stress tolerance (Del Carmen Gijón *et al.*, 2010; Aras and Eşitken, 2018; 2019b). In a previous study, we evaluated M9 and MM106 apple rootstocks under short term salinity stress. MM106 provided more salinity-tolerance on the cultivar "Fuji" compared to M9 (Aras and Eşitken, 2019a). Tworkoski *et al.* (2016) studied drought stress on M9 and MM111 apple rootstocks. They found MM111 highly drought tolerant and attributed its higher tolerance to more extensive root system. It has been found that vigor rootstocks show more drought tolerance by increasing assimilates to root development (Bauerle *et al.*, 2011). Many studies have demonstrated that vigor rootstocks are capable to survive under drought conditions through deeper root systems. There is however, limited knowledge on survival on shallow soils under drought conditions. Understanding plant responses to drought stress under shallow soils is very critical.

The objective of this experiment was to study the influences of two different clonal rootstocks (M9 and MM106) against drought stress in apple plant (*Malus domestica* Borkh) cv. Red Chief under potted condition.

2. Materials and Methods

This study was conducted in pots, in a semi-controlled greenhouse at Yozgat Bozok University. One-year-old, clonally propagated apple cv. Red Chief (*Malus domestica* Borkh) grafted onto M9 and MM106 rootstocks were planted in 10 L plastic pots containing perlite in March in 2019. The experimental design was randomized complete block in three replications. Each plot had three plants per replicate. The plants were placed in a semi-controlled greenhouse where PAR was 35.000-40.000 lux and temperature fluctuated between 25-35°C in the day. Plants were well irrigated and drained until the initiation of the drought experiment. The plants were watered regularly and fertilized once a week with Hoagland's nutrient solution (Hoagland and Arnon, 1950). Apple plants were subjected to drought stress by withholding water for 15 days and 19 days in the greenhouse conditions, while the control treatment was continued watering.

Relative chlorophyll (SPAD) value was measured with a Minolta SPAD-502 chlorophyll meter (Minolta Camera Co, Ltd, Osaka, Japan). The ACM-200 plus Anthocyanin Content Meter was used to measure relative anthocyanin content. Stomatal conductivity and leaf temperature were measured on the youngest fully expanded leaves on upper branches of the plants with leaf porometer (Li-COR).

Leaf relative water content (LRWC) was determined by the procedure of Smart and Bingham (1974). Leaves were collected from the young fully expanded leaves of three plants per replicate. Individual leaves first detached from the stem and then weighted to determine fresh weight (FW). In order to determine turgid weight (TW), leaves were floated in distilled water inside a closed petri dish. Leaf samples were weighted periodically, after gently wiping the water from the leaf surface with the tissue paper until a steady state was achieved. At the end of imbibition period, leaf samples were placed in a pre-heated oven at 72°C for 48 h, in order to determine dry weight (DW). Values of FW, TW, and DW were used to calculate LRWC using the equation given below:

$$\text{LRWC (\%)} = [(\text{FW}-\text{DW})/(\text{TW}-\text{DW})] \times 100$$

The statistical analyses were performed with the statistical software package SPSS, version 20.0. Data were subjected to two-way ANOVA and were separated by the Duncan's test at a significance level of $P < 0.05$.

3. Results

Some responses related with plant physiology were measured to define the effect of drought conditions on apple plants following 15 days and 19 days of drought stress treatment. Both rootstocks were significantly affected under drought conditions. Severe drought stress caused decrease in SPAD value in Red Chief grafted onto both rootstocks. The lowest SPAD value was in M9 (47.36). Moderate stress decreased the value by 13.6 and 3.5 %, respectively. The differences in both treatments and rootstocks were significant for anthocyanin content. Severe and moderate drought stress remarkably declined anthocyanin content in M9 and MM106 (Table 1).

Table 1. Effect of drought stress SPAD and anthocyanin content

Treatments	SPAD			Anthocyanin		
	M9	MM106	Means	M9	MM106	Means
Control	56.20 ^a	59.30 ^a	57.75	9.60	11.20 ^b	10.40 ^B
Moderate Drought	48.56 ^{ab}	57.20 ^{ab}	52.88	10.13	11.31 ^b	10.72 ^B
Severe Drought	47.36 ^b	52.96 ^b	50.16	10.35	14.38 ^a	12.36 ^A
Means	50.71	56.48		10.02 ^A	12.30 ^B	
Rootstocks x Treatments	NS			*		

Means separation within the columns and line by Duncan's multiple range test ($P < 0.05$). NS-not significant, *- $p \leq 0.05$

Leaf relative water content decreased by drought conditions (Table 2). Under severe drought stress condition, LRWC values were found in M9 and MM106 as 64.63 % and 66.89 %, respectively. Moderate stress decreased the value by 4.22 and 15.9 %, respectively. Stomatal conductance was remarkably affected by drought stress. Under severe drought condition, stomatal conductance decreased in Red Chief grafted onto MM106 by 88.26%, while it decreased in Red Chief grafted onto M9 by 82.9%. Leaf temperature was not significantly affected for both rootstocks (Table 2).

Table 2. Effect of drought stress on LRWC, stomatal conductivity and leaf temperature

Treatments	LRWC (%)			Stomatal Conductivity ($\text{mmol m}^{-2} \text{s}^{-1}$)			Leaf Temperature (°C)		
	M9	MM106	Means	M9	MM106	Means	M9	MM106	Means
Control	84.18 _a	82.86 _a	83.52	180.20 _a	205.33 _a	192.76	34.46 ^{NS}	34.76 ^{NS}	34.61
Moderate Drought	80.62 _a	69.67 _{ab}	75.14	50.43 _b	43.00 _b	46.71	34.33	34.63	34.48
Severe Drought	64.63 _b	66.89 _b	65.76	30.80 _b	24.10 _b	24.45	34.73	34.76	34.75
Means	76.48	73.14		87.14	90.81		34.51	34.72	
Rootstocks x Treatments	NS			NS			NS		

Means separation within the columns and line by Duncan's multiple range test ($P < 0.05$). NS-not significant, *- $p \leq 0.05$

4. Discussion

M9 and MM106 have been the leading rootstocks in Turkey apple production. It is known that MM106 is more vigorous than M9 and the roots of MM106 are deeper helping scions to survive under drought conditions by growing deeper in the soil.

SPAD value represents a parameter to evaluate the chlorophyll content. Change in the chlorophyll content is demonstrated as an indicator of plant status under stress conditions and many studies have reported that drought stress decreases the chlorophyll content in leaves (Hamann *et al.*, 2018; Bhusal *et al.*, 2019). In our study, loss in chlorophyll was less in Red Chief on MM106 for both moderate and severe drought stresses compared to the control.

Several studies have showed that anthocyanins are capable to scavenge reactive oxygen species (Efeoğlu *et al.*, 2009; Sperdouli and Moustakas, 2012). Drought stress caused a strong increase in anthocyanin content. In severe drought, anthocyanin content of Red Chief

on MM106 increased by 28,4 % that shows MM106 utilizes anthocyanins as a defense mechanism under severe drought condition. Under moderate drought condition, M9 triggered more anthocyanin accumulation than MM106.

Leaf relative water content is another indicator of drought stress reflecting plant cellular osmotic adjustment under cellular dehydration (Hamann *et al.*, 2018). Decrease in leaf water status prevents chlorophyll functioning (Guerfel *et al.*, 2009; Bhusal *et al.*, 2019). Thus, greater reduction in chlorophyll in M9 may be due to the severe reduction in LRWC under severe drought stress condition. Reduction in LRWC as a result of drought stress was reported in apple (Liu *et al.*, 2012), almond (Alimohammadi *et al.*, 2013), and sweet cherry (Javadi *et al.*, 2017).

Decline in stomatal conductance is a defense mechanism to avoid water loss from leaf surface (Mahouachi, 2009). Some experiments have found that more vigorous rootstocks have greater stomatal conductance than dwarfing rootstocks (Atkinson *et al.*, 2000; Tworkoski *et al.*, 2016). In the present study, MM106, more vigorous rootstock, showed higher stomatal conductance compared to M9 and also more decreased in stomatal conductance under drought conditions. Fernandez *et al.* (1997) stated that abscisic acid (ABA), a responsible phytohormone for stomatal closure, was higher M9 than more vigorous rootstocks that leads to decline in stomatal conductance. Under drought conditions, MM106 showed greater changes in stomata compared to M9 that may be attributed to possessing higher ABA under stress condition.

5. Conclusion

Effects of drought stress on apple plants depend on rootstocks, severity and duration of drought stress. Based on the current study stomatal conductance is a suitable marker for drought stress. When control plants of M9 and MM106 are compared, it is observed that LRWC is greater in M9 that may be a result of drawing more water from roots to leaves and more stomatal closure for water conservation.

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