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Studies on the drought responses of apple trees (*Malus domestica* Borkh.) grafted on different rootstocks

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Summary: The morphological and chemical changes in the leaves of eight apple varieties grafted on M4, M26 and MM106 rootstocks were examined during fruit development in non-irrigated plantation. The aim of the experiment was to examine the defensive mechanisms in apple trees under the mild and serious dryness occurring during fruit development. The content of a relative chlorophyll (SPAD value) of the apple leaves on eastern side of the trees was lower than western and southern sides under drought occurring during fruit development Under sustained drought from first of July to August the SPAD value, the weight of leaves were larger and the stomata density was significantly larger than in the previous periods. There was a close positive significant correlations between SPAD and stoma density both in the group with less sensitive to drought (r=0,8429) and in the large water demanded group (r=0,9209) equally. The rate of increase in SPAD values and the stoma density was slower in the group with drought tolerant, than in the group required good water supply. The varieties being sensitive to water deficite such as Akane, Pink Lady and Red Rome van Well have already responded intensively to short time of drought by the increasing of carbohydrate and antioxidants productions. At 42 days of sustained drought as soon as the level of carbohydrate in the leaves decreased, the antioxidants contents of the leaves rised suddenly in the apple varieties with moderate water requirements as Greensleeves and Idared. Gála apple variety seemed to be able to defend the drought for longer time because there was no change in relatively high level of carbohydrates and antioxidants of their leaves under severe drought during fruit development.

Key words: apple, drought stress, SPAD, carbohydrate, antioxidants

Introduction

The future challenge is to realize less water use in the increasing food production. In intensive apple plantation growing on the world, the limiting factor is the water. In intensive orchard the trees on rootstocks shallow-rooted have sensitive response to water shortage as their decreasing nutrient uptake reduces the fruit size and yield. The water supply of July and Augustus months is the most critical time in terms of the quantity of the crop in the apple orchards. Serious water deficit during the slow fruit growth causes a decrease of fruit size and loss of apple yield. The high temperature results sunburn symptoms on fruits resulting a decrease in marketable value of the product hereby reduces the profitability.

The fruit trees throughout the root system supplement their loss of water resulted by the rate of transpiration of foliage surface. When the available water of soil reduces the dehydration of the root increases the abscisic acid (ABA) synthesis in the root that translocated to the shoot resulting the ABA accumulation in the leaves leading to stomata closure (*Parry* et al., 1992, *Gomes* et al., 2004). The large quantity of ABA in the water stressed leaves produces the discharge of potassium ions from bodygard cells.This causes the stomata closure and the retention of the water in the leaves. The drought-resistant rootstock can compensate for the periodical water shortage of the soil, with deep roots with large suction force, but the rate of the drought tolerance of the varieties depends on an interaction between the rootstock and scions. The plants cannot utilize the dissolved nutrients in water shortage, as a result of this the leaves become smaller, and paler, or the "rolling" leaves, the thinner and weaker offshoot and the fruits being coloured worse are developed (*Zatykó*, 2003). The efficiency of nitrogen and water utilisation of the genotypes showed a frequent coincidence (*Eghball & Maranville*, 1991). Since the chlorophyll contains nitrogen, the water utilisation can be concluded by the measurement of chlorophyll content in the leaves beside the use of nitrogen of the plant.

In water shortage associated with high temperature, due to strong light radiation the chlorophyll of the leaves decays that resultes a decrease in the photosynthesis. The change of chlorophyll content can be related to more factors influencing the photosynthesis There were high positive correlations between the relative chlorophyll content (SPAD) and the photochemical reaction indexes, and the nitrogen demands at an apple (*Perry & Davenport*, 2007), and the measure of drought stress at wheat (*Ommen* et al., 1999), and the efficiency of transpiration at a peanut (*Krishnamurthy* et al., 2007), and the rate of photosynthesis, and the rate of photosynthesis correlated with the stoma conductance at rice (*Kato* et al., 2004) under drought stress. The drought tolerance of grape genotypes was expressed by measure of greenish leaves since a lineal correlation was found between SPAD value and the chlorophyll content of leaves (*Fanizza* et al., 1991). The portable SPAD 502 chlorophyll meter was found suitable for evaluation for chlorophyll content of the leaves and to characterize the state of nitrogen supply of the plant (*Yadava*, 1986, *Berzsenyi & Lap*, 2003).

The first reaction to the water deficiency is to decrease the growth rate, that is attributable a decrease in the cells turgor, and the stomata closing prevents the loss of water in the leaves. Plant water deficiency occurs whenever the atmospheric requirements for transpiration exceeds the rate of root water uptake. This leads to the decrease in transpiration activity which associated with an increase in leaf temperature (*Nobel*, 1999). The radiant temperature of the foliage surface shows that the plant/tree suffers from water shortage, in spite of its sign would be visible (*Giuliani* et al., 2001) and the change of temperature of foliage surface can be measured by the infra-red thermometer. The increase of the time and strenght of dry period make the plants to increase the production of different osmotic compounds such as the glucose, sorbitol, antioxidants (*Sircelj* et al., 2007). Many methods colud be used for the measurement of water status of the plants, but the efficiency of their application depending on the purposes of the examination is different. The laboratory experiments for dessication of the tissues substantially are not coincide that of field ones since the drought occurred in agriculture is often mild. Under the field conditions the stoma conductance and infrared thermography are considered to have a good efficiency, however the methods based on the morphological changes is slightly efficient for the measurement of adaptation to drought and for the testing of genotypes in the plant breeding (*Jones*, 2007). The drought endured until a week already resulted visible symptoms in the vegetable plants such as the leaves turning yellow, droping of leaves and flowers (*Nemeskéri* et al., 2008). The only prolonged soil water deficiency and air drought can produce morphological changes in fruit trees such as falling leaves therefore the tolerance of varieties to drought is rather difficult to establish under field conditions.

The aim of our experiments is to study the defensive mechanisms of apple trees grafted different rootstocks under the mild and severe drought occurring during the fruit development.

Material and methods

Experimental materials

The leaves analysis of eight apple varieties was performed from the stock plantation grown on brown sandy soil, without irrigation at Research and Extension Centre for Fruit Growing, Újfehértó. The row and stem distance was 6m x 4m in the stock plantation. The majority of the varieties such as Akane, Idared, Ozark Gold, and Red Rome van Well trees were grafted on M4 rootstock considered to have a good drought resistance. The investigations were also carried out on Gála and Galaxy Gála trees grafted on M26 half dwarf rootstock and Geensleeves, Pink Lady aple trees on MM106 rootstock considered to be drought-resistant. The responses all of the apple varieties to drought stress was mesaured four times after every 20 days periods during the fruit development.

The leaf measurements concerning on morphological characteristics and biochemical reactions of all varieties were made on three trees of each variety from middle of June until the first of August. Under this period, the microclimate can be characterized by relatively low air humidity, and little moisture, particularly in July was high temperature (*Table 1*).

Table 1: Climate data in stock apple plantation

Period	T min °C	T max °C	Average T °C	Air T min. °C *	Humidity %		Wind speed m/s		Precipitation
					min.	average	average	max.	mm
06.12–07.02	13.7	29.5	22.2	13.3	29	54	2.34	9.03	54.4
07.03–07.24	15.0	30.7	23.1	14.3	28	54	2.77	10.33	28.8
07.25–08.07	13.0	27.6	20.5	12.5	31	55	2.69	10.27	11.9

T=temperature * air temperature on 5 cm from the soil

Experimental methods

Relative chlorophyll content (SPAD) measurement

Yadava (1986) established a close correlation between SPAD values and chlorophyll contents measured on the leaves of different plant species. The use of SPAD 501 chlorophyll meter for the measurement of chlorophyll in the leaves facilitated the assessment of the genotypes under field conditions. In our experiment SPAD 502 portable chlorophyll meter was used to measure the relative chlorophyll content of the leaves on eastern, western and southern side of the apple trees. The leaf measurements carried out from spurs identical development cc. 1–1.2 m high of the trees four times during fruit development. Seven leaves were collected from all tree and 2–2 measurements was made with the chlorophyll meter on all of leaves. Then the fresh leaves from collected samples were used for examination of stomata and other parts of the leaf samples treated by fluent nitrogen in order to block their physiological processes and submitted for chemical analysis.

The examination of stoma density

The skinning epidermis samples were prepared on both sides of the head vein of leaves on abaxial leaf surface where SPAD measurements was carried out all of apple varieties. The stoma density and stomata sizes were measured on skinning epidermis samples used with a light microscope (40×10 in magnification). The number of stomata per mm^2 and stoma sizes was determined in four repetitions.

Analytics examinations

Separation and determination of carbohydrates

The fresh leaves were frozen with liquid nitrogen, powdered and extracted with methanol: H_2O= 80:20, V/V. This suspension was centrifuged at 1500 *g* for ten minutes at -1°C. The clear supernatants were used for overpressured layer chromatographic separations (OPLC chromatograph developed by OPLC-NIT Co., Ltd., Budapest, Hungary). OPLC separations were carried out on TLC and HPTLC silica gel 60 F_{254} precoated chromatoplates (Merck Co.) using acetonitrile: H_2O (85:15, V/V). Staining was performed by aniline-diphenyl amine-phosphoric acid reagent. For densitometric determination a Shimadzu CS-930 TLC/HPTLC scanner (Shimadzu Co., Kyoto, Japan), λ=540 nm was used (*Sárdi* et al., 1999). The data were expressed by µg/g on fresh weight.

Determination of antioxidant capacity

Antioxidant power was measured by the FRAP (Ferric Reducing Ability of Plasma) method at λ=593 nm using tripyridyl-triazine (Benzie and Strain (1996). The antioxidant capacity of samples was expressed by mMol ascorbic acid/liter (mMAS/l).

Statistical methods

The evaluation of the data was made by variancia analysis with SPSS statistical program. The correlation between the stress indicators (SPAD, stoma, carbohydrates) have been explored by regression analysis.

Results

Morphological changes of leaves

The drought symptoms can be seen on the varieties due to a high temperature and longer time (42 days) lasting water shortage during the fruit development in dry year. Single apple made the leaf surface decrease with rolling up in order to reduce the surface for the transpiration. Under the long dry period of July the reactions of apple varieties on M4 rootstock considered to be a good drought-resistant were different; the leaves of Ozark Gold apple became light-green, that of Idared was strongly rolled up and yellow leaves were appeared of Akane, Red Rome van Well and Gala apple tree on M26, respectively.

The leaf lamella, where the stoma examinations and the relative chlorophyll contents (SPAD) measurements were carried out, was thicker at the end of dry period (07.24) compared to the first half of the June. During this time the thickening of cuticle epidermis was came true on the only middle of half leaf. The leaves of Ozark Gold apple tree became thickened in serious prolonged dryness while the intercellular area significantly decreased compared to the early state. The reaction of Akane apple trees due to their leaves thickening was the same. However, no changes were detected in the leaf structure of Idared apple variety during dry period.

Changes in relative chlorophyll content (SPAD) and stoma density

During the fruit development of apple trees that is the most sensitive to water sortage, the changes in the relative chlorophyll content (SPAD) and the stoma density were examined in order to show a relation in drough tolerance of apple varieties. The content of relative chlorophyll (SPAD in the leaves was lower on the eastern (E) side of the apple trees than on western (W) and southern (S) sides under the examined period in dry year (*Table 2*) that presumably related to the strong light illumination. The content of chlorophyll in the leaves decays due to the strong light radiation whereupon the chlorophyll-b is especially sensitive to strong light. In consequence of this the high tolerance to light and larger capacity of potential photosynthesis are characterized by large SPAD values (*Griffin* et al. 2004). At the end of the July and the first of August, the SPAD values and the weight of the leaves were larger, and the stoma density on the leaves was significantly larger under the prolonged dryness compared to the early state (*Table 2*).

Table 2: Relative chlorophyll content and stoma density of apple leaves under drought during fruit development

Time	SPAD/time	SPAD/side*			Average leaf weight (g)	Stomata piece/mm^2	Stomata size μ
		E	S	W			
06.12	45.51 c*	43.66 c	47.70 ab	48.17 ab	0.580	315.80 b	26.52
07.02	47.84 b	47.06 b	48.29 ab	48.18 b	0.636	323.60 b	26.10
07.24	49.11 a	48.86 a	49.41 a	49.,05 a	0.730	367.60 a	26.10
08.07	48.11 a	48.83 a	49.02 a	49.07 a	0.744	362.30 a	26.39
Average	***47.64***	***47.10 b***	***48.60 a***	***48.62 a***	***0.673***	***342.33***	***26.28***

* E=eastern side S=southern side W=western side of the tree
Values in each column having different letters are significantly different at the P<0.05 level using Duncan's multiple range test.

Table 3: Changes in the relative chlorophyll content (SPAD) and stoma density of apple leaves on eastern (E) western (W) and southern (S) side of the trees under drought

Variety/ rootstock	Time	SPAD/side			SPAD average	Stoma piece/mm^2	Stoma size μ
		E	S	W			
Akane (M4)	06.12	37.20	46.88	51.19	45.09 d	255 d	26.44 b
	07.02	48.14	51.11	44.66	**47.97 c**	**357 b**	26.60 b
	07.24	47.58	51.06	47.42	**48.69 c**	**372 b**	24.72 bc
	08.07	46.64	49.98	48.81	48.48 c	355 b	29.37 a
Ozark Gold (M4)	06.12	41.76	44.64	46.15	44.18 e	263 d	27.90 a
	07.02	45.66	45.21	45.54	45.47 e	345 b	25.14 b
	07.24	42.29	45.23	48.48	45.33 ed	361 b	26.33 bc
	08.07	44.24	44.64	42.79	43.89 e	407 a	25.73 b
Red Rome Van Well (M4)	06.12	47.44	49.70	51.81	49.65 c	361 b	27.84 b
	07.02	46.08	49.76	50.41	48.75 c	325 c	25.15 b
	07.24	46.56	50.06	52.11	49.58 c	416 a	25.61 b
	08.07	48.58	48.84	51.80	49.74 c	391 ab	24.50 c
Idared (M4)	06.12	49.84	50.19	52.28	50.77 c	315 bc	25.79 bc
	07.02	52.07	53.94	54.99	53.67 b	307 c	27.63 ab
	07.24	59.11	57.32	56.50	57.65 a	389 a	24.95 c
	08.07	58.61	57.45	59.10	58.39 a	339 b	25.93 bc
Gala (M26)	06.12	41.31	42.29	43.26	42.28 f	297 d	25.14 b
	07.02	43.59	45.36	45.74	**44.90 e**	**330 c**	25.67 b
	07.24	45.31	46.79	47.26	**46.45 d**	**362 b**	26.85 b
	08.07	47.16	48.24	46.54	47.31 cd	337 cb	26.12 b
Galaxy Gala (M26)	06.12	46.39	49.79	46.14	47.44 d	348 bc	24.66 b
	07.02	43.14	46.48	43.77	44.46 ef	324 c	25.56 b
	07.24	45.06	46.03	38.12	43.07 f	319 c	26.76 b
	08.07	42.27	43.41	43.78	43.16 ef	326 c	25.63 b
Greensleeves (MM106)	06.12	49.34	54.44	53.71	52.50 b	402 a	26.52 b
	07.02	49.18	50.81	51.63	50.54 c	317 c	26.18 b
	07.24	53.99	53.72	54.17	53.96 b	346 bc	26.74 b
	08.07	53.59	51.24	51.71	52.18 bc	366 b	27.21 b
Pink Lady (MM106)	06.12	44.87	42.83	44.46	44.05 e	294 d	25.60 b
	07.02	47,08	44.14	46.76	**45.99 d**	**330 c**	24.97 b
	07.24	50.53	47.92	46.54	**48.33 c**	**366 b**	24.60 c
	08.07	49.26	49.49	45.76	48.17 c	389 ab	24.85 bc

Values in each column having different letters are significantly different at the P<0.05 level using Duncan's multiple range test

The drought tolerance of genotypes can be expressed by the change in relative chlorophyll content (SPAD) since a linear correlation was found between SPAD value and the chlorophyll content of the leaves (Fanizza et al. 1991). Based on this finding we supposed that the drought tolerence of apple varieties could also be expressed by changes in SPAD values. The measurements in June can be regarded for the starting water status of the fruit trees. This time the stoma distribution was largest (402 pieces/ mm^2) on the leaves of Greensleeves apple variety and the smallest one (263–255 pieces/mm^2) was in Ozark Gold and Akane apple varieties (*Table 3*). In June the relative chlorophyll content (SPAD) in the leaves was the largest both on W and S sides of the trees, while it was the smallest on the E side except the Pink Lady and Galaxy Gala apples where the SPAD values were near identical greatness on all of three sides (*Table 3*).

Under mild drought lasted until 20 days, the SPAD value was high on the shaded southern (S) side and western side of the tree in Red Rome van Well, Gala and Greensleeves apple varieties whilst the differences between the sides disappeared in the others. The lowest SPAD values weremeasured on all three direction of tree in Ozark Gold apple, and the largest one was in the Idared, Greensleeves varieties. Under the drought for a short time (from June 12 to July 2), the relative chlorophyll content (SPAD) in the leaves was increased significantly, but there was no change in the weight of leaves, in the size and density of stomata compared to the starting state (*Table 2*). However, the apple varieties showed different reaction to the drought; the SPAD and stoma density in the leaves of Akane, Gala and Pink Lady apples was increased but others such as Greensleeves and Red Rome van Well apple trees responded with decrease of stoma density during mild short time (20 days) drought (*Table 3*).

Prolonged drought, until 42 days during fruit development (from June 12. to July 24), the stoma density on the leaves with high relative chlorophyll content (SPAD) increased in Red Rome van Well apple variety contrary to that of Greensleeves apple decreased. SPAD and stoma density was increased significantly in prolonged drought in Akane, Idared, Gala, and Pink Lady apple varieties independently from the rootstock (*Table 3*).

The relative chlorophyll content of leaves in Galaxy Gala trees on M26, however it was low, decreased considerable during increasing dryness meanwhile stoma density did not change.

Changes of carbohydrates and antioxidants

The water potential of the soil is sinking if the available water content decreases in the soil then the turgor of plants decreases gradually. The osmotic potential would able to reduced with salts uptake from the soil, or the transformation of the polysaccharides to water-soluble mono- and disaccharides. As a result of this the water potential of the plants decreases and becomes lower than the water potential of the environment so the plant will be able to absorbe the water for maintenance of their turgor. It means the varieties have been a larger soluble carbohydrate content of the leaves in non-stressed state, where the water potential is lower, and their ability of the water uptake was more guarantee than in which ones the concentration of water-soluble components such as the monosaccharides is smaller. The measurements in June can be considered free of water stress state in the apple trees when the quantity of glucose and fructose in the leaves was large in the Ozark Gold, Akane Idared apple trees on M4 rootstock and Greensleeves on MM106 and Galaxy Gala trees on M26 rootstock.

The changes of carbohydrate content in the leaves is supposed to influence the endurance of dryness in the varieties. Under the short time drought the level of carbohydrate in the leaves remained high in the Ozark Gold, Idared, Akane apple trees on M4 rootstock and Greensleeves on MM106 one. Under this conditions the increase in the level of carbohydrate in the leaves signaled the defence against the dryness for Red Rome van Well trees on M4 and the Pink Lady on MM106. The responses concerning on carbohydrate content in the leaves of Gala and Galaxy Gala apple on M26 rootstock was different from above mentioned apple varieties (*Figure 1*). During prolonged drought the high level of carbohydrate in the leaves decreased significantly and stayed at the same level until the beginning of fruit ripening (the 08. 07) in the first group of apple while the large carbohydrate concentration in the leaves did not change considerable for Red Rome van Well and Pink Lady belonged to the second group of apples what could be defined as defence reaction to the dryness (*Figure 1*).

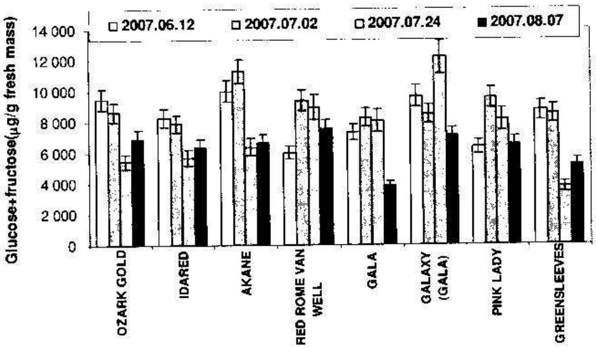

Figure 1: Change of carbohydrate content in the apple leaves under mild (2007.06.12–07.02) and serious drought stress (2007.06.12–07.24)

At the apple trees the drought stress has been treated as a form of active oxygen stress (*Jia* et al., 2003) and the responses to water deficiency and drought as antioxidative reaction was defined (*Šircelj* et al., 2005, 2007). The ascorbic acid gives the large part of the water-soluble antioxidants in an apple leaves. The increase in the ascorbic acid level is an indicator of active stress adaptation in moderate drought lasting until 15 days but its quantity decreases in serious drought (*Sircelj* et al., 2007). Under a mild drought stress the content of antioxidants in the leaves was increased considerable similarly to that of carbohydrates (*Figure 1*) compared to the previous status in the case of Akane, Pink

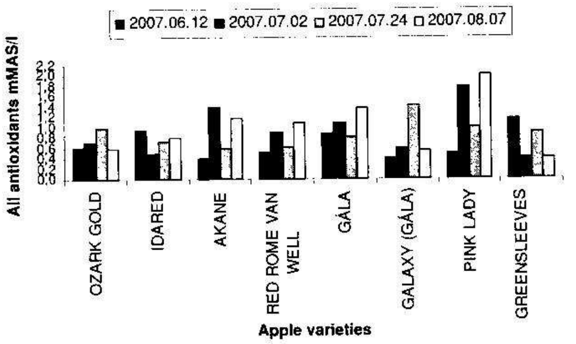

Figure 2: Change of antioxidant capacity of the apple leaves under mild (2007.06.12–07.02) and serious drought stress (2007.06.12–07.24)

Lady and Red Rome van Well apples (*Figure 2*). These apple varieties have already responded sensitively to the water deficiency in mild drought for a short time, by increasing the production of the carbohydrates and antioxidant compounds in their leaves. An other type of responses is to mild drought stress, when in the leaves with large glucose and fructose contents the high level of antioxidants decreased significantly, as this was detected in Greensleeves and Idared apple trees. At the beginning the high glucose and fructose of leaves associated with a relative low quantity of antioxidants provides enough protection for Ozark Gold and Galaxy Gala in a short time drought (*Figures 1* and *2*).

The prolonged drought (06.12-07.24) decreased the level of carbohydrate meantime the rise in antioxidant level in the leaves of Greensleeves and Idared was moderately but it was suddenly concerning on Ozark Gold and Galaxy Gala apple trees (*Figures 1* and *2*). The significant decrease in antioxidant content in the leaves of Akane and Pink Lady apples, and moderate decrease in that of Red Rome van Well apple show that these varieties are not capable to an additional defensive reaction against the drought. The relatively large carbohydrate (7356–8071 µg/g) and all antioxidant activity (0.88–1.08 mMAS/l) in the leaves of Gála apple did not change significantly in the drought being prolonged during the fruit development what demonstrated it is able to defend against the long-lasted drought.

The apple varieties were arranged in three groups based on changes in leaves structure and the reactions of carbohydrate and antioxidants in the leaves, under the shorter and longer periodical dryness occurred during the fruit development. The apple varieties with tolerance to drought such as Gála and Galaxy Gála were classified to the first group, the apples with a moderate sensitivity to water deficiency belonged to the second group (Idared, Ozark Gold, Greensleeves) and that of most sensitive ones such as Akane, Red Rome van Well, Pink Lady apples were arranged into the third group.

Kato et al. (2004) established a close significant correlation between SPAD value and the rate of netto photosynthesis and similar one was between the netto rate of photosynthesis and the stoma conductance. As our results show when the SPAD value was increasing the number of stomata/ mm^2 of the leaf surface also increased in the drought during the fruit development that presumably due to the photosynthesis activity and water retention in the leaves. There was a close significant positive correlation between SPAD values and stoma density in the first group being less sensitive to drought (r=0.8429) and in the group (3) with high water requirements (r=0.9209) equally. The rate of increasing in the stoma density was slower in the less sensitive group than in the large water requirements group.

Discussion

The control of stomatal closure is said to be an efficiently stress indicator during a short time drought (*Mohamed* et al., 2002, *Costa-Franca* et al., 2000) what our results also demonstrated. As our findings the rate of drought tolerance in an apple varieties can be characterized by the changes in stomatal density and thickness of leaf and cuticle epidermis.

The rather vigorous M4 rootstock was considered good drought-resistant that resulted to spread earlier in large scale plantations in the Hungary but the middle vegetative growth rate of trees on it did not satisfy the demands of modern orchards. The half dwarfing MM106 rootstock is considered to have been relatively good drought-resistance (*Sharma & Chauhan*, 2005) however *Hrotkó* (1999) and *Davies & Lakso* (1979) reported the best drought-resistant rootstock is the MM 111. The variety of fruit trees grafted on the drought-resistant rootstock could compensate the water deficiency lasted shorter time without visible dry symptoms due to sucker force and development of roots. The dehydration of the root result the ABA accumulation in the leaves lead to the stomata closure (*Gomes* et al., 2004) in consequently the drought tolerance of rootstock influenced on the stomatal reactions. Since increasing SPAD value associated with an increase in the rate of photosynthesis, and the close correlation was determined between the rate of the photosynthesis and stomatal conductance (*Kato* et al., 2004), the stoma density is probable influenced on photosynthesis rate. *Baker* (2000) reported that the stoma density on abaxiális leaf surface was lower under dry circumstances than in a good water supply. Contrary to Baker's findings our result showed that the stoma density on leaves was increasing in the cumulative dryness and the relative chlorophyll content (SPAD value) also increased. This is presumably related to the fact, that the leaf surface have decreased in drought and so showed larger stomata number refered on unit of surface.

The protective mechanisms start up in the cells to ensure the osmostic adjustment during long-lasted drought periods. *Sircelj* et al. (2007) found an increase in the level of ascorbate, α-tocopherol and sorbitol of apple leaves in moderate drought periods lasted until 15 days but these decreased to the controll level due to a serious drought stress. Jonagold Wilmuta apple trees with large water demand tried to defense the drought lasting 15 days by increase in the content of ascorbate, glutathione, sorbitol, and beta carotene in the leaves. At more serious (23 days) drought the sucrose and the zeaxanthin contents have been increased in their leaves (*Sircelj* et al., 2005). We found contrary results as the sucrose content could not be detected even under severe drought but there was changes in content of glucose and fructose in the leaves of apple varieties. During mild drought stress lasting until 20 days the relative chlorophyll content (SPAD) was growing but the size of stomata and stomata density did not change significantly (*Table 2*). Akane, Gala and Pink Lady apple varieties independently the rootstocks reacted with increasing of SPAD and stoma density (*Table 3*) and increase of glucose+fructose concentration in the leaves to short time drought during fruit development (*Figure 1*).

The adaptive reactions of apple varieties can be characterized well by the changes in the levels of carbohydrate and the compounds functioned as antioxidant under drought occurring during the fruit development. Akane, Pink Lady apples have already intensive responses to mild drought showing the increase in the content of carbohydrate and antioxidants. Further cumulative dryness decreased significantly the content of glucose and fructose and antioxidants in the leaves that showed the protection of these varieties was run down during prolonged drought. Mild drought stress decreased the distribution of stomata on the leaf abaxial surface while increased the concentration of glucose and fructose and antioxidants but has no effects on the relative chlorophyll content (SPAD) in the leaves of Red Rome van Well apple. Under prolonged drought cc. 42 days the high glucose content and SPAD in the leaves of Red Rome van Well apple trees did not change, the stomatal density was increased and the quantity of antioxidants was decresed moderately. This apple variety based on the reaction of dought stress, is similarly to Akane, Pink Lady varieties belonged to the group (3) with the most water demands.

The different factors such as leaf movement and various stomatal density are separately or together contributed to the protection of drought in the Idared and Greensleeves apple varieties. Under the fruit development prolonged (42 days) drought as soon as the level of carbohydrate decreases the antioxidant level rise up suddenly in the leaves of Greensleeves and Idared varieties in spite of high SPAD value the stoma density on the leaves was different they showed modreate response to drought stress

The level of carbohydrate and antioxidants in the leaves of Gála apple did not change significantly under prolonged drought that showed this variety has been able to defense to drgought for a longer time. Greensleeves apple trees on MM106 rootstock would be more tolerated to the prolonged drought compared with the Pink Lady apple based on their adaptive reactions. The apple trees on M4 rootstock based on adaptive reaction showed moderate tolerance to drough in the case of Idared and Ozark Gold varieties and the others as Akane, Red Rome van Well apples were very sensitive to drought. The Gála trees on M26 rootstock was more tolerant to drought than Galaxy Gála apple.

Summarized

We found a close significant positive correlation between SPAD value and stoma density in the group of apples with less sensitive to drought (1) (r=0.8429) and in the most water demanded group (3) (r=0.9209) equally. The rate of increase in stoma density was slower in the group with less sensitive to water deficiency than that of group with large water requirements. Those apple varieties are sensitive to the water deficiency already intensively responded to short time drought by the increase in the production of carbohydrates and antioxidants compounds. The antioxidants content in the leaves of apple varieties with large water demands was considerable decreased under prolonged drought that signaled they not capable onto additional defence. The the level of carbohydrate in the leaves was decreased then the level of antioxidant rised up suddenly in apple varieties with moderate water demands such as Greensleeves and Idared apples under drought lasting for long (42 days). The content of carbohydrates and antioxidants was relatively high and did not change significantly in the leaves of Gála apple variety under long-lasted drought and it was more resistant to drought than Galaxy Gála.

References

Baker, E.A. (2000): The leaf and fruit cuticles of selected drought tolerant plants. Acta Hort. 527: 85–93.

Benzie, I.I.F. & Strain, J.J. (1996): The ferric reducing ability of plasma (FRAP) as a measuring of "antioxidant power": The FRAP assay. Annal. Biochem. 239: 70–76.

Berzsenyi, Z. & Lap, D.Q. (2003): A N-műtrágyázás hatása a kukorica- (*Zea mays* L.) hibridek szemtermésére és N-műtrágya-reakciójára tartamkísérletben (Effect of N fertilisation on the grain yield and N fertiliser response of maize (*Zea mays* L.) hybrids in a long-term experiment) Növénytermelés, 52 (3-4): 389–407.

Costa-Franca, M.G., Pham Thi, A.T., Pimentel, C., Rossiello, R.O.P., Zuily-Fodil, Y. & Laffray, D. (2000): Differences in growth and water relations among *Phaseolus vulgaris* cultivars in response to induced drought stress. Environ. Exp. Bot. 43: 227–237.

Davies, F.S. & Lakso, A.N. (1979): Water stress responses of apple trees. II. Resistance and capacitance as affected by greenhouse and field conditions. J. Amer. Soc. Hort. Sci. 104: 395–397.

Eghball, B. & Maranville, J.W. (1991): Interactive effects of water and nitrogen stresses on nitrogen utilization efficiency, leaf water status and yield of corn genotypes. Commun. Soil. Sci. Plant Anal. 22: 1367–1382

Fanizza, G., Ricciardi, L. & Bagnulp, C. (1991): Leaf greenness measurements to evaluate water stressed genotypes in *Vitis vinifera*. Euphytica, 55 (1): 27–31.

Gomes, A.M.M., Lagoa, A.M.M.A., Medina, C.L., Machado, E.C. & Machado, M.A. (2004): Interactions between leaf water potential, stomatal conductance and abscisic acid content of orange trees submitted to drought stress. Braz. J. Plant Physiol. 16 (3): 155–161.

Guiliani, R., Magnanini, E. & Flore, J,A. (2001): Potential use of infrared thermometry for the detection of water deficit in apple and peach orchards. Acta Hort. 557: 399–405.

Griffin, J.J., Ranney, T.G. & Pharr, D.M. (2004): Photosynthesis, chlorophyll fluorescence, and carbohydrate content of *Illicium* taxa grown under varied irradiance. J. Amer. Soc. Hort. Sci. 129, (1): 46–53.

Hrotkó, K. (1999): Gyümölcsfaiskola. Mezőgazda Kiadó Budapest.

Jia, H.G., Han, Y.O. & Li, D.Q. (2003): Photoinhibition and active oxygen species production in detached apple leaves during dehydration. Photosynthetica. 41, (1): 151–156.

Jones, H.G. (2007): Monitoring plant and soil water status: established and novel methods revisited and their relevance to studies of drought tolerance. J. Exp. Botany 58, (2): 119–130.

Kato, M., Kobayashi, K., Ogiso, E. & Yokoo, M. (2004): Photosynthesis and dry-matter production during ripening stage in a female-sterile line of rice. Plant Prod. Sci. 7, (2): 184–188.

Krishnamurthy, L., Vadez, V., Devi, M.J., Serraj, R., Nigam, S.N., Sheshshayee, M.S., Chandra, S. & Aruna, R. (2007): Variation in transpiration efficiency and its related traits in a groundnut (*Arachis hypogaea* L.) mapping population. Field Crops Res. 103: 189–197.

Mohamed, M.F., Keutgen, N., Tawfik, A.A. & Noga, G. (2002): Dehydration-avoidance responses of tepary bean lines differing in drought resistance. J. Plant Physiol. 159: 31–38.

Nemeskéri, E., Remenyik, J. & Fári, M. (2008): Studies on the drought and heat stress response of green bean (*Phaseolus vulgaris* L.) varieties under phytotronic conditions. Acta Agron. Hung. 56, (3): 321–328

Nobel, P.S. (1999): Physicochemical and environmental plant physiology, 2nd. edn. London: Academic Press

Ommen, O.E., Donnelly, A., Vanhoutvin, S., Oijen, M. & Manderscheid, R. (1999): Chlorophyll content of spring wheat flag leaves grown under elevated CO2 concentrations and other environmental stresses within the 'ESPACE-wheat' project. European J. Agron. 10: 197–203.

Parry, A.D., Griffiths, A. & Horgan, R. (1992): Abscisic acid biosynthesis in roots. II The effects of water stress in wild type and abscisic acid deficient mutant (*notabilis*) plants of *Lycopersicon esculentum* Mill. Planta. 187: 192–197.

Perry, E.M. & Davenport, J.R. (2007): Spectral and spatial differences in response of vegetation indices to nitrogen treatments on apple. Computers and Electronics In Agriculture, 59: 56–65.

Sárdi, É., Velich, I., Hevesi, M. & Klement, Z. (1999): Ontogenesis-and biotic stress dependent variability of carbohydrate content in snap bean (*Phaseolus vulgaris* L.). Z. Naturforsch. **54c**, 782–787.

Šircelj, H., Tausz, M., Grill, D. & Batič, F. (2005): Biochemical responses in leaves of two apple tree cultivars subjected to progressing drought. J. Plant Physiol. 162: 1218–1308.

Šircelj, H., Grill, D. & Batič, F. (2007): Detecting different levels of drought stress in apple trees (*Malus domestica* Borkh.) with selected biochemical and physiological parameters. Sci. Hort. 113 (4): 362–369.

Sharma, D.D. & Chauhan, J.S. (2005): Effect of different rootstocks on root distibution of apple Acta Hort. 696: 167–171.

Yadava, U.L. (1986): A rapid and nondestructive method to determine chlorophyll in intact leaves. HortScience 21: (6): 1449–1450.

Zatykó I. (2003): Az alma aszályérzékenységének tényezői. (The factors influenced on dought sensitivity of apple) Kertgazdaság. 35 (1): 33–39.