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# Growth and Osmotic Adjustment in Two Almond Rootstocks under Water Stress Conditions

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**Abstract**

A greenhouse experiment was conducted in 2010 to evaluate the response of seedlings and cuttings of GF677 hybrid (*Prunus persica* and *P. amygdalus*) rootstock and bitter almond seedlings (*P. dulcis*) to water stress. After determination of field capacity (FC) of the soil used for potting, irrigation treatments were imposed by daily irrigation to FC and 2, 4 or 8 day intervals for 96 days. Delaying irrigation had no significant effect on shoot growth and diameter, but decreased leaf production. Root number and length and root to shoot ratio in GF677 seedlings and cuttings increased with increasing frequency of irrigation. In GF677 seedlings and cuttings, delaying irrigation from 0 to 8 days increased leaf sugar, potassium and proline concentrations significantly, and potassium had a significant role in reducing osmotic potential. GF677 seedlings and cuttings had a greater ability to withstand water stress than bitter almond seedlings. It was concluded that conventional bitter almond rootstocks currently considered most tolerant to drought can be replaced by cuttings of GF677 rootstock.

Water shortage is common in the almond growing areas of southern Iran. Fruit trees express various responses to drought stress and develop a wide range of mechanisms to enable them to retain their metabolic activity under low water potential conditions (30). Osmotic adjustment is one of the crucial processes involved in plant adaptation to drought and allows plant to tolerate temporary or prolonged periods of water shortage (6). Higher solute concentration causes lower tissue osmotic potentials, maintains turgor potential, and improves tolerance to low tissue water potentials. Low osmotic potential and the capacity for osmotic adjustment may also serve as useful criteria for selection and breeding of more drought-resistant species and cultivars (33). Plants subjected to water deficits may accumulate or synthesize sugars (35), amino acids such as proline (26, 36), sodium and potassium ions (10) and organic acids (32). Osmotic adjustment through accumulation of compatible solutes has been reported in many fruit and nut trees such as cherry (20), apple (35), jujube (7), grape (17), peach (23), almond (13) and citrange (18). Delaying irrigation sharply increased the proline concentration in almond leaves (36). Accumulation of soluble sugars in almond cultivars reduced the osmotic potential of the leaves (13)

The use of bitter almond seedlings as a rootstock has traditionally been practiced for almond orchard establishment in Iran. Many growers believe that these rootstocks are most tolerant to drought and soil pathogens. The origin of the majority of them is, however, not known and this causes severe orchard problems due to heterogeneity amongst the trees. The GF677 rootstock has recently been introduced to Iranian growers. If this rootstock performs satisfactorily in the arid and semiarid climate of Iran, it can replace the use of bitter almond seedlings as rootstocks.

The difficulty in rooting this hybrid rootstock has stimulated nurserymen to produce seedlings of GF677. Though drought resistance of vegetative GF677 rootstock has been reported by several researchers such as Alarcon et al. (1) and De Salvador (8), there are no reports of a comparison of drought

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resistance between cuttings and seedlings of GF677. Further, the contribution of various osmotica to osmotic adjustment in the leaves of these plants remains poorly understood.

The objectives of this study were to compare variations in osmotic adjustment in response to water stress 1) between GF677 seedlings and cuttings, and bitter almond seedlings, and 2) to determine the primary solutes that contribute to osmotic potential and osmotic adjustment in these plants. Another objective was to compare the drought resistance of cuttings and of self-pollinated seedlings, because of the difficulty to achieve rooting in cuttings.

## Materials and Method

Two replicate experiments with potted trees were conducted in two consecutive years. Cuttings from 1-year-old shoots and seeds of mature self-pollinated GF677 (*P. persica* × *P. amygdalus*) trees were collected in October 2009 from the Estahban Fig Research Station and seeds of bitter almond (*P. dulcis*) were obtained from the Zarghan Experiment and Research Station of Agriculture in Fars Province, Iran. The hard endocarps of seeds were removed, and the kernels were soaked in water for 48 h, mixed with sand: vermiculite (2:1 v/v) and stratified for 4 weeks at 4°C. The bases of the cuttings were treated with indole-3-butyric acid (3000 ppm in 50 % ethanol) and planted in sand. The stratified kernels and uniform rooted cuttings were subsequently planted in 20-L pots filled with a mixture of leaf mould, sand, and soil (1:1:1, v/v/v). The field capacity (FC) of the soil used for potting was determined according to the protocol described by Richards (22).

One hundred days after sowing the kernels and "sticking" the cuttings, the pots with uniform plants were divided into four groups of eight pots each. The first group was irrigated every day to field capacity (FC). The second, third and fourth groups were irrigated to FC every 2, 4 or 8 days, respectively (at about 25%, 50% or 75% of FC, respectively). The experiment was carried out for 96 days. The maximum and minimum temperatures during the experiment period were 16 and 36°C, respectively, and mean relative humidity was 64%. The use of pots with a relatively large volume minimised any effects of limited soil volume, which can be a problem in drought stress studies. Throughout the course of the experiment no root emergence from pots into the surrounding soil was observed. Stem water potential, the relative water content of plants, and soil moisture content during the experiment have been reported previously (10). The experiment reported in this paper was carried out in 2010 but similar results were obtained in 2008 (data not shown).

Shoot length and leaf number were recorded at the beginning and at the end of the experiment. Plants were harvested at the end of experiment and their leaves, stems and roots were separated. Shoot diameter was measured by digital caliper. Root length and number were determined by Delta-T-SCAN image analysis (Windows Microsoft).

Free proline was extracted from 0.5 g samples of fully expanded and young leaves with 3% sulfusalicylic acid, and estimated by using ninhydrin reagent, according to the protocol described by Bates et al. (3). The absorbance of the fraction with toluene was determined at 520 nm, using a spectrophotometer (Model UV-120-20, Japan).

To determine soluble carbohydrate concentration, 150 mg of dried leaf samples was extracted twice with ethanol. The sample was centrifuged at 3500 rpm for 10 min. and the volume of the supernatant was adjusted to 25 mL. Soluble carbohydrate concentration was measured according to the method of Dubios et al. (9) as modified by Buysee and Mercks (5). In summary, 1 mL of supernatant was transferred to a test-tube and 1 mL of 18% phenol and 5 mL sulfuric acid were added. The mixture was shaken immediately and its absorption was recorded at 490 nm using a spectrophotometer (Model UV-120-20, Japan).

Starch concentration in the leaf samples was measured using anthrone reagent (14). In this method, 5 mL of cold water and 6.5 mL perchloric acid (52%) were added to the residual material used for sugar analysis and mixed for 15 min. About 20 mL of water was then added and the sample was centrifuged. The supernatant was separated and the same procedure was repeated with the precipitate. The supernatants were combined and left for 30 min at 0°C. After filtration, the volumes of supernatants were adjusted to 100 mL. About 2.5 ml of cold anthrone solution (2%) was added, and the sample was heated at 100°C for 7.5 min, then transferred immediately to an ice bath and cooled to room temperature. Absorption at 630 nm was recorded using a spectrophotometer (Model UV-120-20, Japan).

For mineral analysis, a 1 g sample of leaf tissue was ashed in a muffle furnace at 550°C for 5 h. The ash was then dissolved in 10 mL 2N HCl and the volume was adjusted to 100 mL with distilled water. Potassium and sodium concentrations were determined using flame photometry (Model PFP7, Jenway, England).

The contributions of individual solutes to osmotic potential were calculated based on the relative dry weight (RDW) at saturation determined for each sample and by using the van't Hoff relation as given by Nobel (16):

$$\Psi\pi \text{ (MPa)} = \text{RDW} \times \text{C (mg kg}^{-1}\text{ DW)} \times 1/\text{MW (g mol)} \times 0.002437 \text{ (m}^3\text{ MPa mol}^{-1}\text{)}$$

where RDW is equivalent by [dry weight/ (saturated weight – dry weight)], C is the solute concentration and MW is the molecular weight of a given solute. $\Psi\pi$ is calculated for 20°C.

Experimental units were arranged in a completely randomized design with four treatments and eight pots per treatment. The measurements were done on four pots and one plant in each pot (four replications). Analysis of variance was performed using the SPSS software package. Difference among the mean values was compared by least significant difference (LSD) test at $P$ = 0.05.

## Results

Relative shoot length (%) decreased as irrigation was delayed (Fig. 1A) but the differences among treatments (2, 4 or 8-day intervals) were not significant. Plants from GF677 and bitter almond seedlings had greater shoot growth than those of GF677 cuttings. Shoot diameter was not affected by delayed irrigation (Fig. 1B). In GF677 and bitter almond seedlings, leaf numbers under

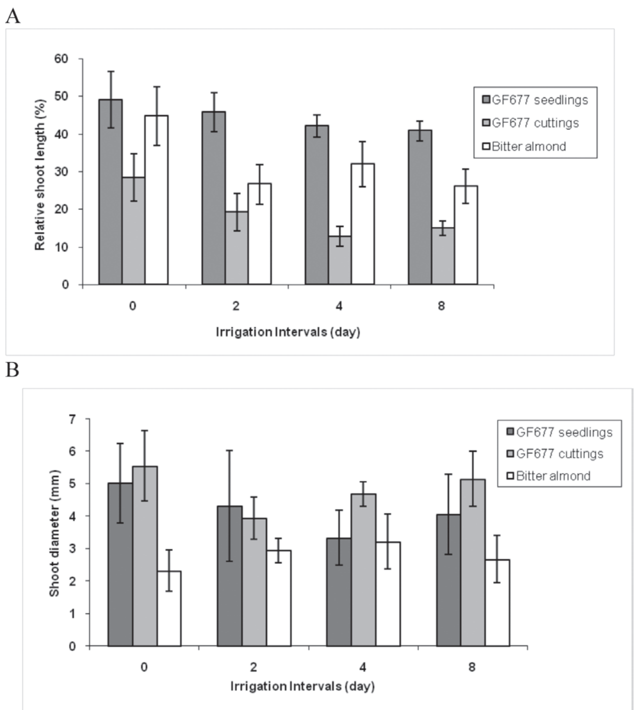


**Fig. 1.** Effects of various irrigation regimes on relative shoot length (A) and shoot diameter (B). Vertical bars are standard deviation (SD) of means.

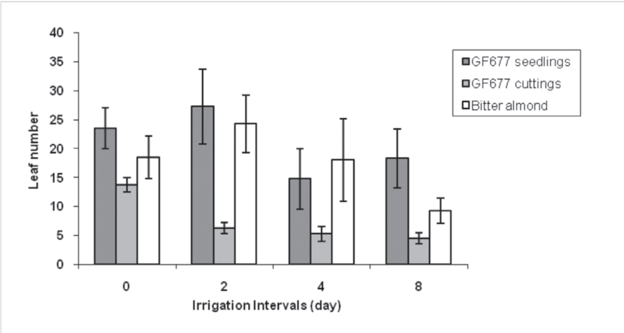


**Fig. 2.** Effect of various irrigation regimes on leaf number. Vertical bars are standard deviation (SD) of means.

2-day irrigation intervals were higher than those of other irrigation intervals. Cuttings of GF677 produced fewer leaves than did seedlings of either GF677 or bitter almond (Fig. 2).

Root length and number were enhanced by delaying irrigation. Root length was greater in GF677 cuttings than in either GF677 seedlings or bitter almond rootstocks, but the differences were significant only for the 4-day intervals (Fig. 3A). Rootstocks irrigated at 2, 4- or 8-day intervals had more roots than did those irrigated daily (Fig. 3B). Root:shoot ratio in GF677 cuttings and bitter almond seedlings increased with increasing water stress, but this effect was not significant in GF677 seedlings. GF677 cuttings had higher root:shoot ratios than the other rootstocks (Fig. 4).

Leaf proline concentration in GF677 seedlings and cuttings increased significantly as irrigation was delayed (Fig. 5). Proline concentration in GF677 sseedlings, cuttings and bitter almond seedlings increased from 0.89, 0.52 and 0.72 mg $g^{-1}$ DW, respectively, in non-stressed plants to 5.98, 2.25 and 1.35 mg $g^{-1}$ DW respectively in the leaves of rootstocks irrigated at 8-day intervals.

When irrigation intervals increased from 0 to 4 and 8 days the concentration of soluble carbohydrates in the leaves of GF677 seedlings increased from 211.6 µg $g^{-1}$ DW to 264.4 and 249.6 µg $g^{-1}$ DW, respectively, and in cuttings from 190.8 µg $g^{-1}$ DW in the control treatment to 219.3 µg $g^{-1}$ DW

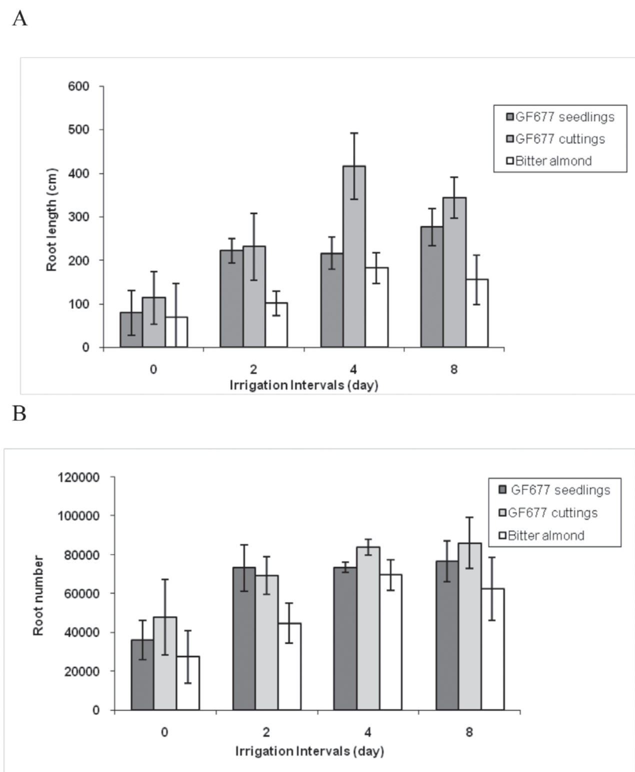


**Fig. 3.** Effects of various irrigation regimes on root length (A) and number of roots (B). Vertical bars are standard deviation (SD) of means.

and 230.98 μg $g^{-1}$ DW under 4 and 8 day irrigation intervals, respectively (Fig. 6). For bitter almond seedlings the differences in soluble carbohydrate concentration between treatments were not significant but the soluble carbohydrates were effective in decreasing osmotic potential (Fig. 6, Table 1). In contrast to the soluble carbohydrate concentration, the starch concentration in the leaves of GF677 cuttings decreased significantly with increasing irrigation intervals. Delaying irrigation had no significant effect on starch concentration in the leaves of seedlings (Fig. 7).

Potassium concentration in the leaves of GF677 seedlings under 2-day irrigation intervals were lower than those in the control seedlings, but increased when irrigation was delayed by 4 or 8 days (Fig. 8). When irrigation was delayed in GF677 cuttings and bitter almond seedlings, potassium concentrations in the leaves increased by 1.4 and 8.5 percent, respectively. However, delaying irrigation did not affect the sodium concentrations in the leaves of GF677 seedlings or cuttings (Fig. 9).

The results of this study showed that both potassium and soluble carbohydrates had signif-

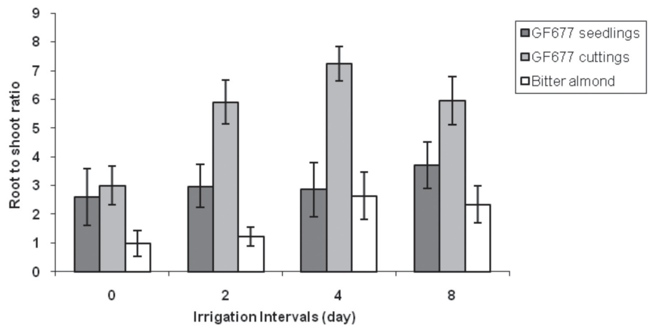


**Fig. 4.** Effect of various irrigation regimes on root to shoot ratio. Vertical bars are standard deviation (SD) of means.

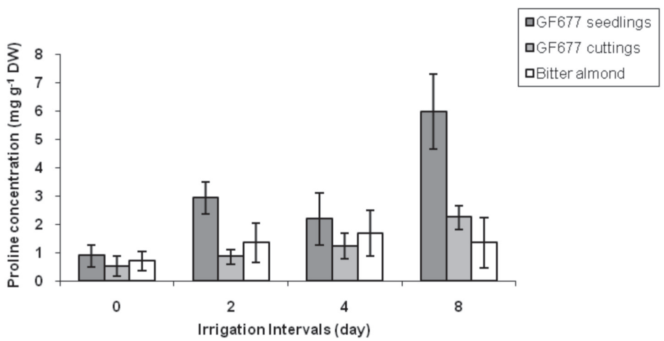


**Fig. 5.** Effect of various irrigation regimes on proline concentration. Vertical bars are standard deviation (SD) of means.

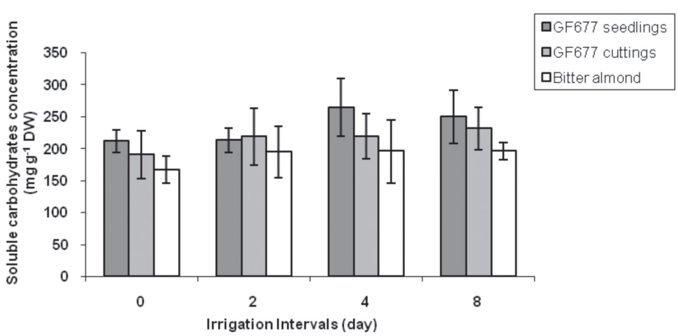


**Fig. 6.** Effect of various irrigation regimes on soluble carbohydrate concentration. Vertical bars are standard deviation (SD) of means.

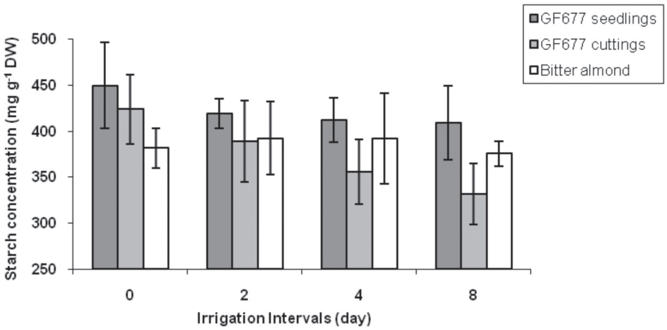


**Fig. 7.** Effect of various irrigation regimes on starch concentration. Vertical bars are standard deviation (SD) of means.

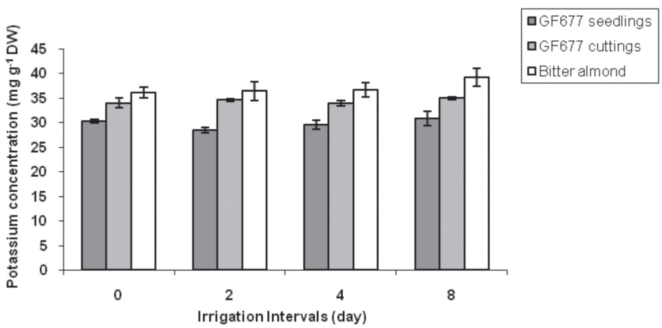


**Fig. 8.** Effect of various irrigation regimes on potassium concentration. Vertical bars are standard deviation (SD) of means.

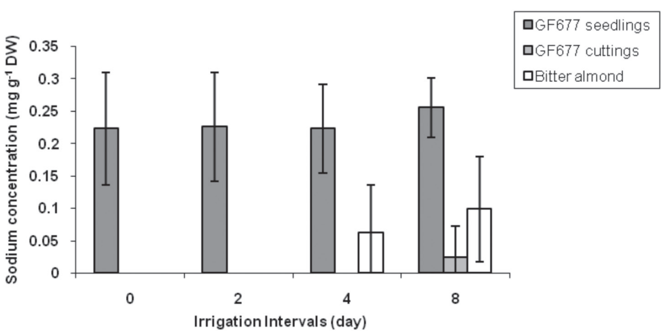


**Fig. 9.** Effect of various irrigation regimes on sodium concentration. Vertical bars are standard deviation (SD) of means.

icant effects in reducing the osmotic potential of leaves from the three rootstocks, but that sodium did not (Table 1).

## Discussion

The high and similar values of stem water potential and relative water content in the control plants of the seedlings and cuttings during the experimental period indicated that plants were adequately irrigated. We noted, however, that stem water potential and relative water content decreased with substrate moisture content.

Cell growth is the most sensitive response to water stress (25). Since cell expansion requires turgor pressure, decrease in turgor because of stress causes a decline in cell expansion (28). In present study, in spite of relative decreases in shoot length and diameter by increasing irrigation intervals, the differences were not significant. It seems that the decrease in turgor pressure was not enough to markedly inhibit cell expansion. Tan and Buttery (29) hypothesized that reductions of seedling height and diameter in peach under water stress were due to the effect of drought on

**Table 1**. The effect of different soluble solutes on decreasing osmotic potential

| | Concentration (mmol $kg^{-1}$ DW) | | Contribution to $\Psi\pi$ (-MPa) | |
|---|---|---|---|---|
| Solute | Control | 8 day interval | Control | 8 day interval |
| | | GF677 seedlings | | |
| Sodium | 9.7 | 11.09 | $7.1 \times 10^{-3}$ | $9.73 \times 10^{-3}$ |
| Potassium | 776.1 | 776.09 | 0.56 | 0.68** |
| Soluble carbohydrates | 202.8 | 239.2 | 0.15 | 0.21* |
| Proline | 7.76 | 51.93* | $5.6 \times 10^{-3}$ | $4.5 \times 10^{-2}$** |
| | | GF677 cuttings | | |
| Sodium | 0 | 1.05 | 0 | $9.7 \times 10^{-4}$ |
| Potassium | 883.6 | 895.1* | 0.75 | 0.83* |
| Soluble carbohydrates | 182.9 | 221.4* | 0.16 | 0.21** |
| Proline | 4.55 | 19.5* | $3.6 \times 10^{-3}$ | $1.8 \times 10^{-2}$** |
| | | Bitter Almond | | |
| Sodium | 0 | 4.29* | 0 | $3.7 \times 10^{-3}$ |
| Potassium | 926.4 | 1004.8* | 0.79 | 0.88* |
| Soluble carbohydrates | 160.2 | 187.6 | 0.14 | 0.16* |
| Proline | 6.28 | 11.76 | $5.3 \times 10^{-3}$ | $1.03 \times 10^{-2}$ |

* and **: significantly different at P = 0.05 and 0.01, respectively (t test, n = 4)

leaf area reduction and a consequence of a smaller photosynthetic surface. Higher shoot growth and leaf number in the seedlings in our study are consistent with this response. The vegetative growth (vigor) in seedling rootstocks, in general, is typically higher than in clonal rootstocks (24).

In contrast with aerial parts, delaying irrigation has often been shown to significantly increase the growth of roots. Drought-tolerant clones of coffee with deep root systems are better able to maintain their leaf water status than are drought-sensitive clones (19). Also, a deeper root system can enhance water use efficiency under drought conditions (11). In this study, root: shoot ratio increased in response to increasing drought severity. This increase may have been due to an increase in root volume, reduced root death, reduction in the rate of leaf development, and/or increase in leaf abscission (2). All but the last factor occurred in this study. Increase in root: shoot ratio is an index of drought resistance under low soil water conditions and reflects a greater reduction of growth in shoots than in roots (4).

Proline contributes to solute accumulation, which increases tolerance to dehydration, presumably by protecting proteins and membrane structures (34). Its role as a scavenger of reactive oxygen species (ROS) under stress conditions has been recently reported (21, 31, 34). Proline accumulation is caused not only by blocking its degradation but also by the activation of proline biosynthesis (21).

Drought can increase sugar concentration in two ways: by reducing growth (12, 23), or by reducing activity of enzymes such as nitrate reductase and $\alpha$-amylase (25). Starch hydrolysis can increase soluble sugar concentration under drought conditions (7, 18). The increase of soluble sugars and decrease of starch in this study under drought conditions confirms previous observations. Soluble sugars may have a protective function, stabilizing proteins and membranes during dehydration (13).

Unlike soluble carbohydrates and amino acids, accumulation of ions such as potassium ($K^+$) and sodium ($Na^+$) are energetically an effective method for plants to adjust osmotic potential because carbon skeletons are not required (15). Our data show that $Na^+$ had almost no role in increasing solute concentration and thereby decreasing osmotic potential. However, $K^+$ concentration increased significantly with drought stress and had the greatest role in decreasing osmotic potential. The uptake and accumulation of large quantities of such ions from soil is, therefore, an advantageous and rootstocks with a low ability to absorb potassium limit the resistance to drought (and low temperatures) of scion cultivars (27). By using this mechanism of inorganic ion accumulation to adjust their osmotic potential, almond rootstocks appear to be able to grow in less favorable conditions.
These critical roles of both potassium and soluble carbohydrates have previously been reported for cherries under water stress conditions (20).

In conclusion, in all three rootstocks potassium and soluble carbohydrates were effective in decreasing osmotic potential. Bitter almond seedlings had lower osmotic adjustment ability under water stress conditions than either GF677 seedlings or cuttings. The osmotic adjustment ability of GF677 cuttings, and their higher root: shoot ratio, indicate that this rootstock might adjust better under drought stress conditions than self-pollinated GF677 seedlings. Nevertheless, these results affirm that GF677 rootstocks could replace bitter almond in the Iranian almond industry as a superior rootstock for use in drought-prone areas.

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