

A New Method for Increasing Water and Nutrient Efficiency in Peach Fruit (*Prunus persica* **var Red Top) in Low Yield Lands**

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Abstract

Water is one of the most significant factors limiting the production of horticultural crops. To confront drought stress, an experiment was designed as a factorial in the form of a randomized complete block. Treatments included placing the pumice bag next to the tree in two levels **—** [no bag (B0), one bag (B1)], different levels of irrigation **—** [50% (I50), 75% (I75), and 100% (I100)] **—** and root inoculation with fungi **—** [*mycorrhiza* (Fm), *Trichoderma* (Ft), and no fungus (F0)]. The highest LA and SLA were measured in the B0I100Ft treatment. The maximum chlorophyll index was measured in the B1I75Ft treatment. The highest yield was measured in the B1I100Fm treatment, which increased yield by 2.1 times compared to the control. Treatments that placed the bag next to the tree and had a sufficient irrigation level yielded more. The highest concentrations of N, Zn, and K were measured in treatments with lower irrigation levels. Only irrigation and bag placement had a significant effect on P. The highest phenols, anthocyanins, antioxidants, and vitamin C were measured in the B0I50F0, B0I75F0, B0I50Ft, and B0I75Fm treatments, respectively. According to the results, the B1I75Fm treatment saved 25% in water consumption, while maintaining good fruit productivity and quality.

Keywords: Irrigation, mycorrhiza, pumice, stress, and Trichoderma

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Introduction

Abiotic stresses significantly impact plant growth and development. Among these stresses, drought stands out as one of the most detrimental due to its diverse adverse effects on plants. It particularly affects the physical structure, internal functioning,

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and biochemical processes of fruit trees. Ultimately, drought severely diminishes their yield, hampers growth indices, and undermines the quality of the produce (Hasanuzzaman et al., 2018). Peach (*Prunus persica*) is native to China. The optimal growth of peach fruit is heavily reliant on receiving an ample and high-quality water supply. However, when peach trees experience drought stress, the growth of the fleshy portion of the fruit is hindered, leading to a decrease in both its size and overall quality. On the other hand, the fruit acts as a sink for absorbing carbohydrates and water when ripe (Eldem et al., 2012).

The term "split root" refers to a technique in plant research or cultivation where different conditions are created for specific segments or parts of the plant's root system, which are isolated from each other. This technique allows researchers or growers to manipulate and study the effects of various environmental factors or treatments on different sections of the root system independently. By creating isolated conditions, it becomes possible to examine the specific impact of different treatments or conditions on the growth, development, or response of the plant's roots. Depending on the plant species being studied and the study's objective, there are different methods to create the split root. The simplest way involves dividing the root into two parts and placing them in separate containers; more complex methods include grafting a second root from another plant (Giertych and Leski, 2023). Split root culture has also been used to study the transfer of nitrogen-15 from roots to stems and leaves (Doi et al., 2022). In addition, unusual water conditions have been used to save water and place part of the root in different Electrical Conductivity (EC) or pH conditions to study growth and yield (Tabatabaie et al., 2004; Xu et al., 2019).

Mineral pumice is an inert aluminosilicate mineral with a volcanic origin, low density, and high porosity. Pumice is free from pathogens and weed seeds. Additionally, it has a low ion exchange capacity and a stable structure (Maucieri et al., 2019). Water quickly percolates into pumice, and due to its high porosity, it has a high water-holding capacity and hydraulic conductivity (Fathoni et al., 2023). Many studies have been conducted on the impact of pumice on the physical properties of substrates for horticultural purposes, such as fruit, plant, and flower production (Crippa et al., 2017). Research has shown that when roots are inoculated with fungi, it can enhance the absorption of water and nutrients by the plants. This symbiotic relationship, known as mycorrhiza, involves a mutually beneficial exchange between the plant and the fungus. The fungi form a network

of hyphae that extend into the soil, increasing the surface area available for nutrient uptake. In particular, mycorrhizal fungi have been found to improve the uptake of certain elements, including sedentary elements such as phosphorus (P), zinc (Zn), and copper (Cu). This increased uptake of water and essential elements by the plant contributes to improved water uptake efficiency, enhanced plant biomass, and overall growth (Marschner and Dell, 1994). Research also indicates that Trichoderma increases water and nutrient uptake and stimulates secondary root formation, enhancing vegetative growth and yield by changing root morphology (Chacón et al., 2007); Pascale et al., 2017). In one study, the root of trifoliate orange was divided into two parts, and one part of the root was inoculated with mycorrhizal fungi, which increased the plant biomass (Wu et al., 2016).

Partial root drying (PRD) is a type of irrigation system. In this method of irrigation, during each irrigation cycle, only one side of the plant root is irrigated (Iqbal et al., 2020). Roots in drying soils during PRD cause the production of the plant hormone abscisic acid (ABA), which is conveyed through xylem vessels to maintain shoot functionality (Kang and Zhang, 2004). Increased ABA concentrations cause partial closure of stomatal apertures and a reduction in leaf development, while the roots in the moist portion of soil absorb sufficient water to sustain the plant shoots (Iqbal et al., 2019).

Considering the challenges related to water shortage, it is crucial to provide suitable and innovative solutions to improve the performance, quality, and quantity of horticultural products. This research aimed to create favorable conditions for fruit tree growth by combining PRD, split root, and root inoculation with fungus.

Materials and Methods

The experiment was conducted in the split root research garden of Shahed University, Tehran, Iran. The longitude of the experimental site was 51˚ 20', the latitude was 35˚ 33', and the altitude was 1037 m. In May 2017, seedlings of the Redtop peach variety, which were grafted onto *GF677*

Fig. I. Schematic of bag placement

Table 1 Chemical properties of soil used in the experiment

ЕC	рH	ገር	TNV	CEC
(ds/m)		(%)	'%)	(meq/100g)
		∩ 1		

rootstock, were planted in three rows. The rows were three meters apart, and the trees in each row were two meters apart.

The first factor was Bag placement (B) (Fig. I). The B treatments were B0 and B1, indicating no bag and one bag, respectively. The bag was filled with pumice and placed on the left side of the trees in a pit with a depth of 60 cm and a distance of 40 cm from the tree trunk. Composite bags with a 40-liter capacity were placed in the pits. At 20 cm from the top of the bag, three holes were made to remove excess water and to signal the root to move into the bag. The purpose of placing the bag was to minimize water wastage, making water only available for the roots of the plant.

The second factor was Irrigation (I). Irrigation treatments were I50, I75, and I100, indicating 50%, 75%, and 100% field capacity irrigation, respectively. In the 50% irrigation treatment, one dripper with a flow rate of eight liters per hour was used. In the 75% treatment, two drippers with flow rates of eight and four liters per hour were used, and in the 100% treatment, two drippers with a flow rate of eight liters per hour were used. The irrigation water was prepared with several types of fertilizers in specific amounts, with the compositions and quantities provided in Table 2.

The third factor was root inoculation with fungi (F). Fungi treatments were labeled as F0, FM, and FT, indicating no fungi, *Mycorrhiza glomus*, and *Trichoderma harzianum*, respectively. For the mycorrhiza-treated plants, 250 g of fungus was mixed with the pumice in the bag; in treatments without bag placement, the fungus was added directly to the tree planting pit. The mycorrhiza was a mixture of several pure *Glomus* mycorrhiza strains, produced by Turan Biotech Company. For each plant treated with *Trichoderma harzianum*, 4.5 g was mixed with the pumice bag. In treatments without bag placement, the fungus was added directly to the tree planting pit. The *Trichoderma harzianum* strain was obtained from Hayat Sabz Fanavaran Company under the brand name Trichomix-HV.

A 5000-liter tank and an underwater pump were installed to supply water to the trees. A 200-liter tank was placed beside the main water tank to supply macro and micronutrients as needed. Water was delivered to the drippers through 2 inch polyethylene pipes and 16 mm side pipes, and both water and nutrients were transferred to the bag and trees through the macaroni pipe. The EC and pH values of the main tank were automatically and regularly adjusted by a measuring device to 1.7 dS/m and 7, respectively.

The treatments started on the first day of planting the trees (May 2017). In total, 27 treatments were conducted in three replications. Two rows were considered as guard rows. Data collection started in 2019.

To measure the leaf area (LA), 20 leaves were selected from each treatment in June 2019, and measurements were taken using a leaf area meter (WINAREA-UT-11). To determine the specific leaf area (SLA), after measuring the leaf area, the leaves were placed in an oven at 75 °C for 48 hours to dry completely. The dried weight (DWt) of the leaves was then measured with a digital scale. SLA was calculated using the following formula (Cornelissen et al., 2003):

$$
SLA = \frac{LA(cm^2)}{DWt(g)}
$$

Table 3

ANOVA for the effect of bag placement, fungi inoculation, and irrigation levels on physiological, quantitative, and qualitative characteristics of peach cv. 'Red Top' in split root culture.

ns, ** and *: non-significant, significant at p≤0.01 and p≤0.05, respectively

Dried leaf samples were milled to measure N, P, K, and Zn. For K and P measurements, 0.5 g of powdered leaves was placed in digestion tubes. In the next step, 10 ml of 65% nitric acid was added to each tube and kept at room temperature for 12 hours. The tubes were then heated to 60 °C for three hours, followed by 110 °C for another three hours. After cooling, 20 ml of distilled water was added to each tube, and the solution was passed through filter paper to reach a final volume of 100 ml. The amount of K in the plant samples was measured using a flame photometer (Prisma Tech Flame Photometer PTFP-5), and P was measured with a spectrophotometer (SHIMADZU UV-1205). Atomic absorption spectroscopy (Analytik Jena contrAA300) was used to measure Zn.

To determine N content, 0.5 g of dried and milled leaves was placed in digestion tubes. Potassium

sulfate (2.5 g), copper sulfate (0.5 g), and 10 ml of 98% sulfuric acid were added to the tubes. The initial temperature was set at 50 °C and was increased by 30 °C every 30 minutes until it reached 380 °C. The samples were held at this temperature for three hours. After cooling, 20 ml of distilled water was added to each sample, and the solution was filtered and diluted to a final volume of 100 ml. Nitrogen content was measured using the Kjeldahl method (Tabatabaei, 2013).

Chlorophyll Index: Five leaves from each tree were randomly selected in June at 12 noon, and the chlorophyll index was measured using a chlorophyll meter (Minolta SPAD-502, Japan). The average total chlorophyll of the five leaves was calculated by the device.

Fig. II. Means comparison of bag placement effect on LA, SLA, chlorophyll, yield, fresh fruit weight, dry fruit weight, phenol antioxidant, and vitamin C. Means followed by the same letters in each column are not significantly different at a 5% level of probability according to Duncan's multiple range test.

Fig. III. Effect of irrigation level and bag placement (a) and fungi inoculation and bag placement (b) on N.

Fig. IV. Effect of bag placement (a) and irrigation level (b) on P.

At maturity, the fruits from each tree were harvested separately and weighed. Three fruits per tree were randomly selected in July, weighed, and then placed in an oven at 75 °C for 72 hours. Once the dried weight of the fruits stabilized, the average fresh and dry weights of the three fruits were calculated.

Vitamin C, Antioxidant, Anthocyanin, and Total Phenol: To assess these parameters, three fruits were randomly harvested from each tree. The method of Barakat et al. (1973) was used to measure vitamin C. Antioxidants were quantified using the DPPH assay (Prevc et al., 2013). The differential pH method was employed to determine total anthocyanin (Cheng and Breen, 1991), and phenolic compounds were measured using the Folin-Ciocalteu reagent (Slinkard and Singleton, 1977)**.** Treatment B0I100F0 was used as the control.

Statistical Analysis

A three-factor randomized complete block design was applied for this experiment. The data were analyzed using analysis of variance (ANOVA) via the PROC ANOVA procedure in SAS version 9.4. Mean separation was performed using Duncan's multiple range test.

Results

As a result of the ANOVA, most of the characteristics were significantly affected by bag placement, irrigation level, and fungal inoculation (Table 3).

LA, SLA, and Chlorophyll Index

Bag placement, root inoculation with fungi, and different irrigation levels had a significant effect on LA and SLA. The highest LA (Table 4) was measured in the B0I100Ft treatment, which increased LA by 59% compared to the control. The maximum SLA (Table 4) was also observed in the B0I100Ft treatment. The chlorophyll index was influenced by all factors, with the highest index recorded in the B1I100Ft treatment. Bag placement notably increased LA, SLA, and the chlorophyll index (Fig. II).

Yield

As seen in Table 4, the highest yield was measured in B1I100Fm (13.11 kg). The B1I100Fm treatment increased the yield fourfold compared to the control. In the treatments where the bag was placed next to the tree, yield increased relative to the same treatments without bag placement. In treatments inoculated with mycorrhizal roots, yield increased compared to the same treatments without inoculation. The highest fresh and dry fruit weights (Table 4) were observed in the B1I75Fm and B1I75F0 treatments, with increases of 40% and 46%, respectively, compared to the control. Placing a bag next to the tree increased fresh and dry fruit weights (Fig. II e-f).

Elemental Analysis

According to the ANOVA results, the interaction of the three factors had no significant effect on N (Table 3), but the interaction of irrigation levels and bag placement, and of fungal inoculation and bag placement, had a significant effect on N. With the interaction effect of bag placement and irrigation levels, the highest N content was measured in the B0I50 treatment (Fig. IIIa). With the interaction effect of bag placement and fungal inoculation, the highest N content was found in the B1Fm treatment (Fig. IIIb). The highest K content in leaves (Table 4) was observed in the B0I75F0 treatment, which increased K by 25% compared to the control. The interaction of the three factors had no significant effect on P content in leaves; however, bag placement and irrigation levels had a significant effect on P content. At different irrigation levels (Fig. IV), the highest P content in leaves was measured under full irrigation. For bag placement (Fig. IV), the highest P content was measured in treatments with one bag placement, with an average of 5.26. The interaction of the three factors had a significant effect on Zn content in leaves. Treatments B0I50Ft and B0I50Fm (Table 4) had the highest Zn levels in leaves, as these treatments were subjected to the highest drought stress.

Table 4

Mean comparisons of interaction effects of bag placement, different irrigation levels, and root inoculation with fungi on growth and physiological characteristics of peach cultivar Redtop on GF677 rootstock.

Table 4.

Continue:

*Means followed by the same letters in each column are not significantly different at a 5% level of probability according to Duncan's multiple range test. In Table, B0 and B1 represent no bag placement and one bag placement, respectively; I50, I75, and I100, respectively represent 50, 75, and 100% irrigation and F0, FM, and FT represent without inoculation, inoculatio with mycorrhizal fungi, and inoculation with *Trichoderma* fungi.

Quality Characteristics

Based on the comparison of mean chart results in Table 4, the sample labeled B0I50F0 showed the highest total phenolic content, indicating a 3% increase in phenol levels compared to the control.

In treatments with bag placement and fungal inoculation, total phenolic content decreased as irrigation levels increased. The highest and lowest antioxidant capacities (Table 4) were measured in B0I50Ft and B1I100F0 treatments, with mean

comparisons of 93% and 16%, respectively. In treatments with one bag placement, antioxidant capacity decreased as irrigation levels increased.

For total anthocyanin, a similar trend was observed, as levels decreased with increasing irrigation and bag placement. In most cases, total anthocyanin was further reduced by fungal inoculation. The highest total anthocyanin content (Table 4) was observed in the B1I50F0 treatment, with anthocyanin levels decreasing as irrigation levels increased in treatments with one bag placement.

The highest vitamin C content (Table 4) was measured in the B0I75Fm and B0I100F0 treatments. In most treatments, the presence of fungi increased vitamin C content. The main effect of bag placement showed that treatments without bag placement had the highest levels of vitamin C, antioxidant capacity, and total phenolic content (Fig. II).

Discussion

As drought stress intensifies, photosynthesis tends to decrease. However, this decline in photosynthesis occurs at a slower rate compared to the reduction in leaf growth. The plant's initial reaction to drought is a reduction in leaf area, primarily due to hindered leaf expansion caused by a drop in photosynthesis (Anjum et al., 2011). In most treatments, root inoculation with mycorrhiza increased LA compared to noninoculated treatments. Under drought stress, decreases in leaf area and cell volume lead to a reduction in SLA. As SLA decreases and leaf thickness increases, the density of photosynthetic cells per unit area rises (Pessarakli, 2019). The increase in LA and SLA in treatments where a bag was placed next to the tree and 75% irrigation was applied, compared to treatments with the same irrigation level but without bag placement, suggests a reduction in drought stress due to bag placement. The drought-induced reduction in LA is associated with suppressed leaf development, resulting from reduced photosynthesis (Nonami, 1998; Pessarakli, 2019).

The use of *Trichoderma* enhances P uptake, a crucial factor in cell division and the regulation of photosynthetic substances, by modulating plant hormones and reducing stress damage. Root inoculation with *Trichoderma* has been shown to increase leaf area and chlorophyll index in tomatoes (Chacón et al., 2007). Plant growth depends on the balance between the root system and shoot interactions, as resources move between these parts based on their source-sink relationship. Water deficit severely hampers plant growth and development more than any other abiotic stress (Anjum et al., 2011). In pistachio, root inoculation with mycorrhiza under drought stress increased LA and chlorophyll index compared to non-inoculated conditions (Abbaspour et al., 2012). Drought stress directly or indirectly impairs plant physiological activities, leading to the production of reactive oxygen species (ROS), an early defense response known as the oxidative burst. The production of ROS causes chlorophyll decomposition, leading to structural deterioration in chloroplasts and the disintegration of thylakoid (Sharifa and Muriefah, 2015). During drought, many plant species exhibit chlorophyll reduction, with the degree of decrease depending on the stress severity and duration. For instance, mild drought stress reduced the chlorophyll index in sesame by approximately 14.30% and 2.02% under optimal irrigation conditions. Root inoculation with mycorrhizal fungi has been shown to improve the chlorophyll index (Askari et al., 2018). Treatments with one bag placed next to the tree, 75% and 100% irrigation, and root inoculation with fungi showed higher chlorophyll than the same treatments without bag placement, suggesting reduced drought stress due to bag placement.

In peaches, as in other plants, a decrease in photosynthesis and sucrose production is linked to reduced carbon dioxide absorption. Sucrose, the main sugar transported through the phloem, provides energy for growth and development in swollen buds. Under drought stress, sorbitol production increases relative to sucrose and starch. In almonds, an increase of more than two percent in the sorbitol-to-sucrose ratio causes fruit drop (Moing et al., 1997). Drought stress inhibits dry matter production primarily by restricting leaf expansion and development, resulting in reduced light interception (Nonami,

and * Significance at the probability level of 1 and 5% **

1998). There is a positive correlation between LA and chlorophyll with yield, fresh fruit weight, and dry fruit weight (Table 5). Fungal associations with plant roots improve nutrient absorption, with mycorrhizal fungi enhancing nutrient uptake and dry matter production compared to nonmycorrhizal plants under water stress conditions (Allen et al., 2010). Treeby et al. (2007) linked fruit weight loss under stress to reduced fruit cell volume and decreased photosynthetic material compared to full irrigation. Continuous drought stress, particularly during fruit growth, can reduce fruit yield. Bag placement was shown to increase fresh and dry fruit weight, likely due to improved water and nutrient absorption.

Although Zn levels were generally similar across treatments, drought-stressed treatments exhibited the highest Zn concentrations. The increase in Zn and N in treatments B0I50Fm and B0I50Ft may be attributed to reduced growth and lower consumption of these nutrients. Drought stress increases K concentration in the root, enhancing drought resistance. Studies on sesame indicate that mycorrhizal inoculation increases N, P, and K in leaves, while drought stress decreases these nutrients (Askari et al., 2018). Our results showed no significant interaction effect among the three factors on P levels. P ions adhere to clay particles under drought stress, making them less accessible to roots (Marschner and Dell, 1994). While the fungus did not significantly affect leaf P, it improved this index compared to treatments without fungi. Increased leaf P due to bag placement may result from fertigation, which irrigates the bag. The primary function of mycorrhizal fungi is to enhance P and Zn uptake from the soil, although a decrease in shoot P due to mycorrhizal fungi has been observed in soybeans (Lambert and Weidensaul, 1991). Drought stress-induced changes in nutrient dynamics, including increased K and decreased P in *Amaranthus* leaves, have been reported (Sarker and Oba, 2018). *Trichoderma* and mycorrhizae improve nutrient absorption by enhancing root development. *Trichoderma* further aids nutrient uptake by releasing gluconic and citric acids, which lower soil pH and increase the solubility and availability of macro- and micronutrients (Azarmi et al., 2011). Higher N concentrations in B0I50Ft and B0I50Fm and Zn in B0I50Fm and B0I50Ft treatments may result from reduced growth under drought stress, which increases nutrient concentration due to reduced dilution.

Water stress leads to ROS production, necessitating antioxidants to mitigate these stress effects and enhance plant tolerance. Antioxidants play a critical role in balancing free radical production and scavenging (Bettaieb et al., 2011). Under drought stress, there is a strong correlation between increased phenolic content and

antioxidant production in plants. Compounds such as vitamin C, anthocyanins, and phenolics serve as natural antioxidants that counteract free radicals from drought stress, preventing tissue damage (Fischer et al., 2013). Increases in phenolic content, vitamin C, and anthocyanins under drought stress have been observed (Sarker and Oba, 2018); An et al., 2020). According to Table 5, there is a high positive correlation among phenols, antioxidants, anthocyanins, and vitamin C, aligning with findings from Zhang et al. (2023)and Grace et al. (2019).

Bag placement reduced antioxidant capacity, vitamin C, and total phenolic content. In most treatments, the fungus increased vitamin C content, as has been observed with mycorrhizal inoculation (Wahb-Allah et al., 2014).

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Conclusion

This study demonstrates the significant impact of drought stress on the physiological and biochemical traits of plants, particularly in relation to leaf area, chlorophyll content, and antioxidant compounds. Results indicate that bag placement and root inoculation with *Trichoderma* and mycorrhizal fungi help mitigate drought stress by improving nutrient uptake, enhancing chlorophyll content, and boosting antioxidant activity. Bag placement reduced drought severity, particularly at moderate irrigation levels, by enhancing nutrient availability and water absorption. The use of beneficial fungi increased vitamin C and phenolic content, helping plants combat oxidative stress.

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