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Influence of drought stress on growth and mineral uptake of GF677 (peach and almond hybrid) rootstock under *in vitro* conditions

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ABSTRACT

The effect of drought stress induced by polyethylene glycol (PEG-6000) on the growth and mineral uptake of GF677 (peach and almond hybrid) rootstock was investigated *in vitro* using solid and liquid mediums. Plantlets of the GF677 rootstock were subcultured into the Murashige and Skoog (MS) proliferation medium containing 1 mg/l BA (6-Benzyladenine) and 0.1 mg/l NAA (naphthaline acetic acid) under four different drought stress conditions: 0 (control), 1, 2, and 3 percent polyethylene glycol. After six weeks, results indicated that the highest drought level reduced fresh weight, dry weight, shoot length, and proliferation rate, with the reduction being greater in the solid medium than in the liquid medium. Leaf abscission was greater in the solid medium than in the liquid medium. In the liquid medium, the GF677 rootstock absorbed more nitrogen (N) than in the solid medium. Drought stress had no effect on phosphorus (P) uptake. Potassium (K) uptake increased when drought levels were evaluated in both mediums, but was greater in the liquid medium than in the solid medium. Calcium (Ca), magnesium (Mg), and iron (Fe) uptake decreased in both mediums as drought levels increased. The GF677 rootstock was capable of uptake of N and K at a high concentration. Mineral uptake was greater in a liquid medium than in a solid medium. In conclusion, the GF677 rootstock exhibited a high capacity for N and K uptake under drought stress.

Highlights

- *In vitro* drought stress induced by PEG on peach and almond hybrid rootstock growth and mineral uptake was studied using solid and liquid media.
- In the Murashige and Skoog proliferation medium with 1 mg BA and 0.1 mg NAA, rootstock plantlets were subcultured under four different drought stress conditions: 0, 1, 2, and 3 percent PEG.
- The results showed that the highest level of drought reduced fresh weight, dry weight, shoot length, and proliferation rate.
- Potassium uptake increased with drought levels in both liquid and solid media, but more so in the liquid.
- Finally, the GF677 rootstock had high N and K uptake capacity under drought stress.

1. Introduction

Drought stress is one of the most important problems in fruit tree production in arid and semi-arid areas, which reduces the growth and yield of fruit trees. Therefore, evaluation of rootstocks of fruit trees to stresses and their ability to grow in these conditions is very important to identify resistant and tolerant rootstocks (Kozlowski, 2002; Yadav et al., 2021).

GF677 is a hybrid of peach (*Prunus persica* L.) and almond (*Prunus amygdalus* Batsch), which is widely used in the world. One of the advantages of this drought - resistance rootstock is calcareous soils, high soil moisture, and iron deficiency (Antonopoulou et al., 2005).

More than 80% of plant tissue is water, and its deficiency quickly reveals severe side effects in plants. This is the most important factor limiting the growth and development of plants and reduces plant height due to drought stress (Zhang et al., 2006). Under drought stress conditions, the water potential of the plant is more negative than in normal conditions, and water uptake by the plant is difficult (Anjum et al., 2011). Plants in

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response to drought stress show responses that vary depending on the intensity of stress, duration of stress, genotype, age, and developmental stage of the plant at the time of stress (Chartzoulakis et al., 2002). Therefore, different plant species show a wide range of drought resistance mechanisms that lead to morphological, physiological, and biochemical adaptations (Anjum et al., 2011).

Polyethylene glycol (PEG) is a flexible, non-toxic polymer that can cause negative osmotic pressure. It also has no tendency to react with chemicals and biology, and this property has made polyethylene glycol one of the most useful molecules for generating negative osmotic pressure in scientific experiments (Sivritepe et al., 2008). This substance is not absorbed by the plant, and its concentration remains constant throughout the stress period. Therefore, it is known as the best treatment for osmotic stresses compared to other osmolytes, such as mannitol, salt, etc. (Georgieva et al., 2004). The difference between liquid and solid mediums is that in the solid medium, a jelly-like substance such as agar is used, but in the liquid medium it lacks it. Agar as a gel-producing agent in culture mediums is one of the factors reducing the water potential of the culture medium and inducing drought stress (Al-Khayri and Al-Bahrany, 2004).

The use of *in vitro* culture techniques to select plant species that tolerate salinity and drought is very common because it is possible to have more control compared to field conditions (Habibi and Amiri, 2013). Under field conditions, plants are exposed to variable climatic conditions that can affect research results. Plant tissue culture techniques can overcome these limitations and allow the plant to grow under controlled nutritional and climatic conditions, and it is possible to perform experiments under the same conditions throughout the year (Habibi and Amiri, 2013). Also, evaluating the salinity tolerance of plants in field and greenhouse conditions is expensive and requires a lot of time. For example, in a study, increasing drought stress levels induced by PEG-8000 reduced fresh callus weight, relative growth rate, and water content in two date palm genotypes (Al-Khayri and Al-Bahrany, 2004). The enzymatic and antioxidant responses of two banana genotypes were investigated under drought stress induced by PEG-6000. With the increasing drought, the activities of catalase, glutamine, ascorbate peroxidase, and superoxidase superoxide increased, as well as lipid peroxidation (Chai et al., 2005). In a study, the *in vitro* response of Gisela5 sweet cherry rootstock to different concentrations of PEG-8000 showed that shoot dry weight, shoot length, water content, and leaf chlorophyll content of explants decreased with increasing drought levels. Malondialdehyde (MDA) and antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, peroxidase, and catalase increased significantly with increasing drought stress. In addition, the concentrations of potassium (K), calcium (Ca), iron (Fe) and manganese (Mn) in the explant tissue also decreased (Sivritepe et al., 2008).

The GF677 rootstock is widely used around the world as a drought-resistant rootstock for stone fruits. However, no definite report has been found about the mineral uptake potential of this rootstock under drought stress conditions. Therefore, the purpose of this study was to investigate the growth responses of the GF677 rootstock and to evaluate the potential of mineral uptake under drought stress *in vitro* and compare it in solid and liquid mediums.

2. Materials and methods

One-year-old buds were used as plant material in this experiment to prepare explants. After disinfection, plant samples were transferred to Moraschig and Skock (MS) medium with 0.5 mg/l BAP. After budding, they were transplanted to MS basal medium. Baseline compounds included macro elements, microelements, vitamins, and iron, 30 g/l sucrose, 1 mg/l benzyl adenine (BA), and 0.1 mg/l naphthalene acetic acid (NAA).

Drought stress was performed in solid and liquid mediums. To prepare the drought treatment mediums, after calculating the required amount of PEG-6000 (0, 1, 2, and 3%) for each treatment level, add it to the prepared MS medium and mix well, and then label the containers. The pH was measured. The pH of the culture medium was adjusted from 5.7 to 5.8. Then 7 g/l of agar was added to the culture medium and completely dissolved by stirring and continuous heating on the heater. The medium was then distributed in culture dishes and autoclaved for 15 minutes at 121 ° C and 15 pounds per square inch. To apply drought stress in liquid conditions, the steps for preparing the liquid culture medium were the same as for preparing the solid culture medium; the difference is that agar was not added after adjusting the pH. Also, a cellulose filter (sorbol) was used to establish and prevent the explants from drowning in the liquid medium, and the explants were placed in the middle of the filter. Three uniform explants (approximately 1.5 cm) were planted in each culture dish. The storage conditions of all cultures (establishment stage, filling stage, and salinity treatment) in the growth chamber were at 25±2 °C with 16 hours of light and a light intensity of 30002500 lux.

This study was conducted in a completely randomized design (CRD) in the tissue culture laboratory of the Department of Horticultural Sciences, Zanjan University. Six weeks after drought stress, growth parameters (fresh weight, dry weight, shoot height, proliferation rate, number of new leaves, leaf abscission) and mineral concentrations in GF677 explants were measured. At the end of the stress period (sixth week), the shoot length of explants was measured by a ruler (with an accuracy of 0.1) in centimeters. To measure fresh weight, explants were removed from the culture dishes and washed with distilled water (to remove MS medium from the bottom of the explants). Then, the fresh weight of explants was measured with a digital balance with an accuracy of 0.0001. After measuring the fresh weight, the explants were placed in a paper bag and placed in an oven at 65 ° C for 48 hours, after which time they were taken out of the oven and their dry weight was measured with a digital

balance with an accuracy of 0.0001. The proliferation rate was obtained by counting shoots produced in the sixth week. Each replication was counted to determine the number of new leaves produced in each treatment (Ghaleb et al., 2010).

Dried explants were used in the oven to measure mineral concentration. First, all dried explants of each treatment were completely pulverized with the help of a laboratory mill and passed through a 0.5 mm sieve, and then 0.3 g of the powdered sample was extracted by the wet ashing method with sulfuric-salicylic acid and oxygenated water. After extraction, total nitrogen (N) by the Kjeldahl method, phosphorus (P) by a calorimetric method by a spectrophotometer (Cecil.Series 2, England), potassium (K) by flowmeter (Jenway PFP7, England), calcium (Ca), magnesium (Mg), and iron (Fe) were measured by atomic absorption spectrometry (Varian-Specter AA 20, Australia) (Emami, 1996).

The obtained data were analyzed using MSTAT-C statistical software, and the means were compared at a 5% probability level using the Duncan multiple range test.

3. Results and discussion

Increasing the drought level of solid and liquid mediums caused more weight loss in GF677 explants. According to Table 1, the highest fresh weight loss was achieved at the 3% dry level with 450 mg. The highest fresh weight, with 1013 mg, was observed in the control treatment. In the liquid medium, the highest fresh weight loss was achieved at the dry level of 3% with 600 mg. The highest fresh weight, at 1147 mg, was observed in the control treatment. In the liquid medium, no significant difference was observed between levels of 2 and 3% PEG-6000 (Table 2). In the solid medium, fresh weight at different levels of drought than liquid medium was more affected by different levels of PEG-6000.

Table 1. Influence of drought levels on growth parameters of GF677 rootstock after six weeks on solid MS basal medium

Drought levels (%)	Fresh weight (mg)	Dry weight (mg)	Shoot length (cm)	Proliferation rate (shoot/month)	Leaf number (leaf/explant)	Leaf abscission (Number)
0	1013 a	91.67 a	5 a	5 a	14 a	0.7 c
1	830 b	82.67 b	4.16 b	3.33 ab	13 ab	3.04 b
2	627.7 c	65 c	3.5 c	2 bc	11 b	5.04 a
3	450 d	45.33 d	3.16 c	1 c	8 c	5.7 a

Means in each column with similar letters are not significantly different.

Table 2. Influence of drought levels on growth parameters of GF677 plantlets after six weeks on liquid MS basal medium

Drought levels (%)	Fresh weight (mg)	Dry weight (mg)	Shoot length (cm)	Proliferation rate (shoot/month)	Leaf number (leaf/explant)	Leaf abscission (Number)
0	1147 a	98.33 a	5.33 a	4.66 a	13.67 a	0.7 c
1	983.3 a	88.33 a	5.16 a	4 a	12 ab	1.37 b
2	763.3 b	68.33 b	4.16 b	2.33 b	11 bc	1.7 b
3	600 b	56.67 b	3.5 c	1.66 b	10 c	2.7 a

Means in each column with similar letters are not significantly different.

The dry weight of GF677 explants decreased with increasing drought levels in both mediums. In the solid medium, the highest dry weight loss was achieved at 3% dry surface with 45.33 mg. The highest dry weight, at 91.67 mg, was observed in the control treatment (Table 1). In the liquid medium, the highest dry weight loss was achieved at 3% dry surface, with 56.67 mg. The highest dry weight, with 98.33 mg, was observed in the control treatment (Table 2). Dry weight at different levels of drought in the solid medium was more reduced than in the liquid medium.

According to Table 1, in the solid medium, the highest shoot height was obtained in the control treatment at 5 cm. The lowest shoot height of 3.16 cm was observed in the 3% PEG-6000 treatment. There was no significant difference in shoot levels of 2 and 3% in terms of shoot height. In the liquid medium, the highest shoot height was obtained in the control treatment at 5.33 cm (Table 2). The lowest shoot height of 3.5 cm was observed in the 3% PEG-6000 treatment. There was no significant difference between shoot control and the 1% level in terms of shoot height (Table 2).

Table 2 shows that in the solid medium, the lowest proliferation rate with one shoot per month was obtained in the 3% PEG-6000 treatment. The highest proliferation rate, with 5 shoots per month, was observed in the control treatment. In liquid medium, the lowest proliferation rate of 1.66 shoots per month was obtained in the 3% PEG-6000 treatment (Table 2). The highest proliferation rate, with 4.66 shoots per month, was observed in the control treatment.

In solid mediums, the highest and lowest number of new leaves, with 14 and 8 leaves, were observed in the control treatment and 3% PEG-6000 treatment, respectively. There was no significant difference between the control treatment and 1% treatment and also between 1% and 2% treatments (Table 1). According to Table 2, in the liquid medium, the highest and lowest number of new leaves, with 13.67 and 10 leaves, were observed in the control treatment and 3% PEG-6000 treatment, respectively.

Leaf abscission increased with increasing drought levels in the solid medium. Table 1 shows that the lowest leaf abscission was in the control treatment. The highest leaf abscission was observed at drought levels of 2 and 3%, which did not differ significantly. In the liquid medium, the lowest leaf abscission was obtained in the control treatment. The highest leaf abscission was observed with a 2.7 number of leaves on the dry surface of 3% (Table 2). In the solid medium, leaf abscission was

more affected by different levels of PEG-6000 than in the liquid medium.

GF677 rootstock growth parameters in the solid medium were more affected by different drought levels than in the solid medium (Tables 3 and 4). This is because increasing PEG levels reduces the water availability to the plant by creating a negative osmotic pressure (especially osmotic stress) (Al-Khayri and Al-Bahrany, 2004). Under these conditions, the water potential of the plant's environment is more negative than under normal conditions, and water uptake by the plant is difficult. These results are also obtained under drought stress in date palm (Al-Khayri and Al-Bahrany, 2004), raspberries (Georgieva et al., 2004), banana genotypes (Chai et al., 2005) and Gisela5 cherry rootstock (Sivritepe et al., 2008). Therefore, growth reduction indicates the effect of osmotic stress induced by polyethylene glycol on GF677 growth parameters (Macar et al., 2009). The effect of osmotic stress, which occurs immediately after increasing the PEG concentration in the explant culture medium, significantly reduces growth (Shibli and Al-Juboory, 2002). Fresh weight and dry weight loss in plants under drought stress seem to be due to inhibition of cell development and growth due to the reduction of turgor pressure (Al-Khayri and Al-Bahrany, 2004). The researchers reported that under drought stress conditions, plant dry matter production also decreased due to reduced water availability.

Also, the growth response of the branches to water shortages is highly dependent on the plant genotype (Munns, 2002). Growth inhibition may play a role in adapting to drought stress. Also, when plants do not have enough access to water, the amount of growth inhibitors, including abscisic acid, in the plant increases. On the other hand, some researchers have reported a decrease in the amount of growth-promoting hormones, such as auxins, gibberellins, and cytokinins, in the plant due to a lack of water (Macar et al., 2009). On the other hand, a decrease in growth hormones and an increase in growth inhibitors could be the reasons for this decrease in growth (Xu et al., 2001). Drought stress in solid and liquid conditions reduced the vegetative growth of GF677 rootstock, but this rootstock was able to continue its growth and did not dry out, which indicates the resistance of this plant to drought stress and the appropriate internal mechanism of this plant to cope with drought stress (Figure 1 and Figure 2).

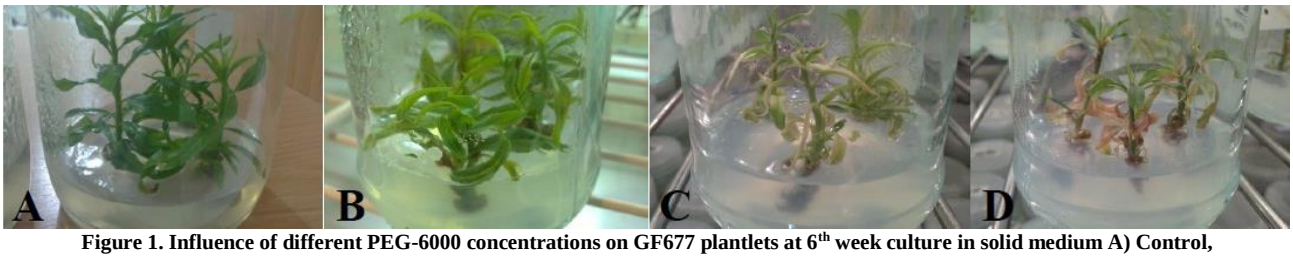

Figure 1. Influence of different PEG-6000 concentrations on GF677 plantlets at 6th week culture in solid medium A) Control, B) 1%, C) 2%, D) 3%.

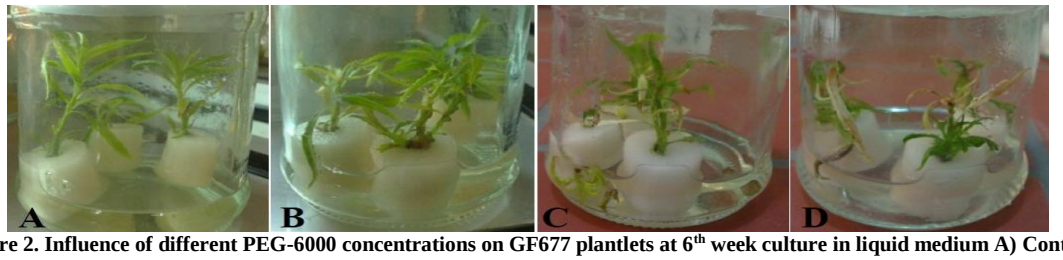

Figure 2. Influence of different PEG-6000 concentrations on GF677 plantlets at 6th week culture in liquid medium A) Control, B) 1%, C) 2%, D) 3%.

As can be seen in Table 3, in the solid medium, the highest N concentration was obtained in the control treatment with 4.73%. The lowest tissue N concentration, with 4.5%, is observed on the dry surface of 3%. There was no significant difference between control and 1% treatments, and also between 1% and 2% treatments. In the liquid medium, the highest N concentration was obtained in the control treatment, at 5.33%. The lowest tissue N concentration is observed at 1.5% on the dry surface of 3%. There was no significant difference between control and 1% treatments and between 2% and 3% treatments (Table 4). GF677 rootstock in liquid medium uptakes more N at different levels of drought than in solid medium.

According to Tables 3 and 4, it can be seen that drought stress had a small effect on P uptake, so that with increasing drought levels, P uptake decreased in both mediums, which was not significant.

In the solid medium, with increasing drought, tissue K concentration in GF677 rootstock increased as the highest concentration of K was observed in the treatment of 3% with 0.8%. There was no significant difference in K concentration between the control treatment and levels of 1 and 2% (Table 3). According to Table 4, with increasing drought, tissue K concentration in the GF677 rootstock increased as the highest concentration of K was observed in the treatment of 3% with 1.11%. There is no significant difference in K concentration between 1 and 2% levels. In

general, the GF677 rootstock was more capable of adsorbing K in the liquid medium than in the solid medium.

According to Table 3, the concentration of Ca in the GF677 rootstock tissue decreased with increasing dry levels in the solid medium. The lowest concentration of calcium on the dry surface was 3% with 0.47%. The highest tissue Ca concentration was in the control treatment, at 1.22%. In the liquid medium, the lowest concentration of Ca on the dry surface was 3% with a tolerance of 0.56%. The highest tissue Ca concentration was in the control treatment with a 1.48%.

At GF677 rootstock, the lowest Mg concentration in tissue was at a 3% dry level, at 641.2 ppm. The highest concentration of Mg was in the control treatment with 679.5 ppm. There was no significant difference in Mg uptake between levels 2 and 3% of PEG-6000 (Table 3). GF677 had the lowest Mg concentration in the tissue at a dry surface of 3% at 624 ppm. The highest concentration of Mg was in the control treatment, with 955 ppm.

According to Table 3, the highest concentration of Fe in the solid medium was obtained in the control treatment with 283.7 ppm, and the lowest concentration of Fe, with 280.9 ppm, was obtained at the dry level of 3%. In the liquid medium, the highest concentration of Fe was in the control treatment, with 300.7 ppm, and the lowest concentration of Fe, with 296.3 ppm, was obtained at the dry level of 3%. There was no significant difference in Fe concentration between 1 and 2% levels (Table 4). Fe uptake at different dry levels of PEG-6000 in the solid medium was more reduced than in the liquid medium.

Table 3. Influence of different PEG-6000 concentrations on mineral uptake of GF677 plantlets after six weeks in solid MS basal culture medium

Drought levels (%)	Nitrogen (N) (%)	Phosphorous (P) (%)	Potassium (K) (%)	Calcium (Ca) (%)	Magnesium (Mg) (ppm)	Iron (Fe) (ppm)
0	4.73 a	0.67 a	0.72 b	1.22 a	679.5 a	283.7 a
1	4.68 ab	0.66 a	0.75 ab	1.03 b	652.3 b	282.5 ab
2	4.6 b	0.64 a	0.78 ab	0.7 c	645 c	281.6 bc
3	4.5 c	0.63 a	0.8 a	0.47 d	641.2 c	280.9 c

Means in each column with similar letters are not significantly different.

Table 4. Influence of different PEG-6000 concentrations on mineral uptake of GF677 plantlets after six weeks in liquid MS basal culture medium

Drought levels (%)	Nitrogen (N) (%)	Phosphorous (P) (%)	Potassium (K) (%)	Calcium (Ca) (%)	Magnesium (Mg) (ppm)	Iron (Fe) (ppm)
0	5.33 a†	0.68 a	0.81 c	1.48 a	955 a	300.7 a
1	5.25 a	0.58 a	0.88 bc	1.2 b	665 b	298.7 ab
2	5.16 b	0.69 a	0.96 b	0.83 c	655.7 c	298 b
3	5.1 b	0.69 a	1.11 a	0.56 d	642.7 d	296.3 b

Means in each column with similar letters are not significantly different.

Drought stress, in addition to the negative effects on growth, causes a lack of nutrients in the plant, and one of the most harmful effects of drought stress is a disorder in the process of nutrient uptake, which reduces growth and yield (Hu and Schmidhalter, 2005). Because water is the most important molecule in the plant growth medium, any factor that reduces water uptake reduces the uptake and availability of elements by the plant, and the uptake of elements in these conditions mainly depends on water availability (Amiri and Arzani, 2006). In this experiment, drought stress induced by PEG also affected the uptake of high-consumption and low-consumption basic elements. Nitrogen, Ca, Mg, and Fe uptake decreased significantly with increasing dry levels. While K consumption has increased. The potential of elements' uptake in a liquid medium was higher than in a solid medium. No change in phosphorus uptake was observed in solid or liquid mediums (Tables 3 and 4).

Reduction of mineral uptake has also been reported in an olive (Brito et al., 2003), an apple (Molassiotis et al., 2006), and a Gisela5 cherry rootstock (Sivritepe et al., 2008) under in vitro drought stress. The mechanisms of mineral uptake and transport in plants are more or less a function of the amount of water availability in the medium, and in the case of a lack of moisture, the intensity and amount of mineral uptake are affected (Sivritepe et al., 2008). The availability of an element for the plant is also affected by other available elements, as well as available water (Molassiotis et al., 2006). The K uptake increased in both mediums with increasing dry levels. Reports from various researchers also confirm that the uptake of K increases during drought stress, and its reason is the mechanism of uptake of this ion (Hu and Schmidhalter, 2005). During drought stress, in order to increase drought resistance, it increases K uptake by consuming energy. Increasing K uptake has a positive effect on ATP and NADP production, more protein synthesis, and the most important issue during drought stress is increasing plant water uptake (Cakmak, 2005). If drought stress is associated with K deficiency, these damages will be more severe because K induces dehydration tolerance in plants, and this element is mainly considered as an important osmotic regulator in plants (Hu and Schmidhalter, 2005). Under drought stress, the transfer of K ions ultimately prevents damage to plant cells. If the drought stress is severe, then complete plasmolysis is performed and the contents of the cells leak out, resulting in the death of the cells (Hu and Schmidhalter, 2005). There are some reports that the plant needs K ion. The K accumulation in plant leaves during long periods of drought indicates the role of this ion in regulating stomatal function and increasing the activity of antioxidant enzymes in leaves (Cakm 2005).

In this experiment, GF677 was the capable rootstock for K uptake under drought stress conditions, and the results of other studies have shown that increasing the K concentration under drought stress conditions reduces the drought effects (Hu and Schmidhalter, 2005)

4. Conclusion

Vegetative growth of GF677 rootstock was reduced in both culture mediums under drought stress, but this rootstock was able to continue the growth, which indicates the appropriate internal mechanism of this plant to cope with drought stress. GF677 was a capable rootstock for N and K uptake and was able to uptake high concentrations of these elements. Also, by comparing drought stress in solid and liquid mediums, it can be concluded that the exerting of drought stress by polyethylene glycol in liquid medium is more accurate than in solid medium because the liquid culture system provides a more uniform medium for growth and mineral uptake. In the solid medium, agar induces more drought stress in the culture medium because it reduces the water potential, which affects plant growth. Therefore, it is recommended to use a liquid medium with drought stress to study the drought tolerance of plants.

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