Responses of apple tree cultivars to drought: carbohydrate composition in the leaves

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Abstract: The changes in the composition of carbohydrates in the leaves of several apple cultivars were investigated during fruit development in an intensive plantation and in a model experiment. Analytical measurements were performed on leaves collected from the eastern, western, and southern sides of trees four times: on days 57, 73, 99, and 120 after flowering. Simultaneously, the shoots collected from these varieties were exposed to mild, moderate, and severe water stress induced by polyethylene-glycol solution, modeling drought. Under short-term drought, a close significant correlation (R = 0.8348) was found between the degree of leaf rolling and the carbohydrate (glucose + fructose) content 73 days after flowering. During mild drought, water-intensive cultivars exhibited significant leaf rolling and high accumulation of carbohydrates, while those with moderately rolled leaves contained less glucose and fructose. During severe drought, it is the sucrose content of leaves that can be considered a stress indicator, while during temporary dry periods, it is the changes in the glucose + fructose content of leaves. The early stage of slow fruit development was more suitable for screening the drought tolerance of apple trees based on the changes in morphology and the carbohydrate composition of the leaves. The results can be used to select apple trees with good adaptability to drought in breeding programs.

Key words: Apple, carbohydrates, polyethylene-glycol, drought stress

1. Introduction

Among abiotic stresses, drought is the most significant restrictive factor for plants, causing severe loss of crop in fields and fruit plantations. In apple plantations, water sufficiency during slow fruit development is of critical importance, determining crop yields (Caspari et al., 2004; Naschitz and Naor, 2005). In intensive plantations, shallow-rooted stocks are widely used. These rootstocks are very sensitive to soil water shortage, since decreased absorption of nutrients results in reduced fruit size and crop yields. The extent of the damage also depends on the drought stress tolerance of varieties.

During short-term drought periods, the strategy of avoiding water deficit in plants is based on restricting transpiration by stomata closure. Under drought stress, abscisic acid is transported from the roots to the leaves alone or conjugated with glucose (Sauter et al., 2002) where it induces stomatal closure through a signaling system in the guard cells of chloroplast (Yokota et al., 2006). Long-term stomatal closure causes a partial or total reduction in transpiration together with a reduction of the atmospheric CO_2 diffusion to the intercellular space (Singh and Raja Reddy, 2011). As a result of this process, photosynthetic

activity decreases and plant growth is retarded. Leaf rolling is one of the defense mechanism to prevent water loss. It produces a favorable microclimate on the leaf surface so that photosynthesis and growth may continue (Matthews et al., 1990). Simultaneously with leaf rolling, soluble carbohydrates accumulate for osmotic adjustment under drought stress (Kadioglu and Turgut, 1999; Kadioglu and Terzi, 2007), which prevents the stomata from closing during rolling. Thus, a higher rate of photosynthesis can be maintained (Ludlow, 1980).

Prolonged or intensifying drought causes plants to increase their production of various osmolytes. Organic compounds, such as sugars, proline, and glycine-betaine as the primary osmotica, provide osmotic adjustment during drought (Fernadez et al., 1997; Šircelj et al., 2007). The soluble sugars are considered to contribute to osmotic adjustment in response to drought for apple (Lakso et al., 1984; Wang and Stutte, 1992), cherry (*Prunus* spp.; Ranney et al., 1991), and peach (Escobar-Gutiérrez et al., 1998). A strong correlation has been established between the accumulation of carbohydrates and osmotic stress tolerance (El-Tayeb, 2006), although the quantity of carbohydrate components varies in the stress reactions of different plant

species (Pelleschi et al., 1997; Šircelj et al., 2007; Hudak et al., 2010). A great significance is attributed to the sucrose concentration in apple leaves during flowering and fruit set (Vemmos, 1995). Depending on the foliage levels of trees, the intensity of biochemical processes can be different under abiotic stresses. The upper foliage levels of trees are more exposed to drought, high temperature, and UV radiation; therefore, due to a higher glucose content, osmoregulation is more active than on the middle and lower levels (Jie et al., 2010). Seasonal differences in the carbohydrate content of the stems of apple trees have also been shown (Sivaci, 2006). Water stress conditions for the fruit trees are rather difficult to provide and control in the field because of experimental and ecological conditions. Nevertheless, these difficulties can be overcome by inducing drought in laboratory experiments, wherein the results correspond with those of field experiments.

Few studies have examined changes in the carbohydrate content and composition of the leaves in apple trees in response to drought during the stages of development. It may be hypothesized that the differences in the drought stress reactions of apple trees can be better studied by examining the osmotic adaptability of leaves situated on the middle level than on the upper one. The purpose of our research was to investigate the drought tolerance of apple tree cultivars by tracking the quantitative changes in the carbohydrate components of leaf samples taken from fields and model experiments.

2. Materials and methods

2.1. Field experiment
The experiments were conducted in the Debrecen-Pallag experimental apple plantation of the University of Debrecen over a period of 8 weeks (16 June–18 August) in a dry (2007) and a wet (2008) year. This period coincided with the slow fruit development stage of the apple trees, when the distribution of precipitation and relative humidity was rather variable (Table 1). The trees of the studied cultivars (11-year-old Idared, Jonagold, Remo, and Gala, grafted on M 26 rootstock) were trained using a slender spindle system and spaced at 4 m between rows and 1.5 m within rows. The rows were oriented in a north-south direction. The soil type was sand with 1% humus content. Pest and disease managements were carried out according to the rules of integrated plant production and weeds were controlled by the regular tilling of interrows and rows.

Three trees were chosen for each variety in the experimental area. For each cultivar, seven leaf samples were collected from shoots of identical developmental stages found at about 1.0–1.2 m in height on the eastern, western, and southern sides of the trees. The first sample was taken 57 days after the end of flowering (AF1), while

further samples were taken 73 days (AF2), 99 days (AF3), and finally 120 days (AF4) after flowering. The rolling of the leaves was measured with a protractor and defined by the horizontal angle parameter in the AF2 and AF4 stages. In each case, the collected leaf samples were kept at –80 °C until the chemical analysis was carried out; after that, the composition and the amount of carbohydrates were determined.

2.2. Drought model experiment
Concurrently with the field measurements, on four occasions, 1-year-old shoots of identical development and containing at least 10 leaves were collected from the eastern and western sides of the trees of each cultivar to model drought. Drought stress was induced by solutions of polyethylene glycol (PEG 6000) in concentrations of 2%, 5%, and 7% (PEG2, PEG5, and PEG7). For each cultivar, two of the collected shoots were put into separate wide-necked bottles containing 200 mL of the PEG solution or distilled water (control). The treatment of the four varieties and the sampling took place simultaneously; thus, every cultivar was under the same degree of stress. The shoot samples of the experiment were maintained at daytime/night temperatures of 25/10 °C with a 16 h/day photoperiod of white fluorescent light (400 μmol m^{-2} s^{-1}) in the laboratory for 24 h. The leaves from the lower, middle, and upper parts (treated as separate groups) of the shoots were removed and kept at –80 °C until the chemical analysis. In the drought model experiment, the results of the examination of the leaves in the middle of the shoots were compared with the results of the field experiment.

2.3. Determination and separation of carbohydrates
The methods used for measurement of soluble carbohydrates (monosaccharides and sucrose) were as described by Sárdi et al. (1999, 2006). The preparation of samples for chemical analysis was carried out according to Nemeskéri et al. (2010a), and then carbohydrates were separated by overpressured layer chromatography (NIT Co., Ltd., Hungary).

2.4. Statistical methods
The data of the experiments were evaluated by variance analysis, using SPSS 13.0 for Windows. The average values of the treatments were compared at $P < 0.05$ using Duncan's multiple range test. The connection between the degree of leaf rolling and carbohydrate content was established by regression analysis.

3. Results

3.1. Field experiment
The early (AF1–AF2) and the later (AF3–AF4) periods of slow fruit development were characterized by drought (Table 1). However, the AF2 and AF3 periods were also characterized by variable precipitation and warm weather. This fluctuation in the precipitation supply in field

Table 1. Meteorological data during the slow fruit development of apple trees.

Years	Climatic factors	Fruit development			
		AF1*	AF1–AF2	AF2–AF3	AF3–AF4
2007	Tmin (°C)	12.3	13.7	15.0	13.0
	Tmax (°C)	28.9	29.5	30.7	27.5
	Average T (°C)	21.2	22.2	23.1	20.5
	Precipitation (mm)	0.0	54.4	28.8	11.9
	RH% (min)	25.0	29.0	28.0	31.0
	RH% (average)	60.0	54.0	54.0	55.0
2008	Tmin (°C)	14.6	15.9	15.3	15.8
	Tmax (°C)	24.6	28.0	26.7	27.6
	Average T (°C)	18.5	22.0	21.0	21.7
	Precipitation (mm)	8.2	42.5	118.8	13.8
	RH% (min)	62.2	46.0	43.0	42.0
	RH% (average)	80.3	52.0	78.1	65.4

*From 57 (AF1), 73 (AF2), 99 (AF3), and 120 days (AF4) after the end of flowering.
Tmin = minimum temperature, Tmax = maximum temperature, RH% = air humidity.

conditions can be regarded as the alternation of drought stress and rewatering.

During a short period of drought (16 days), the changes of the green color of the leaves of fruit trees were not visible, although the cultivars had already responded by leaf rolling. In the meantime (AF2), the carbohydrate (glucose and fructose) contents of leaves intensively increased concurrently with the degree of leaf rolling (R = 0.8348; P < 0.01). In a longer period of drought (37 days and AF4), the carbohydrate content was significantly lower and, until a 60° leaf rolling, the increase was slow-paced (R = 0.5838) (Figure 1). During a late period of fruit development (AF3 and AF4), the carbohydrate content of the leaves relative to the aggregate of glucose and fructose significantly decreased in comparison with the initial (AF1) stage. However, in a drought year (2007), the decrease in carbohydrates could be attributed to mainly the fructose contents of the leaves, while it was due to the glucose content in a mildly dry year (2008) (Table 2).
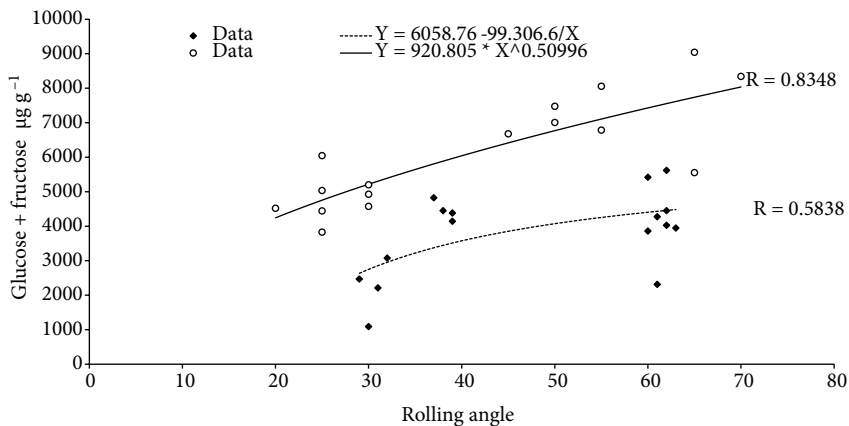
Figure 1. Relationship between the rolling angle of leaves and carbohydrate content (glucose and fructose) in the leaves of apple trees under a short period of mild drought (AF2 = o) and prolonged drought conditions (AF4 = ◆).

Table 2. Changes in carbohydrate content in the leaves of the apple trees under fruit development.

Time*	2007				2008			
	Glucose (μg g^{-1})	Fructose (μg g^{-1})	Glucose + fructose (μg g^{-1})	Sucrose (μg g^{-1})	Glucose (μg g^{-1})	Fructose (μg g^{-1})	Glucose + fructose (μg g^{-1})	Sucrose (μg g^{-1})
AF1	6326.752 a	1914.772 a	8241.524 a	-	5098.86 a	3002.05 a	8100.90 a	-
AF2	6996.949 a	2018.964 a	9015.913 a	-	4037.20 b	2682.14 a b	6719.35 b	-
AF3	5783.837 b	1556.954 b	7340.791 b	-	3843.44 b	2376.08 b	6219.52 b	-
AF4	5149.411 b	1113.891 b	6263.301 b	1846.56	2154.42 c	1630.67 c	3785.08 c	853.83

Values in a column having different letters are significantly different at the P < 0.05 level using Duncan's multiple range test.
*For times, see Section 2.

3.2. Drought model experiment

The carbohydrate content of leaves collected from the middle level of apple trees in the plantation was compared with the carbohydrate content of the middle leaves of shoots collected from the same level and exposed to drought stress during fruit development, at the same four times as those of the field experiment in 2008. In the middle leaves of shoots kept in the control (distilled water) solution, the glucose and fructose content significantly decreased only in the last stage of fruit development (AF4) compared to the initial (AF1) stage (Table 3), which corresponds to the plantation results (Table 2). In the AF3 state, there was a significant increase in the glucose level already under mild (PEG2), while only in the fructose level under medium drought stress (PEG5) conditions. This indicates that mild water deficiency stress first causes a rise in the glucose production, then the intensifying stress increases fructose production.

3.3. Reactions of cultivars in field experiment

There were differences in the response of apple cultivars to drought during fruit development. The highest degree of leaf rolling was observed on the leaves of the Idared and Jonagold varieties, while the lowest degree was observed with the Gala and Remo varieties (Table 4). During this period, there were changes in the leaf tissues; the reduction of thickness in the cuticle and the blade of a leaf as well as intercellular spaces was most noticeable in the leaves of Idared cultivar, while Gala and Remo cultivars had thick leaf-blades.

In a short period (16 days and AF2) of mild drought, the difference between the cultivars was better characterized by the aggregate amount of glucose and fructose than by either the glucose or the fructose content (Figures 2a–2c). In the wet period (AF3) following the drought during fruit development, the carbohydrate content (glucose and fructose) significantly decreased in the leaves of the Idared and Jonagold trees, while it increased in the Gala and Remo cultivars (Figure 2c). The change in fructose and glucose content in the leaves of Remo trees was similar to that of the carbohydrate content (glucose + fructose) when the dry and wet periods occurred alternately during the slow fruit development (AF1–AF4). The fructose and glucose content in the leaves of other apple tree cultivars changed differently (Figures 2a and 2b). No amounts of maltose, galactose, or sucrose could be detected in the leaves until the last stage of fruit development (AF4) after

Table 3. Changes in carbohydrate content in the leaves of apple shoot induced by water stress in the dry model trial (2008).

Time*	Glucose (μg g^{-1})				Fructose (μg g^{-1})			
	Control	PEG2	PEG5	PEG7	Control	PEG2	PEG5	PEG7
AF1	775.066 b	739.011 b	614.258 b	465.342 c	774.287 b	828.421 b	662.121 b	681.388 b
AF2	979.665 a	793.239 b	505.669 c	689.295 b	830.506 a b	728.039 b	867.199 a b	986.326 a
AF3	985.818 a	1640.104 a	904.401 a	1397.990 a	1062.877 a	984.883 a b	1128.623 a	1356.028 a
AF4	324.724 c	485.908 c	248.383 d	337.291 c	423.390 c	537.024 c	780.698 b	448.996 c

PEG2, PEG5, PEG7 represent the solution of polyethylene glycol (PEG 6000) in concentrations of 2%, 5%, and 7%. Values in a column having different letters are significantly different at the P < 0.05 level using Duncan's multiple range test. *For times, see Section 2.

Table 4. Changes in the rolling angle of leaves of apple cultivars under mild drought (AF2) and prolonged drought conditions (AF4).

Cultivars	AF2, ° angle	AF4, ° angle	Cultivars, average ° angle
Gala	27.50 ± 2.88 c	38.25 ± 0.96 b	32.88 ± 6.08 b
Remo	25.00 ± 4.08 c	30.50 ± 1.29 c	27.75 ± 4.06 b
Idared	52.50 ± 8.66 b	61.50 ± 1.29 a	57.00 ± 7.48 a
Jonagold	61.25 ± 7.20 a	61.25 ± 0.66 a	61.25 ± 4.95 a

Values are the average of 3 independent plant samples with standard deviation. Values in each column having different letters are significantly different at the P < 0.05 level using Duncan's multiple range test.
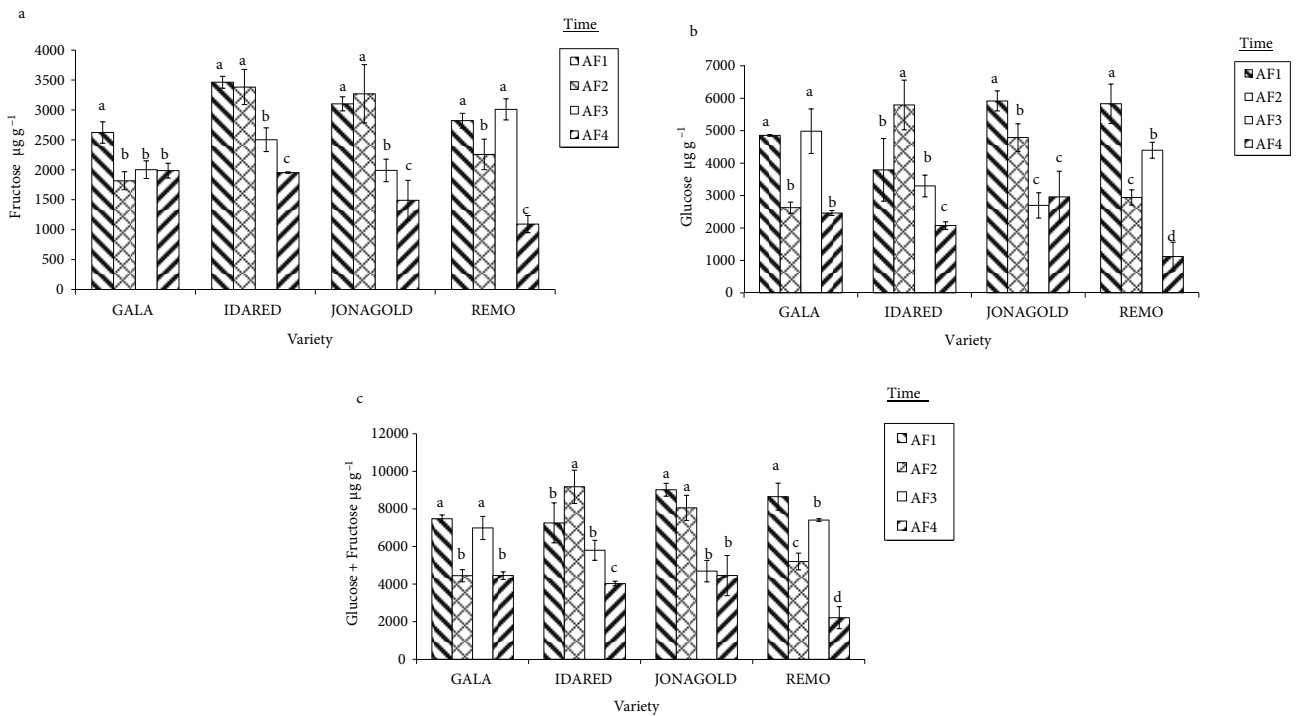
Figure 2. Changes in the fructose **(a)**, glucose **(b)**, and glucose and fructose **(c)** contents in the leaves of apple trees during fruit development. The values are the average of measurements made on 3 different occasions; the vertical bars indicate the standard error (±SE). Different letters indicate significant differences at the P < 0.05 level according to Duncan's multiple range test.

a long period of drought. In the case of the Idared and Jonagold cultivars, the amount of galactose was identical; only the sucrose content in the leaves showed a significant difference between the cultivars (Figure 3).

3.4. Reactions of cultivars in drought model experiment
Leaves in the drought model experiment had lower glucose and fructose levels than those from the plantation, but there were demonstrable differences between the cultivars. Leaves collected during mild drought (AF2) and exposed to water stress treatment responded by leaf rolling and by the

yellowing of the intervein areas. Under mild drought stress (PEG2), moderate leaf rolling and yellowing occurred in the case of the Remo and Gala cultivars. Under similar stress, the Jonagold cultivar responded by very strong leaf rolling and by the browning of a larger leaf surface than the Idared cultivar. Under severe drought condition (PEG7), the leaves of nearly all apple cultivars grown under stress for 24 h rolled significantly, then dried and became brittle.

In the shoot samples collected during short (16 days and AF2) and extended periods of drought (37 days and
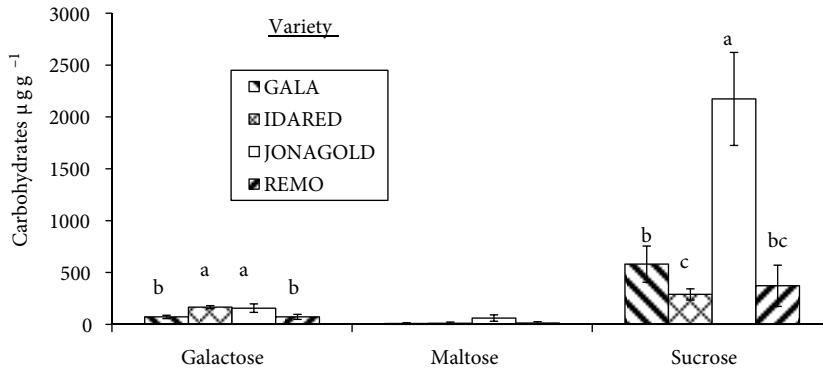
Figure 3. Changes in other carbohydrate contents in the leaves of apple varieties under prolonged drought conditions (AF4). The values are the average of measurements made on 3 different occasions; the vertical bars indicate the standard error (±SE). Different letters indicate significant differences at the P < 0.05 level according to Duncan's multiple range test.

AF4), the difference between cultivars is shown most of all by the fructose content in the leaves exposed to lack of water (Figure 4a). During the early period of fruit development (AF1), mild drought stress (PEG2) resulted in a significant rise in the fructose content of the leaves of the Idared shoots compared to the control. In later periods (AF2–AF4), moderate (PEG5) and severe (PEG7) drought stress induced a significant increase in fructose content. The Remo cultivar responded differently; in the leaves of the shoots collected during both mild (AF2) and prolonged (AF4) drought, a significant increase in the fructose content occurred due to water stress conditions of various degrees of severity (Figure 4a). The difference between cultivars is best detected on the basis of the changes in the glucose content of leaves during the wet period (AF3) following a period of drought (Figure 4b). At this time, the difference between cultivars was significant in the first place in mild (PEG2) and also in severe (PEG7) drought stress conditions. Severe drought stress (PEG7) significantly increased the accumulation of glucose in comparison with the control in all varieties except the Remo cultivar.

The field research and laboratory drought model experiments brought similar results. In both experiments, the glucose-fructose content of the control leaves was identical in the cultivars, and thus their responses indicated the extent of their drought tolerance. In a short period of drought, both in the field experiment and under mild water stress in the model experiment, the leaves of the Remo and Gala cultivars showed lower glucose and fructose contents and slighter leaf rolling than those of the Jonagold and Idared cultivars.

4. Discussion

In an intensive apple plantation, the use of dwarfing stocks is advantageous for the harvest, but their uptake of water and nutrients can be diminished by poor water supply. As a result of water shortage, smaller-sized, less pigmented or rolled leaves, thin and weak shoots, and poorly colored apple fruits have been grown (Zatykó, 2003). On a drought-tolerant rootstock, the varieties are able to compensate for a short period of water shortage due to the development and absorbing power of the roots. It is only in extended drought that apple varieties on a drought-tolerant rootstock show morphological changes (Nemeskéri et al., 2009); this is in contrast to the varieties on the semidwarf M 26 rootstock, which responded to short-term drought by leaf rolling.

Leaf rolling has been observed in numerous species, such as rice, maize, wheat, and sorghum, as a response to water deficit or other abiotic stresses (Kadioglu et al., 2012), but it has not been studied in apple trees. According to the results, the leaf rolling that occurred in apple tree cultivars grown under drought conditions was related to the carbohydrate content of the leaves. This finding indicated a significant connection between the degree of leaf rolling and the carbohydrate content (glucose and fructose) of the leaves, but the process was influenced by the duration and severity of drought. Kadioglu and Turgut (1999) also showed that in rolled leaves, the quantity of sugar crystals, including glucose, fructose, and sucrose, is connected with the degree of leaf rolling in *Ctenanthe setosa*. Rolling quickly reduces the effective leaf area and transpiration and is a useful drought-avoidance mechanism. During the rolling of leaves, photosynthetic activity can be maintained, more or less, due to the accumulation of soluble sugars and opened stomata under drought conditions. Therefore, the degree of leaf rolling can be used as a criterion in the visual
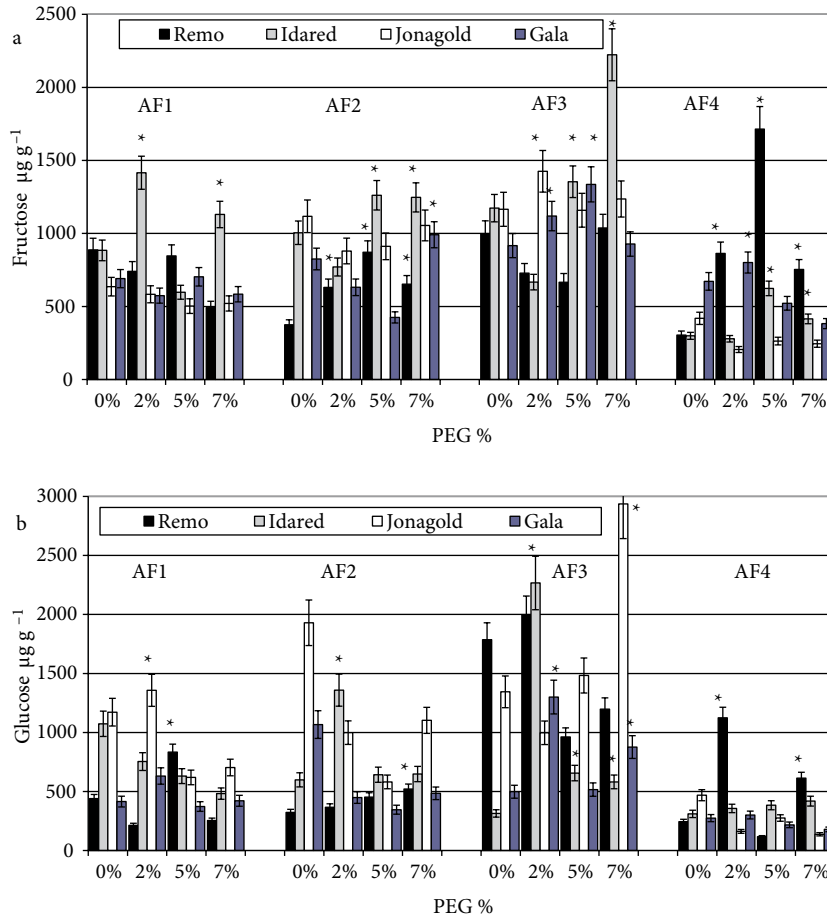
Figure 4. The fructose contents **(a)** and glucose content **(b)** in the middle leaves of apple shoots under mild water stress (PEG 2%), moderate water stress (PEG 5%), and severe water stress (PEG 7%) conditions in the various stages of fruit development (AF1, AF2, AF3, AF4). The asterisk indicates a significant increase compared to the control.

scoring of varieties being screened for drought resistance (Loresto et al., 1976).

In intensive apple plantations, the difference between varieties manifests itself in the avoidance of drought and in the degree of leaf rolling. Even in short periods of drought, in water-intensive cultivars such as Idared and Jonagold, the carbohydrate content significantly increased in connection with the degree of leaf rolling (R = 0.7311; P < 0.05). In cultivars more tolerant of drought, represented by Gala and Remo (Nemeskéri et al., 2010b), it was only an extended period of drought that resulted in a significant connection between the degree of rolling and carbohydrate content (R = 0.8961, P < 0.01; unpublished data). The drought sensitivity of Idared and Jonagold apple cultivars has also been proved by the high antioxidant level in the leaves expressed by the antioxidant capacity of water soluble substances (ACW) under drought during the slow fruit development (Nemeskéri et al., 2010b). They established that the ACW content (70.23 µg mg⁻¹) was

the highest in the leaves of Idared cultivar, while a lower (53.64 µg mg⁻¹) but gradually rising level of ACW was recorded in the leaves of Jonagold cultivar under drought conditions. The higher drought tolerance of Remo and Gala apple cultivars was also confirmed by a lower level of ACW content (48.55–45.46 µg mg⁻¹) (Nemeskéri et al., 2010b) and lower carbohydrate content in their leaves in comparison with the Idared or Jonagold cultivars under drought.

The data published so far show considerably contradictory results regarding the connection between the changes in the carbohydrate content of the leaves and the water stress responses of apple cultivars. Under drought conditions, a reduction in the sucrose and starch contents of apple leaves has been detected (Wang and Stutte, 1992; Xu et al., 2001), while others (Li and Li, 2005) showed an increase in the sucrose, glucose, and fructose content. Šircelj et al. (2007) did not find any changes in the glucose and fructose contents of the apple leaves under drought.

Šircelj et al. (2005) detected an increase in the sucrose level of apple leaves in severe (23 days long) drought; however, our findings showed that only an extended period (37 days) of drought causes an increase in the sucrose level of the leaves of the apple cultivars, which was the highest for the water-intensive Jonagold cultivar.

Sugar accumulation during apple growth is considered to be the result of the translocation of sorbitol and sucrose from photosynthetic leaves (Bieleski, 1969; Loescher and Everard, 1996); however, during relatively longer periods of water deficit, the accumulation of sorbitol and sucrose accelerated in the leaves (Chaves et al., 2003; Bianco and Francaviglia, 2012). According to our results, the correlation between the accumulation of carbohydrate in the leaves and leaf rolling was strong when drought occurred during the early stage of slow fruit development (73-79 days after flowering), but later (120 days after flowering) it became weaker, probably due to the carbohydrate mobilization. Therefore, it is advisable to carry out the screening of the drought tolerance of apple trees based on the changes in the morphology and the carbohydrate composition of the leaves in the field and the laboratory during the early stage of slow fruit development. During this period, the translocation of sugars from the leaves to fruit may be even slower.

Because of the diversity of the glucose and fructose accumulation of varieties, we have concluded that the differences between cultivars are best shown by the quantitative changes in the glucose-fructose content in the leaves found on the middle level of trees and the middle part of shoots. In the different stages of fruit development, the glucose and fructose content of the leaves differed in quantity, which may be due to the age difference between the examined plant parts, such as leaf samples taken from branches and shoots. Nevertheless, the relative difference between the varieties was identical in the plantation and in the model experiment control treatment. During severe, prolonged drought, the extent of the decrease in the fructose and glucose content in the leaves of the varieties was different, but the sucrose level increased in the leaves of all varieties. In the case of greater water shortage, which rarely occurs in nature, sucrose can be regarded as the best indicator compound characterizing drought tolerance. That sucrose can also be a good indicator characterizing the drought tolerance of apple cultivars is confirmed by the results of Borókay and Sárdi (1999), obtained in a similar model experiment with bean plants. When interpreting the results, it must be taken into consideration that in model experiments, the tree shoots that had previously been exposed to various natural stress in the plantation (see also the changes depending on the picking time of gathered samples) experienced the PEG treatments of various intensity as repeated drought stress.

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