

Full Length Research Paper

Influence of soil drought stress on photosynthesis, carbohydrates and the nitrogen and phosphorus absorb in different section of leaves and stem of Fuji/M.9EML, a young apple seedling

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Drought is the most significant limiting factor for plant cultivation. Greenhouse-grown 1-year-old potted M.9EMLA apple trees (*Malus domestica* Borkh.) (after growing for 6 weeks) were subjected to drought stress by withholding water for an additional six-week period. The photosynthesis, carbohydrates and the nitrogen (N), phosphorus (P) absorb in the upper, middle and lower leaves of the seedlings were determined. The results revealed that drought stress obviously decreased the photosynthesis and carbohydrates contents in the leaves at all sections of shoot compared with the control. Sorbitol and glucose concentrations increased over time and was kept at a higher level during the drought stress period whereas sucrose concentration declined. N content in the leaves kept a flat trend and was higher than that of the control while P concentration decreased compared with the control. These results demonstrated that drought stress prevented N and P absorption and decreased RWC of apple leaves, which caused the decline of photosynthesis and transpiration. Thus, the direct phenotype was the slowed growth which was specifically expressed in decreased plant height, number of leaves, average leaf area and stem diameter.

Key words: *Malus domestica* Borkh, sorbitol, water deficit, transpiration.

INTRODUCTION

Drought is the most significant limiting factor for plant agriculture worldwide, which can cause serious losses of yields and productivity in most crop plants, including apple in arid and sub-arid regions. The degree of these effects depends on its impact on the plant physiological and biochemical, as well as molecular biological process and the ability of plant to adapt to drought stress (Bulbotko, 1973; Atkinson et al., 2000; Massonnet et al., 2007). The active accumulation of solutes within cells, named as osmotic adjustment, represented an aspect of the adaption ability. During drought, organic compounds

such as polyols, sugars, proline and glycine-betaine comprise the bulk of solutes for osmotic adjustment (Fernandez et al., 1997; Sircelj et al., 2007; Philip et al., 2003).

In the stressed apple, cherry and peach, carbon partitioning in the leaves and shoot was shown to be higher during drought stress. Ranney et al. (1991) found that after the Nemaguard peach was irrigated daily at rates of 100, 67 and 33% of evapotranspiration (ET) in greenhouse, the sorbitol accumulation in both mature leaves and shoot tips of the stressed plant started from the second week of treatment and reached up to 80% of total solutes in osmotic adjustment. It was also found that sucrose content was up to 8-fold lower than sorbitol content and accumulated only occasionally. Using potted 2-year-old Jonathan/M₇ apple tree, Wang and Stutte, (1992) concluded that sorbitol, glucose and fructose concentration increased while sucrose and starch levels decreased

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Abbreviations: ET, Evapotranspiration; Pn, photosynthesis; E, transpiration; RWC, relative water content; DPS, data processing system.

significantly as water stress developed, strongly suggesting that sugar alcohol and monosaccharide are the most important osmotic parameters for adjustment; and also, that sorbitol is a primary carbohydrate in the cell sap and accounted for >50% of total osmotic adjustment.

For fruit crops in particular, the accumulation of sorbitol has been documented in cherry and apple trees subjected to water deficit (Ranney et al., 1991; Wang and Stutte, 1992). Recently, this response has also been noted in peach (Bianco et al., 2000) and all of these studies indicate that sucrose plays a minor role in osmotic adjustment related to sorbitol. In apple Melrose on several rootstock, Ferree et al. (2001) found that soil drought increased magnesium (Mg) and manganese (Mn) content and reduced potassium (K) and boron (B) content, but had no effects on the levels of N and P while both photosynthesis (Pn) and transpiration (E) were higher under drought stress.

In general, little is known about the effects of soil drought stress on the carbohydrates and the N, P absorb in different section of leaves of apple plants. The aims of this study are to elucidate the effects of drought on shoot growth and leaf area, the levels of Pn, E, relative water content and carbohydrates, and N, P absorption in different section of leaves at the shoot of 1-year-old apple trees.

MATERIALS AND METHODS

Plant materials and experimental design

M.9EMLA root stock was selected as the plant material for this study because it is the most widely planted dwarfing apple root stock and more tolerant of soil moisture deficit (Fernandez et al., 1997) compared to the other rootstocks.

On March 8, 2000 (OARDC) and 2005 (BUA) respectively, Fuji apple trees M.9 EMLA with all roots pruned to 5 cm were planted in 5.4 L containers filled with a sandy silt loam soil. Trees were cut back and trimmed to a single unbranched shoot. The experiments were carried out during a six-week period on 20 uniform plants selected from more than 100 plants. Soil drought treatments were withheld from apple plants from April 22 to May 24. Prior to the beginning of the experiment, the pots were put into tubs (1.5 x 1 x 0.25 m) filled with water. Pots were left submerged for several hours until the soil volume was thoroughly saturated. A layer of fine gravel covered the top of the soil to retard evaporation of water. Day and night temperatures were about 21.6 and 10°C respectively, and plants were grown under natural sunlight and day length during the treatment period.

Two treatments were employed, as follows: T1(ck): Watered-plants were well watered weekly by immersion of the pots in tubs containing water for two hours on the day before sampling (the same as was done at the beginning of the treatment period). T2: drought-plants were drought stressed by providing no water from April 24 to May 22. The treatments were arranged as a 2 factorial (well watered versus drought stressed) in a randomized block design with 10 replicate trees (pots) for each treatment. On May 22, all pots were put into tubs filled with water to fully saturate the soil and initiate a recovery period for the drought stressed plants. Drought treatments were established in March. Soil was saturated with water just prior to the first sampling date (April 19) and just after May, 22 sampling date. The sampling plants were divided into three parts, the upper, middle and lower parts. The leaves that lied at the central part in each section of the samples were also tested.

Methods

Growth analysis

Leaf area was calculated using the formula:

$$\text{Leaf area} = 0.76 (\text{length} \times \text{width}) + 2.0$$

10 replicate plants were sampled.

Pn and E

The measurements were made weekly using a portable gas analyzer (Analytical Development Corp.LCA-2) with an air supply unit and a Parkinson leaf chamber; the leaf area is 6.25 cm². Once a week, on Sunday morning, at 9:00 O'clock, the measurement was taken. Only the leaves on the younger branches were selected; six leaves behind the newly expanded three were sampled.

Relative water content (RWC)

RWC was calculated as (fresh weight - dry weight)/ (turgid weight - dry weight) × 100. The leaves sampled were the same as Pn and E.

Carbohydrate analysis

Leaf tissue used for carbohydrate analysis was the remainder of the 10 leaves used for RWC analysis. The plastic vials containing leaf samples were immediately frozen at -80°C and then freeze dried (Model 24449, Virtis). Dried, ground tissue was simultaneously extracted and derivatized for analysis of carbohydrates by gas-liquid chromatography (WATERS, Amer.) using an automated injector as described by Streeter and Strimbu (1998). The Suger-Pak300 X -65 mm was used.

N and P content

The dried plant material was finely pulverized and analyzed for N and P. Total N was determined using the Dumas dry combustion method using an ANCA-SL Elemental Analyser coupled to a 20 - 20 Tracermass spectrometer (Europa Scientific Ltd., grewe,UK). The plant material was dry-ashed at 500°C for 3 h, solubilized in 3 M HCl, dried and solubilized again in 1 M HNO₃ before filtering. The concentration of P in the resulting solution was determined spectrophotometrically using a molybdophosphate blue method (Murphy and Riley, 1962).

Statistical analysis

The data processing system (DPS) system was used for analysis of variance and EXCEL was used for calculation of correlation coefficients.

RESULTS

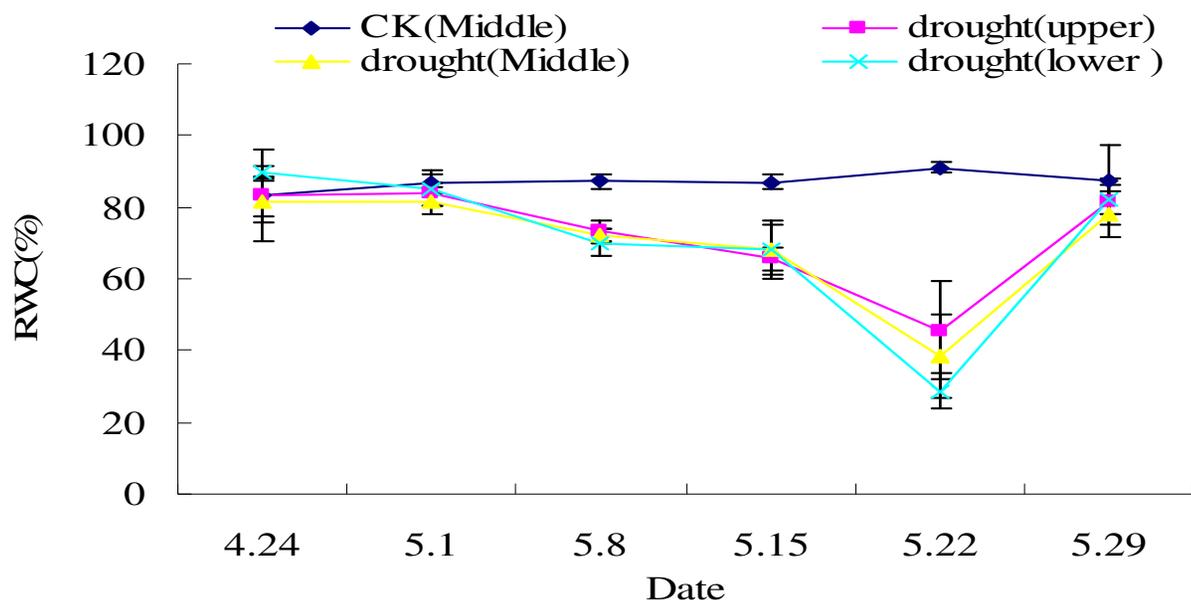
Plant growth

The drought treatment had a great effect on plant growth, as well as on plant height and number of leaves. Also, it

Table 1. Effects of soil drought on growth of apple trees.

Treatment	Plant height (cm)			Number of leaves			Average leaf area (cm ²)			Stem diameter (mm)
	Initial	Final	Change	Initial	Final	Change	Lower	Middle	Upper	
T1	70.6 ± 1.6	94.5 ± 2.0	23.9 ± 1.7	29	43	14	40.7 ± 1.0	60.1 ± 0.6	82.8 ± 3.8	8.8 ± 0.1
T2	59.8 ± 0.8	65.4 ± 1.2	5.6 ± 0.6	27	33	6	33.7 ± 1.2	34.3 ± 2.0	28.3 ± 2.1	8.2 ± 0.1

Initial, April 24 - May 8; final, May 9 –May 22; T1, watered, T2, drought.

**Figure 1.** Effect of soil drought on relative water content (RWC) of leaves in different parts of shoot on apple trees.

resulted in significantly smaller stem diameter and average leaf area at the end of the drought treatment period (Table 1). The average leaf area at all section of the shoot declined remarkably, especially at the middle and upper part of the shoot, which was only 1/2 to 1/3 of the control. This demonstrated that the soil drought severely inhibited the growth of apple seedlings.

Effects on RWC, Pn and E

Plants in all four treatments had similar RWC one week after the initiation of drought treatments (Figure 1). However, the trend toward lower RWC in stressed plants was apparent two weeks later. On May, 22, RWC in drought-stressed plants was significantly lower than that of control. The RWC

of leaves at different sections of the shoot kept an equal level, but decreased to the minimum at the end of drought. After a recovery period, RWC ascended to the level of CK. Photosynthetic activity and transpiration was obviously lowered in drought-stressed plants for essentially all of the sampling dates. Thus, drought had the most significant impacts on these parameters of plant

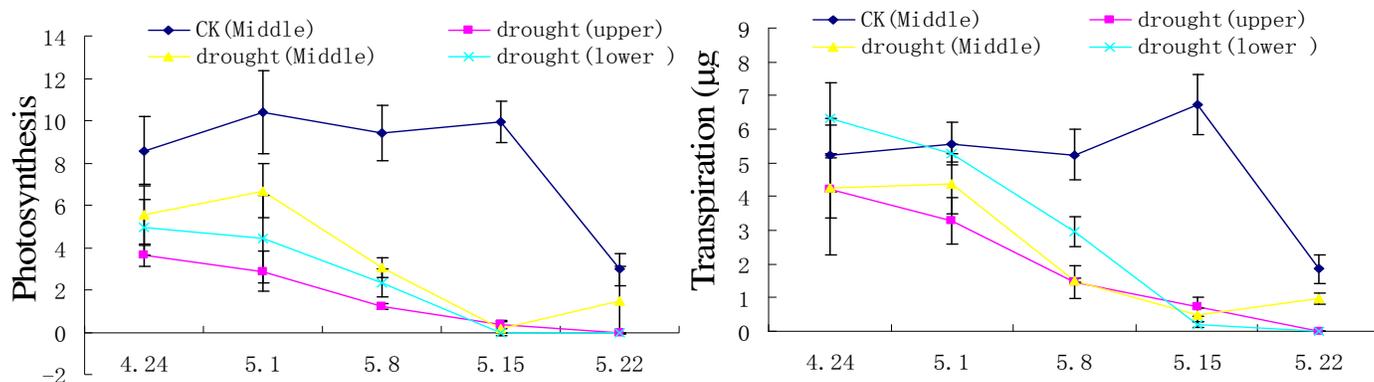


Figure 2. Effect of soil drought on photosynthetic activity (Pn) and transpiration (E) of leaves in different parts of shoot on apple trees.

function. There is a tendency that Pn and E descended during the drought treatments and they were remarkably lower than that of well-watered plants. For leaves at different sections of shoot, the photosynthesis of the leaves at the middle section was higher than that of other parts, while the transpiration at the lower section was higher. At the last two weeks, they both declined to zero. It is interesting to note that both the photosynthesis and the transpiration of drought stressed plants did not recover after fully re-watering (data not shown).

Carbohydrate composition of leaves

At the medium-term of treatments, the leaf sucrose concentrations under drought stress were significantly lower than that of control, but after that, it increased quickly (Figure 2). However, after the plants were fully re-watered, the leaf sucrose concentrations declined sharply to the level of control. The sucrose of leaves at upper, middle and lower section kept a similar trend under drought stress. Glucose concentration in leaves of plants under drought stressed showed random variations over time with no clear trends (Figure 3). In contrast, glucose in leaves on drought-stressed plants showed a trend toward increasing concentrations over time. For plants subjected to upper drought stress, leaf glucose concentration increased slightly and then descended heavily and until May, 15 it was higher than that of other treatments. However, at the lower and middle drought, leaf glucose concentration was flat over time except May, 22. It is noticeable that leaf glucose concentration at upper section of seedlings was higher than that of other sections, which demonstrated that the osmotic adjustment ability was higher in the new leaves.

There was an anomalous switch in leaf fructose concentration. At May 8, the leaf fructose concentration was lowest in all the treatments. Differently, the leaf sorbitol content declined slightly and then rose at the following two weeks. But probably due to the severe stress level, it

reduced to a minimum at May, 22. While after re-watering, the leaf fructose content in all section was to increase especially at the middle and lower part of shoot, it was higher than that of the upper shoot. It is noticeable that, for the period from 5/8 to 5/22, the sucrose and sorbitol concentrations in the drought treatment were mirror images in terms of concentration changes (Figure 3). As expected, the major carbohydrate in the leaves was sorbitol and glucose. In general, effects of treatments on sorbitol concentration were complex but plants in drought stress had higher sorbitol and glucose concentrations.

Treatment effects on N, P content of leaves

Generally, there was an anomalous switch in leaf P content. As Figure 4 shows, during the drought period (from 4.24 to 5.22), N content in the leaves under upper drought kept an increasing trend. Especially in the first week, the N content increased quickly. On the contrary, the N content in the leaves under upper drought decreased remarkably. After re-watering, the leaf N content declined severely and was remarkably lower than that at other sections and the control. Leaf P content descended sharply at the upper and middle drought, but under upper drought, kept a higher level compared with other treatments. After re-watering, leaf P content at upper and middle section of shoot had recover but was lower than that at upper shoot.

DISCUSSION

Drought is a major factor limiting growth and development in higher plants. Since drought is a common occurrence in many environments, many perennial plant species have developed mechanisms to cope with a restricted water supply. Plants can avoid drought stress by maximizing water uptake (e.g., tapping ground water by deep roots) or minimizing water loss (e.g., stomatal closure, small leaves). In this study, the results showed that drought

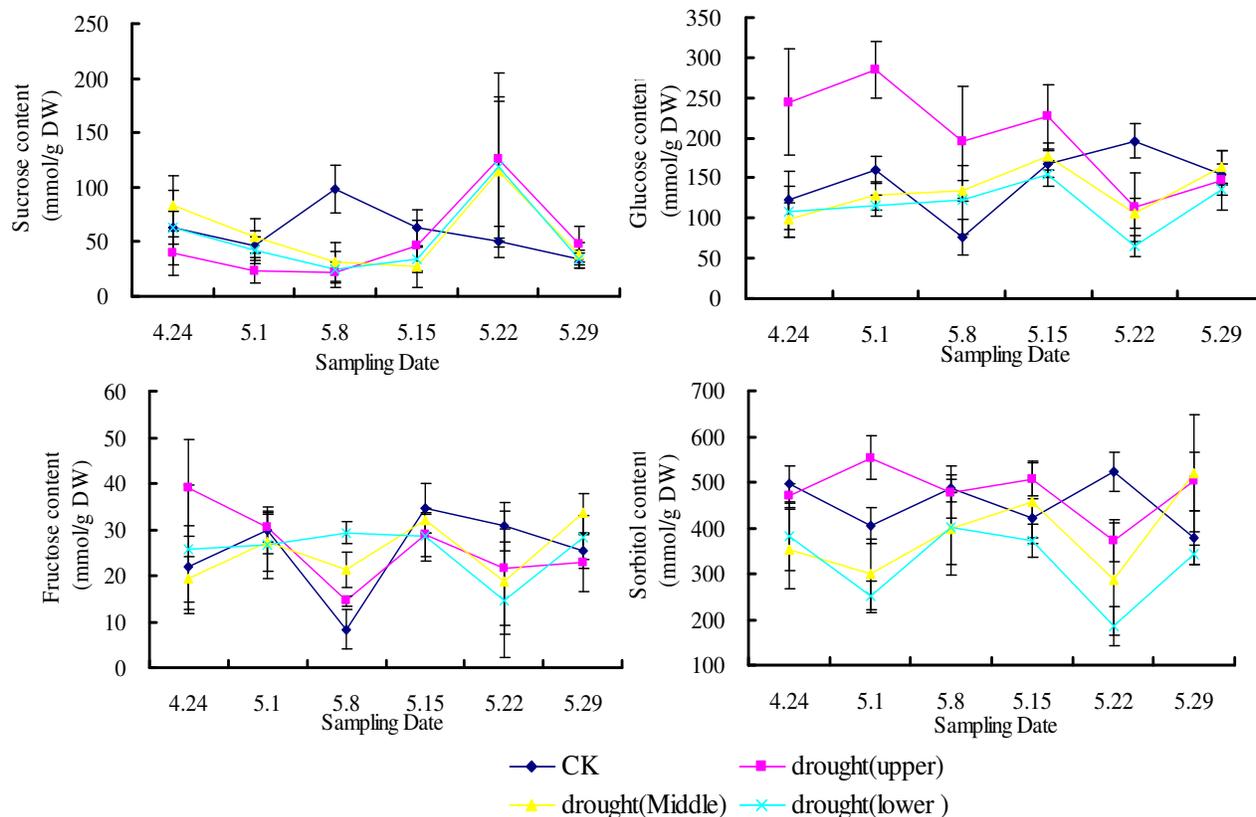


Figure 3. Effect of soil compaction and drought on sucrose, glucose, fructose and sorbitol concentration of leaves in different parts of shoot on apple trees.

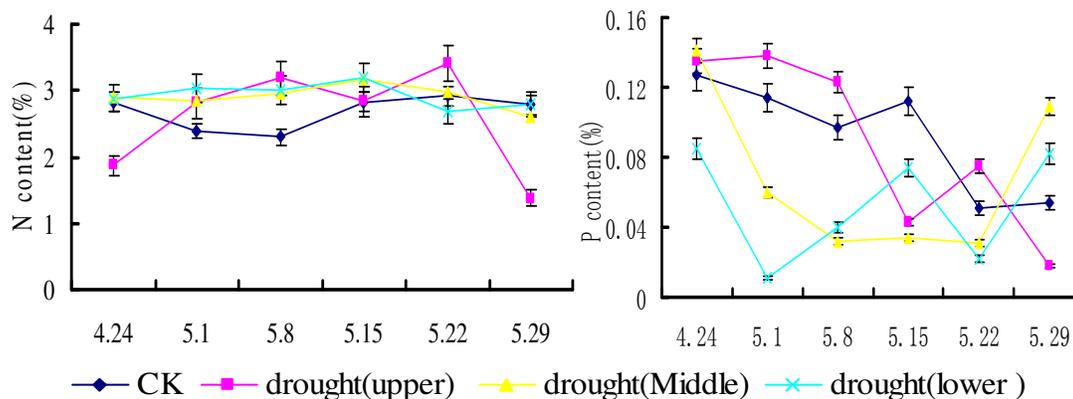


Figure 4. Effect of soil drought on N and P concentration of leaves in different sections of shoot on apple trees.

had major effects on plant growth (Table 1), which had a significantly smaller stem diameter and average leaf area at the end of the drought treatment period. In the meantime, we found that drought remarkably depressed Pn and E. Similar effects of drought on growth and Pn of different plants have been reported previously (Sircelj et al., 2007; Fischer and Maurer, 1978).

During the period of decreased photosynthetic rates in

the drought-stressed leaves when photosynthetic production was insufficient to meet demand, breakdown of soluble carbohydrates could sustain metabolism. In theory, species possessing both drought-avoidance mechanism and ability to acclimate by active osmoregulation would be at an advantage, because of increased flexibility in response to changing environmental conditions. In addition, osmotic adjustment may provide an ecological advantage

for young seedlings by maintaining metabolic activity under suboptimal conditions during establishment when roots have not reached deep soil water. In apple, sorbitol is the major product of photosynthesis and is the major translocated form of carbon (Loescher et al., 1982). Sorbitol is also an important reserve carbohydrate in apple leaves (Chong and Taper, 1971). From currently available data, we found that drought had an unexpected and large effect on sorbitol concentration in leaves and especially, there was a higher sorbitol concentration at the upper section in response to drought stress. This demonstrates that the leaves at the upper section have higher osmotic adjustment ability. This result confirms earlier findings that soluble sugars accumulate in leaves during drought stress (Munns et al., 1981) and that starch decreases under water deficit in leaves of many fruit trees (Wang and Stutte, 1992; Rodrigues et al., 1993).

In addition, we also discovered that drought increased glucose and fructose concentrations and decreased the sucrose content in upper leaves as the deepening of drought stress, but the mechanisms underlying this phenomenon could not be deduced from the analyses carried out in this study. The depression of sucrose concentration occurred in concert with a marked depression of shoot growth (Table 1) so it is possible that the relative lack of sucrose availability in stressed plants plays a role in reducing plant growth, thereby maintaining turgor pressure for a longer period during drought.

Drought stress also can affect the mineral nutrient absorb. It is well known that N and P are the important mineral nutrient elements which can promote cell division and growth and the increase of plant leaf area and thus indirectly promotes plant photosynthesis. From Figure 4, we can see evidently that N content in all leaves at different section kept a flat trend, while P content decreased obviously except the upper leaves at the beginning of the drought stage. These resulted directly in slow growth and an obvious decline in photosynthesis and respiration. Similar effects of drought on the soybean and maize has been reported (Purcell and King 1996; Samuel et al., 2006).

In summary, we conclude that drought stress prevented N and P absorb and decreased RWC of apple leaves, which caused the decline of photosynthesis and transpiration. Thus the direct phenotype was the slowed growth which was specifically expressed in decreased plant height, number of leaves, average leaf area and stem diameter. In order to adopt or avoid the drought stress, the apple also had some physiological shift just like the carbohydrate content change in the leaves at any section of the shoots. However, the drought-resistant mechanism of higher plants is a complex and long process, yet to be further studied.

REFERENCES

Atkinson CJ, Policarpo M, Webster AD, Kingswell G (2000). Drought

- tolerance of clonal *Malus* determined from measurements of stomatal conductance and leaf water potential. *Tree Physiol.* 20(8): 557-563.
- Bulbotko GV (1973). The effect of the physical properties of soils on the development of the root system of apple trees. *Soviet Soil Sci. USSR*, 5: 219-224.
- Bianco LR, Rieger M, Sung JS (2000). Effect of drought on sorbitol and sucrose metabolism in sinks and sources of peach. *Physiol. Plant*, 108: 71-78.
- Chong C, Taper CD (1971). Daily variation of sorbitol and related carbohydrates in *Malus* leaves. *Can. J. Bot.* 49: 173-177.
- Fernandez RT, Perry RL, Flore JA (1997). Drought response of young apple trees on three rootstocks. II. Gas exchange, chlorophyll fluorescence, water relations, and leaf abscisic acid. *Plant Physiol. Biochem.* 122(6): 841-848.
- Fernandez RT, Perry RL, Flore JA (1997). Drought response of young apple trees on three rootstocks: growth and development. *Plant Physiol. Biochem.* 122(1): 14-19.
- Ferree DC, Schmid JC, Palmer JW, Wunsche JN (2001). Apple rootstock reaction to soil compaction. *Acta Hort.* 557: 111-115.
- Fischer RA, Maurer R (1978). Drought resistance in spring wheat cultivars. I. Grain yield responses. *Aust. J. Agric. Res.* 29(5): 897-912.
- Loescher WH, Marlow GC, Kennedy RA (1982). Sorbitol metabolism and sink-source interconversions in developing apple leaves. *Plant Physiol.* 70: 335-339.
- Massonnet, EC, Serge R, Erwin D, Regnard JL (2007). Stomatal Regulation of Photosynthesis in Apple Leaves: Evidence for Different Water-use Strategies between Two Cultivars Catherine. *Ann. Bot.* 100(6):1347-1356
- Munns R, Weir R (1981). Contribution of Sugars to Osmotic Adjustment in Elongating and Expanded Zones of Wheat Leaves During Moderate Water Deficits at Two Light Levels. *Aust. J. Plant Physiol.* 8(1): 93-105.
- Murphy J, Riley JP (1962). A modified single solution method for the determination of phosphate in natural waters. *Anal. Chem. Acta.* 27: 31-36.
- Philip JJ, Jo Rytter EA, Detwiler JW, Travis TW, McNellis (2003). Plant Molecular Biology Rootstock effects on gene expression patterns in apple tree scions, 53(4): 493-511.
- Purcell LC, King CA (1996). Drought and nitrogen source effects on nitrogen nutrition, seed growth, and yield in soybean. *J. Plant Nutr.* 19(6): 969-993.
- Ranney TG, Bassuk NL, Whitlow TH (1991). Osmotic adjustment and solute constituents in leaves and roots of water-stressed cherry (*Prunus*) trees. *J. Am. Soc. Hortic. Sci.* 117: 816-823.
- Rodrigues ML, Chaves MM, Wendler R, David MM, Quick WP, Leegood, Stitt M, Pereira JS (1993). Osmotic adjustment in water stressed grapevine leaves in relation to carbon assimilation. *Aust. J. Plant Physiol.* 20: 309-321
- Samuel B, Feila B, Jampatongb S, Stampa P (2006). Effects of pre-anthesis drought, nitrogen fertilizer rate, and variety on grain yield, yield components, and harvest index of tropical maize. *Agric. Water Manage.* 81: 41-58.
- Sircelj H, Tausz M, Grill D, Batic F (2007). Detecting different levels of drought stress in apple trees (*Malus domestica* Borkh.) with selected biochemical and physiological parameters. *Sci. Hortic.* 113(4): 362-369.
- Streeter JG, Strimbu CE (1998). Simultaneous extraction and derivatization of carbohydrates from green plant tissues for analysis by gas-liquid chromatography. *Anal. Biochem.* 259: 253-257.
- Wang Z, Stutte G W (1992). The role of carbohydrates in active osmotic adjustment in apple under water stress. *J. Am. Soc. Hort. Sci.* 117: 816-823.