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## Effects of Drought Stress on Pigment, Ascorbic Acid and Free Amino Acids Content in Leaves of Two Apple Tree Cultivars

By

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**Key words:** Drought stress, oxidative stress, ascorbate, free amino acids, pigments, apple tree.

### Summary

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Photosynthetic pigments, ascorbic acid and free amino acids content in leaves of *Malus domestica* Borkh. cv. 'Elstar' and cv. 'Jonagold wilmuta' subjected to mild drought or no drought were measured by HPLC in order to detect drought stress. Water deficit ( $\Psi_L$  of approximately  $-1.9$  MPa) increased ascorbic acid and zeaxanthin and decreased total chlorophyll (a+b), neoxanthin and  $\beta$ -carotene in leaves of cv. 'Jonagold wilmuta'. The results indicate that mild drought induced oxidative stress in leaves of cv. 'Jonagold wilmuta'. Drought ( $\Psi_L$  of approximately  $-2.0$  MPa) had no statistically significant effects on oxidative stress indicators measured in leaves of cv. 'Elstar'. The free amino acid content increased in leaves of stressed plants in both cultivars. The most pronounced difference was found between control and stressed leaves in Orn, Arg and Pro content in cv. 'Elstar' and Pro and Arg content in cv. 'Jonagold wilmuta'. The results indicate that the tolerance to water deficit depends on the apple tree cultivar.

### Introduction

Drought has been shown to affect different physiological and biochemical parameters in plants. Among other effects drought may trigger an increased formation of ROS which are highly reactive and bring about cellular damage. Increased ROS formation affects plant metabolism in different ways: it stimulates biosynthesis or degradation of various important molecules. Changes in the ratios

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of photosynthetic pigments have already been suggested as early indicators of oxidative stress (DARRAL & JAGER 1984). It is also already known that oxidative stress affects AA and carotenoid content in plants. Ratios involving  $\beta$ -carotene are often used to show significant metabolic disorders. Under many environmental circumstances zeaxanthin is produced from violaxanthin, and therefore its accumulation could possibly be used as an indicator of stressed tissue (ALSCHER & CUMMING 1990).

Drought is also known to affect the amino acid metabolism. Total FAA in leaves are often increased in water stressed leaves (HSIAO 1973). This may be the result of de novo synthesis and/or protein degradation. The adaptive significance of FAA accumulation during stress is still uncertain, but it appears that their major role is most likely in osmotic adjustment.

In order to detect drought stress in two apple tree cultivars important in Slovenia we analysed AA, photosynthetic pigments and FAA in leaves by HPLC.

Abbreviations:  $\Psi_L$  = leaf water potential, AA = ascorbic acid, FAA = free amino acids, JW = 'Jonagold wilmuta', E = 'Elstar', ROS = reactive oxygen species, V+A+Z = violaxanthin + antheraxanthin + zeaxanthin

## Materials and Methods

The experiment was carried out in summer 1997 on 4-year-old JW and E on M9 rootstock. At the end of June water stress treatment was initiated on half of the trees by withholding irrigation. A polyethylene cover was used for protection of the surface under the non-watered trees from rain. Leaf samples for measurements of the chosen biochemical stress parameters were collected when the midday  $\Psi_L$  of stressed JW reached  $-1.9$  MPa and the midday  $\Psi_L$  of stressed E reached  $-2.0$  MPa.  $\Psi_L$  was determined using the pressure chamber technique (TURNER 1988). Sampled leaves were immediately frozen in liquid nitrogen, lyophilised and stored at  $-20^\circ\text{C}$  until analysis. A Spectra Physics HPLC system was used for the analyses of AA (method developed by TAUSZ & al. 1996), the photosynthetic pigments (method developed by PFEIFHOFFER 1989) and FAA (method adapted from VANCE & ZAERR 1990).

T-test was used to compare the means (calculated from 5 replicates) of the concentrations of photosynthetic pigments, AA and FAA extracted from the leaves of control and stressed plants.

## Results and Discussion

AA and carotenoids play an important role in protecting the photosynthetic apparatus against the destructive effects of light and ROS. Their contents increase with the intensity of slowly developing oxidative stress. In Table 1 data for AA and carotenoids are listed. AA content was found to be higher in the leaves of stressed JW compared to control JW. The total carotenoid content was not significantly different in leaves of stressed plants. Unchanged contents of total carotenoids in plants exposed to drought were already reported (ZHANG & KIRKHAM 1996). Our results indicate that the physiological levels of carotenoids in leaves of apple trees subjected to the stress conditions used in the experiment might be high enough to protect from photo-oxidation and drought stress induced ROS.

Table 1. Ascorbic acid and photosynthetic pigments content (mg/g DW) in leaves of control and drought stressed JW and E

	AA	N	V	A	Z	V+A+Z	L	$\alpha$ -car	$\beta$ -car	total C	chl a+b
E-S	1.87	0.097	0.08	0.020	0.014	0.111	0.48	0.096	0.310	1.20	3.27
E-C	2.71	0.104	0.07	0.018	0.008	0.100	0.53	0.107	0.324	1.27	3.48
JW-S	3.22 <sup>s</sup>	0.103 <sup>s</sup>	0.07	0.018	0.067 <sup>s</sup>	0.150 <sup>s</sup>	0.51	0.100	0.335 <sup>s</sup>	1.35	3.46 <sup>s</sup>
JW-C	1.61 <sup>s</sup>	0.116 <sup>s</sup>	0.07	0.019	0.016 <sup>s</sup>	0.108 <sup>s</sup>	0.54	0.114	0.368 <sup>s</sup>	1.35	3.70 <sup>s</sup>

<sup>s</sup> statistically significant differences between control and stressed plants

E-S = Elstar stress, E-C = Elstar control, JW-S = Jonagold stress, JW-C = Jonagold control, AA = ascorbic acid, N = neoxanthin, Z = zeaxanthin, V = violaxanthin, L = lutein,  $\alpha$ -car =  $\alpha$ -carotene,  $\beta$ -car =  $\beta$ -carotene, total C = total carotenoids, chl a+b = chlorophyll a+b

Slowly developing oxidative stress can cause an increase in the  $\beta$ -carotene content (ALSCHER & CUMMING 1990) but in our experiment  $\beta$ -carotene in leaves of stressed JW was lower than in the control. The total pool of xanthophyll cycle pigments, known as protectants of photosynthetic membranes increased in stressed JW, mainly on account of increased zeaxanthin. The results indicate that the increase in zeaxanthin may have been due to biosynthesis from  $\beta$ -carotene, which is the biosynthetic precursor of zeaxanthin. It has already been reported that an increase in V+A+Z can be accompanied by decreases in  $\beta$ -carotene content (DEMMIG-ADAMS & al. 1988).

The contents of pigments and AA did not indicate oxidative stress in E. Obviously a response to water deficit conditions depends on apple tree cultivar.

13 of the 18 FAA examined were found in the tested samples. In Table 2 data for single and total FAA (i.e. the sum of all studied amino acids) are listed. They show an increase in the content of total FAA in the stressed leaves of both tested cultivars. FAA are known as important constituents of osmoregulation in leaves of many species (MORGAN 1984) and their rise during slowly developing stress is correlated with increasing drought tolerance of the plant. Drought increased Glu, Pro and Arg in both cultivars, Orn in stressed E and Asn in stressed JW. Drought stress induced increase of Glu, Pro, Arg, Asn and Orn has already been reported for ponderosa pine (VANCE & ZAERR 1990). Arg is together with Asp predominant as transport and storage form of nitrogen in apple trees. It appears to play a role in long-distance transport (MIFLIN & LEA 1977). The accumulation of Arg with drought may reflect the refixation and possible sequestering of endogenous  $\text{NH}_3$ , a product of proteolysis (VANCE & ZAERR 1990). Glu is the common substrate for Pro, Orn and Arg. As other FAA did not significantly increase in stressed leaves, de novo synthesis from Glu may be the source of the accumulated Pro, Orn and Arg. We found the most pronounced differences between control and stressed leaves in Orn, Arg and Pro content in E and Pro and Arg content in JW. These results are not in agreement with results of some other authors (HSIAO 1973, VANCE & ZAERR 1990) who reported that the level of Pro might be insensitive to mild drought stress.

Table 2. Free amino acid contents (nmol/g DW) in leaves of control and drought stressed JW and E.

	Asp	Ser	Asn	Glu	Pro	Ala	Val	Phe	GABA	Om	His	Arg	Lys	total
E-S	397	445	1769	3704 <sup>s</sup>	224 <sup>s</sup>	1067	718	105	898	161 <sup>s</sup>	147	2081 <sup>s</sup>	182	11897 <sup>s</sup>
E-C	314	499	1503	3148 <sup>s</sup>	166 <sup>s</sup>	946	798	90	851	88 <sup>s</sup>	145	1395 <sup>s</sup>	146	10088 <sup>s</sup>
JW-S	364	490	1163 <sup>s</sup>	3004 <sup>s</sup>	544 <sup>s</sup>	1262	554	104	337	104	156	553 <sup>s</sup>	193	8828 <sup>s</sup>
JW-C	353	469	734 <sup>s</sup>	2679 <sup>s</sup>	179 <sup>s</sup>	1008	785	0	442	80	124	290 <sup>s</sup>	163	7307 <sup>s</sup>

<sup>s</sup> statistically significant differences between control and stressed plants

E-S = Elstar stress, E-C = Elstar control, JW-S = Jonagold stress, JW-C = Jonagold control

Our results indicate that the tolerance to water deficit depends on the apple tree cultivar. For better understanding of responses of apple tree cultivars on drought stress analyses of additional biochemical parameters are necessary.

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