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EFFECT OF ROOTSTOCKS ON LEAF WATER POTENTIAL, WATER RELATIONS, ANTIOXIDANT ACTIVITIES AND DROUGHT TOLERANCE IN FLEMISH BEAUTY PEAR UNDER WATER STRESS CONDITIONS

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SUMMARY

A study was conducted to investigate the effect of two *Pyrus* seedling rootstocks [Kainth (*Pyrus pashia* Buck and Ham.), Shiara (*Pyrus serotina* Redh.) and three clonal quince (*Cydonia oblonga* Mill) rootstocks [BA 29, Quince A and Quince C] on growth, water relations and antioxidant activities of one-year-old Flemish Beauty pears at -0.5 bars and -10.0 bars (water-stress) of soil moisture levels. Plant height, shoot and root dry weights of plants on different rootstocks decreased markedly by water stress. Plants on Kainth and BA 29 rootstocks were less affected under water stress than those on other rootstocks. Plants on Kainth and BA 29 rootstocks maintained higher leaf water potential and showed less reduction in stomatal conductance and photosynthetic rate. These plant accumulated more soluble sugar, ascorbic acid and proline content in their leaves in comparison to plants on other rootstocks under water stress conditions. The antioxidant enzymes (catalases, peroxidases and superoxide dismutase) activities increased significantly under water stress and increase was higher in plants on Kainth and BA 29 rootstocks than those on other rootstocks. The results suggest that plants on Kainth and BA 29 rootstocks exhibit better protection mechanism against oxidative damage under water stress conditions than those on other rootstocks.

***Key words*: Antioxidant enzymes, Flemish Beauty pear, water relations, water stress.**

INTRODUCTION

Pear (*Pyrus communis* L.) is an important fruit crop in temperate regions of India and grown successfully in Himachal Pradesh, Jammu and Kashmir and Uttarakhand. In these areas, pear often develops variable moderate water stress due to low precipitation during summer season, and results in decreased yield. Plant respond to water stress at morphological, anatomical and cellular levels by modifications to avoid the stress or increase tolerance (Bray 1997). The extent to which the photosynthetic capability is maintained during periods of water stress and the ability of rapid recovery of photosynthesis after rewatering also play important role in plant adaptation to drought environments (Yordanov *et al.* 2003). Under water stress conditions, the accumulation of osmolytes such as soluble sugar and proline takes place, which act as osmotic buffers in plant to drought stress.

Abiotic stress conditions cause the production and accumulation of reactive oxygen species (ROS) (Apel and Hirt 2004) which often induces cellular damage. In order to avoid the harmful effects of ROS, plants have evolved an effective scavenging system comprised of non-enzymatic antioxidants (tocopherol, ascorbate, carotenoids) and enzymatic antioxidants, such as catalase (CAT), peroxidase (POD) and superoxide dismutase

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(SOD). As a major scavenger, SOD catalyzes the disproportion of superoxide radicals to yield molecular oxygen (O_2) and hydrogen peroxide (H_2O_2), whereas, CAT and POD decompose H_2O_2 to water and oxygen (Sairam *et al*. 2005). Use of drought tolerant rootstock(s) can help in improving cropping and quality of fruits under water stress conditions. A better understanding of tolerance response of plants on different rootstocks to water stress will help to enhance irrigation management practices. The present investigation was conducted to analyse role of physiological processes and antioxidant enzymes activity to combat water stress and to screen out promising rootstock(s) of Flemish Beauty pear for drought tolerance.

MATERIALS AND METHODS

The experiment was conducted at an experimental orchard of the Department of Fruit Science, University of Horticulture and Forestry, Nauni, Solan (H.P) during 2005-2006. One-year-old uniform 'Flemish Beauty' pear plants grafted on two *Pyrus* seedlings [Kainth (*Pyrus pashia* Buck and Ham.) and Shiara (*Pyrus serotina* Redh.)] and three clonal quince (*Cydonia oblonga* Mill) rootstocks (BA 29, Quince A and Quince C) were used as experimental material. The experimental plants were grown in a plastic greenhouse under ambient environmental conditions during February 10, 2005 in pots (60 cm diameter), filled with 3:1 (V: V) mixture of sandy loam soil and farm yard manure. Soil water potential was monitored throughout the experimental period. The plants were subjected to -0.5 bars (control) and -10.0 bars (water-stress) treatments, 60 days after planting. Under control irrigation was applied when soil water potential reached -0.5 bar, and in water stress treatment, irrigation was applied at -10.0 bar soil water potential. The soil water regimes were maintained from March to August. The quantities of water applied per irrigation to bring the soil moisture from –0.5 and –10.0 bars to field capacity were 4.10 and 17.50 liters, respectively.

Plant height, shoot and root dry weight of plants were determined at the end of the experiment. After measuring plant height, the plants were separated into roots and shoots, dried in hot-air oven at 75 ^{0}C for 48 hours, and dry weight of shoots and roots were recorded. Leaf water potential (ψ_w) was measured using pressure chamber apparatus and stomatal conductance (g_s) and photosynthetic rates (Pn) were measured using portable photosynthesis meter (LI-6400, USA) between 10:00 to 11:00 AM. Soluble sugar was determined by the anthrone method (Wu and Xia 2006). Ascorbic acid content was estimated as described by Mukherjee and Chaudhari (1983) and free proline was extracted from 0.5 g of fresh leaf samples in 3% (w/v) aqueous sulphosalicyclic acid and was estimated by ninhydrin reagent (Bates *et al.* 1973).

Fresh leaf samples were homogenized in 5 ml of phosphate buffer (0.1 mol l^{-1}, pH 7.8), centrifuged at 4,200 xg for 10 min at 4^{0}C, and the supernatant was used for assays of CAT, POD and SOD activities. Catalase (CAT) activity was determined as per the method described by Thimmaiah (2001), and one unit of catalase was defined as that amount of enzyme which breakdown 1μ mole of H_2O_2 under standard assay condition. The concentration of H_2O_2 was calculated using the extinction coefficient 0.036/ìmol/ml. POD activity was determined using the method of Chance and Maehly (1955). SOD activity was analyzed using the method of Stewart and Bewley (1980) and was expressed as unit g $^{-1}$ fresh weight (fwt.). One unit of SOD was defined as the amount of enzyme that inhibited 50% nitro blue tetrazolium by light. The experimental data were subjected to analysis of variance (ANOVA) in accordance with the procedure described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Plant height, shoot and root dry weights of plants on different rootstocks decreased significantly with water stress (Table 1). Drought stress caused great reduction in vegetative growth of pear, particularly during early period of growth (Teng *et al.* 1999). Plant height and shoot dry weight were maximum in plants grafted on Kainth rootstocks, whereas root dry wt. was highest in plants on BA 29 rootstocks at -0.5 and -10.0 bars. The drought tolerance of rootstocks can be measured in terms of growth status under drought stress (Levy *et al.* 1978). In this study, we observed that the growth inhibition caused by water stress was least in plants on Kainth, followed by plants on BA 29 rootstocks than those on other rootstocks at -10.0 bars of soil moisture

Table 1. Effects of rootstocks on growth and plant biomass production of Flemish Beauty under two soil moisture regimes.

Rootstocks	Plant height (cm)		Mean	Shoot dry wt. (g plant^{-1})		Mean	Root dry wt. (g plant^{-1})		Mean
	-0.5 bars	-10.0 bars		-0.5 bars	-10.0 bars		-0.5 bars	-10.0 bars	
Kainth	136.6	120.8	128.7	142.4	110.1	126.3	116.3	95.2	105.8
Shiara	132.2	106.6	119.4	128.4	92.7	110.6	104.8	68.4	86.6
BA 29	126.9	112.4	119.7	122.0	100.4	111.2	120.6	104.2	112.4
Quince A	124.1	102.7	113.4	120.8	78.6	99.7	108.4	60.8	84.6
Quince C	117.7	95.2	106.5	106.6	67.4	87.0	96.4	45.2	70.8
Mean	127.5	107.5		124.0	89.8		109.3	74.8	
$CD_{0.05}$		**Plant height**			**Shoot dry wt.**			**Root dry wt.**	
Moisture levels (M)		4.46			7.48			5.24	
Rootstocks (R)		4.85			8.24			5.68	
M X R		8.87			14.26			10.80	

tension. Similarly, Chandel and Chauhan (1991), reported less reduction in growth and vigour of Starking Delicious apple on drought tolerant rootstocks under water stress conditions.

Significant differences in the leaf water potential (ψ_w), stomatal conductance (g_s), and photosynthetic rate (Pn) of plants were detected at -0.5 and -10.0 bars of soil moisture tension (Table 2). Plants on Kainth showed highest leaf water potential (ψ_w) which was statistically at par with those on BA 29 rootstocks at -10.0 bars. Leaf water potential has been used as a sensitive indicator of plant water stress (Naor 1998). The ψ_w, g_s and Pn were highest in plants on Quince C rootstocks

Table 2. Effects of rootstocks on leaf water potential (ψ_w), stomatal conductance (g_s) and photosynthetic rate (Pn) in Flemish Beauty pear under two soil moisture regimes.

Rootstocks	ψ_w (-MPa)		Mean	g_s (mmol m^{-2} s^{-1})		Mean	Pn (μmol m^{-2} s^{-1})		Mean
	-0.5 bars	-10.0 bars		-0.5 bars	-10.0 bars		-0.5 bars	-10.0 bars	
Kainth	0.67	1.50	1.09	4.32	3.58	3.95	18.60	14.20	16.40
Shiara	0.71	1.82	1.27	4.46	2.68	3.57	17.94	11.61	14.78
BA 29	0.69	1.52	1.11	4.28	3.47	3.88	19.34	14.22	16.78
Quince A	0.74	1.87	1.31	4.72	2.90	3.81	19.20	12.66	15.93
Quince C	0.80	1.92	1.36	4.78	2.36	3.57	20.07	11.18	15.63
Mean	0.72	1.73		4.51	3.00		19.03	12.77	
$CD_{0.05}$		**ψ_w**			**g_s**			**Pn**	
Moisture levels (M)		0.18			0.36			0.58	
Rootstocks (R)		0.20			0.42			0.62	
M X R		0.38			0.89			1.26	

at -0.5 bars. However, plants on Kainth rootstocks showed highest g_s and Pn at -10.0 bars. The higher rates of Pn in plants on these rootstocks could be attributed to their higher stomatal conductance (g_s) at -10.0 bars. The drought tolerant plant control stomata function to allow carbon fixation under drought stress conditions, thus improving photosynthetic efficiency, or open stomata rapidly when water deficit is relieved (Yordanov *et al.* 2003). The Pn of plants on Kainth and BA 29 rootstocks recovered fully after rewatering, while the plants on Quince C rootstocks showed less recovery in the photosynthetic rate (data not given).

The data presented in Table 3 reveal that plants irrigated at -10.0 bars accumulated significantly higher soluble sugar, ascorbic acid and proline contents in their leaves than those irrigated at -0.5 bars. Plants when subjected to water stress accumulate soluble sugar, that is used for osmotic adjustment (Rhodes *et al.* 1986). The results of present study indicate that plants on Kainth and BA 29 rootstocks accumulated more ascorbic acid and proline contents in their leaves at -10.0 bars in comparison to those raised on other rootstocks. The lowest ascorbic acid and proline contents were recorded in the leaves of plants on Quince C rootstocks. However, differences in ascorbic acid and proline content in plants on Shiara, Quince A and Quince C rootstocks were non-significant under water stress conditions. Ascorbic acid, a non-enzymatic antioxidant, is associated with H_2O_2 scavenging (Sairam *et al.* 1998), which also react with superoxide radicals ($O_2^{\cdot-}$), hydroxyl radicals ($OH^{\cdot-}$) and lipid hydroperoxidase (Reddy *et al.* 2004). A role for increased ascorbic acid content in amelioration of oxidative stress has also been reported by Reddy *et al.* (2004). Increased proline level is another common response of plants upon osmotic stress. Proline has a function of osmotic adjustment in plants, but it also protects enzyme and membranes against oxidative stress (Agarwal and Pandey 2004). The enhanced accumulation of soluble sugars, ascorbic acid and proline content in plants on Kainth and BA 29 rootstocks thus, can be expected to protect the plants from oxidative stress during drought stress.

In the present study, water-stressed plants showed higher activities of CAT, POD and SOD in their leaves than well-watered plants regardless of rootstocks used. Sofo *et al.* (2005) also reported that water stress induces stimulation of antioxidant system in olive trees. The activities of CAT and SOD were highest in plants grafted on Kainth under water stress conditions, whereas, POD activity was highest in plants on BA 29

Table 3. Effects of rootstocks on soluble sugars, ascorbic acid and proline content of Flemish Beauty pear under two soil moisture regimes.

Rootstocks	Soluble sugars (%)		Mean	Ascorbic acid (μmol g^{-1} fw)		Mean	Proline (μmol g^{-1} fw)		Mean
	-0.5 bars	-10.0 bars		-0.5 bars	-10.0 bars		-0.5 bars	-10.0 bars	
Kainth	3.35	3.87	3.61	9.14	14.20	11.67	0.68	1.27	0.98
Shiara	3.14	3.51	3.33	8.80	11.10	9.95	0.62	0.98	0.80
BA 29	3.62	4.29	3.96	9.16	13.92	11.54	0.65	1.23	0.94
Quince A	3.95	4.39	4.17	8.98	10.84	9.91	0.57	0.88	0.73
Quince C	4.20	4.50	4.35	9.10	10.48	9.79	0.55	0.83	0.69
Mean	3.65	4.11		9.04	12.11		0.61	1.03	
$CD_{0.05}$		Soluble sugars			Ascorbic acid			Proline	
Moisture levels (M)		0.10			0.87			0.18	
Rootstocks (R)		0.13			0.94			0.20	
M X R		0.26			1.60			0.42	

Table 4. Effects of rootstocks on antioxidant enzymes activities of Flemish Beauty under two soil moisture regimes.

Rootstocks	CAT ($U\ min^{-1}\ g^{-1}$ fw)		Mean	POD ($U\ min^{-1}\ g^{-1}$ fw)		Mean	SOD ($U\ g^{-1}$ fw)		Mean
	-0.5 bars	**-10.0 bars**		**-0.5 bars**	**-10.0 bars**		**-0.5 bars**	**-10.0 bars**	
Kainth	4.50	7.51	6.01	0.63	1.75	1.19	4.20	8.25	6.23
Shiara	3.85	4.75	4.30	0.76	1.26	1.01	3.90	5.65	4.78
BA 29	4.19	7.20	5.70	0.86	1.84	1.35	4.60	7.95	6.28
Quince A	3.48	4.62	4.05	0.55	1.20	0.88	4.20	5.75	4.98
Quince C	3.16	4.41	3.79	0.51	1.15	0.83	4.80	5.70	5.25
Mean	3.84	5.70		0.66	1.44		4.34	6.66	
$CD_{0.05}$		**CAT**			**POD**			**SOD**	
Moisture levels (M)		0.36			0.11			0.16	
Rootstocks (R)		0.41			0.14			0.17	
M X R		0.84			0.28			0.34	

rootstocks (Table 4). However, there were non-significant differences in CAT, POD and SOD activities of water stressed plants on Kainth and BA 29 rootstocks. Furthermore, plants on Quince C rootstocks showed lowest activities of antioxidant enzymes under stress conditions. The ability of plants to up regulate antioxidant enzymes activities is an important attribute linked to drought tolerance. The ROS produced by drought stress often induces cellular damage (Apel and Hirt 2004) and the higher antioxidant enzymes activities were related to detoxification of ROS (Goicoechea *et al.* 2005). Superoxide dismutase reacts with superoxide radicals to produce H_2O_2 and H_2O_2, like other ROS can be expected to be responsible for lipid peroxidation, so plants need to destroy it by CAT and POD (Sairam *et al.* 2005). When plants on Kainth and BA 29 rootstocks had higher antioxidant enzymes activities, cellular and photooxidative damages would be decreased. However, observations on ROS metabolism of rootstocks seedlings would be required to explain, how precisely grafted pear plants on different rootstocks responded variably to water stress in this study. The results of present study are in conformity with the findings of Champakan *et al.* (2005), who observed higher activities of CAT, POD and SOD in the leaves of drought tolerant genotypes of coconut compared to susceptible ones.

This study concludes that among the five rootstocks used, Kainth and BA 29 rootstocks have an ability to maintain growth and water balance of Flemish Beauty pear during water stress. The protection mechanism against oxidative damage by maintaining higher proline, ascorbic acid and antioxidant enzymes activities in plants on these rootstocks could be involved in drought tolerance. However, further studies on water stress tolerance potentials of non-grafted rootstocks seedlings are required for better understanding of this stress mechanism imparted by Kainth and BA 29 rootstocks on grafted scion cultivar.

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