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The response of transgenic strawberry plants overexpressing a drought induced gene to water stress

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Summary

Transgenic strawberry plants expressing a chitinase gene were evaluated for their performance during water stress. Transgenic and non-transgenic plants were assigned to three different soil water contents (SWC). They were kept under well-watered, moderately watered or stressed water conditions. At final stage of experiment, dry matter components, leaf area, photosynthesis rate, water-use efficiency (WUE) and water use per leaf area (WULA) were measured. Transgenic lines showed vigorous growth as compared with non-transgenic plants. Leaf area (LA), leaf dry matter (LDM), root dry matter (RDM) and total dry matter (TDM) of well-watered and water-stressed plants of transgenic lines were significantly higher than those of non-transgenic plants. The WUE increased significantly in transgenic lines, while water use (WU) per leaf area reduced in transgenic plants relative to control plants. Photosynthetic rates were not different between transgenic and non-transgenic plants. Soil water contents significantly affected dry matter production, and photosynthetic rates. Transgenic plants also showed vigorous growth in comparison to non-transgenic plants when grown *in vitro*. Shoot, root and total fresh and dry weight of *in vitro* transgenic lines were significantly higher than those of non-transgenic plants.

Introduction

Cell dehydration is a common factor in major environmental stresses such as drought, high salinity and cold temperature. Although water is essential for growth and development of all plants, a high diversity in adaptation to water availability exist among different plant species. In one extreme, resurrection plants become desiccated and completely air-dry during drought spans and upon rehydration they revive and resume growth. In another extreme, some higher plant species are adapted for life in the water. In response to environmental stresses, many plant species express a similar set of genes and produce common proteins. The precise functions of most of these proteins are not well known.

Many stress-induced genes have been identified and isolated from wild species, which demonstrate excellent tolerance to biotic and abiotic stresses (Leckband and Lorz, 1998; Oldach et al., 2001). Some of these genes have been introduced into cultivated plants in order to improve their performance under stress conditions or to study the gene function (Chalavi et al., 2003).

Chitinases are proteins of 25-35 KDs molecular weight and their expression is induced by biotic and abiotic stresses in higher plants (Hong and Hwang, 2006). These proteins are widely distributed in bacteria, fungi, animals, and plants (Park et al., 2000; Merecedes Dana et al., 2006; Punja and Zhang, 1993). Plant chitinases are endo-chitinases and are able to hydrolyze chitin of hypha walls, it is speculated that these enzymes are involved in plant defense against fungal attack and resistance to abiotic stresses (Chalavi et al., 2003; Schickler and Chet, 1997; Mercedes Dana et al., 2006). Moreover, the induction of chitinase gene by osmotic stress (Chen et al., 1994) implies that in addition to the known function in plant defense against fungal diseases, this protein might play other roles in plant metabolism (Kasprzewska, 2003). In recent years, there are some reports indicating the involvement of chitinases in flower formation (Takakura et al., 2000; Neale et al., 1990; Lotan et al., 1989), fruit ripening (Peumans et al., 2002; Robinson et al., 1997), embryogenesis (De Jong et al., 1992), seed germination (Caruso et al., 1999), and insect resistance (Van der Westhuizen et al., 1998). Chitinases are also assumed to be involved in freezing tolerance (Yeh et al., 2000; Hiilovaara et al., 1999). Other reports indicated the role of chitinases in drought tolerance (Yu et al., 1997) and enhancement plant fungal defence mechanisms (Karasuda et al., 2003). Clendennon et al. (1998) hypothesized that a class III banana pulp-specific chitinase is a storage protein. Due to its expression pattern and deduced amino acid sequences the authors dismissed the possibility of its involvement in plant defensive mechanism. On the other hand, the principal role of a *Lupinus albus L.* class III chitinase, constitutively expressed in vegetative organs and developing seeds, assumed to be in antifungal activity (Regalado et al., 2000).

Water use efficiency (WUE), dry matter production per unit of consumed water, is one of the factors which evaluate plant physiological behavior under favorable or stressful environmental conditions. It has been shown that WUE increases during water stress for plant species that could cope with other environmental conditions (Raeini-Sarjaz and Barthakur, 1997).

In the present study, we hypothesized that transgenic strawberry plants constitutively expressing a drought induced chitinase gene may have improved tolerance to water stress. We therefore produced transgenic strawberry lines overproducing chitinase protein. These strawberry transgenic lines were phenotypically normal. The growth parameters of strawberry transgenic lines, J_A and J_M, with non-transformed one, J, were compared.

Materials and methods

Plant material:

Production of transgenic plants:

The production of transgenic strawberry cv. Joliette plants have been described eleswhere (Chalavi et al., 2003). Briefly, the coding region of a chitinase gene from *Lycopersicon chilense (*a wild tomato), induced by drought and abscisic acid (Chen et al., 1994), was placed under the transcriptional control of cauliflower mosaic virus (CaMV) 35S of binary pBin19 vector (Bevan, 1984). The T-DNA of the pBin19 vector contains a kanamycin (Kan) selection marker gene. The resulting vector was introduced into *Agrobacterium tumefaciens* strain LBA4404 (Hoekma et al., 1983) in a mix electroporation. *In vitro* shoots of strawberry (cv. Joliette), were transferred by stipule transformation method (Chalavi et al., 2003). Trandgenic shoots were recovered and rooted on MS (Murashige and Skoog, 1962) medium containing 100 mgl^{-1} kanamycin for selection and 500 g ml^{-1} carbenicillin for killing *Agrobacterium*. Positively trandgenic

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plantlets, identified by PCR (polymerase chain reaction), Northen and Southern blot analysis (CHALAVI et al., 2003), were propagated and used in this experiment.

In vitro grown plants

To evaluate the effect of chitinase gene on growth enhancement of strawberry (cv. Joliette), two transgenic lines along with non-transformed shoots, as control, were grown on half-strength MURASHIGE and SKOOG (1962) salts and B5 vitamins (GAMBORG et al., 1968), solidified with 0.6% of agar. The culture was kept at 26 °C under 16 h photoperiod and 50-70 µmol m^{-2} s^{-1} light intensity. After 2 months, plants were removed from culture medium, washed with deionized water, blotted with paper towels and the fresh weight of shoots and roots were recorded. To measure the dry weight, shoots and roots were dried in an oven at 60 °C for 72 h.

Soil grown plants

Strawberry transgenic and control (non-transformed) *in vitro* rooted plantlets were transferred to sterile soil and acclimated for 2 weeks under plastic cover. After 6 weeks, plants were transplanted in 2-L pots containing 1.6 L of composite soil made up of soil, sand and vermiculite (75, 12.5 and 12.5%, respectively).

Potted plants were randomly assigned to different soil water contents (SWC) as described elsewhere (RAEINI-SARJAZ and CAHALAVI, 2011); well-watered (W_0), moderately-watered (W_1), and water-stressed (W_2). Two transgenic lines, J_A and J_M, and control strawberry plants were grown in pots under a completely randomized design. Five pots (replications) were used for each treatment. Well-watered plants were initially watered to 100% soil water content (SWC) and re-watered when soil water dropped to 80%. Soil water content of W_1 and W_2 plants initially were kept to 80% and 50% SWC and water was supplied when SWCs reached 60% and 30%, respectively. The total amount of the supplied water to each plant was calculated at the end of the experiment. Plants were fertilized uniformly with 20-20-20 NPK fertilizer every week during experiment.

Gas exchange measurements:

After three weeks, when plants had 4 extended leaves, all plants were watered to 100% SWC. One hour later, when plants were acclimatized to new condition, instantaneous leaf photosynthesis rates (P_i) were measured on newly developed leaves of all plants of different long-term soil water contents and transgenic lines using a photosynthesis measurement system, Li-6400 (LiCor Inc., Lincoln, Nebraska, USA). The photon flux density was kept constant at 800 µmol m^{-2} s^{-1} and mean CO_2 concentration was closed to 400 ppm. After one day, when soil water content dropped to 60%, photosynthesis rates were measured again on the same leaves.

Plant shoot and root measurements:

After four weeks, the plants' shoot and root components were harvested and weighed. Leaf area of each plant was measured using a leaf area meter, LI-3100 (LiCor Inc., Lincoln, Nebraska, USA). Plants growth components were dried in an oven at 60 °C for 72 h to determine leaf dry matter (LDM), petiole dry matter (PDM), root dry matter (RDM) and total dry matter (TDM) with an electronic balance of ± 0.001 g precision. Water use efficiency (WUE) of each treatment was calculated as total dry matter production (TDM) to total used water (WU) as transpiration, by plant, and evaporation from pot surface.

Statistical methods

Data presented as mean ± STD. Fresh and dry weight data were evaluated using a two factor analysis of variance (ANOVA) procedures for soil grown plants and one-way ANOVA for *in vitro* plants. When the main treatment effects showed significant differences, the Tukey's post hoc tests were used to determine if significant differences exist between transgenic lines and water treatments. Associations between different growth components were determined using Pearson Product-Moment Correlation Coefficients. The accepted level of significance was $p<0.05$.

Results

In vitro experiment

Shoot, root and total fresh weight (SFW, RFW and TFW, respectively) of *in vitro* plants were significantly higher ($p<0.001$) in transgenic plants relative to nontransgenic ones (Tab. 1). SFW of J_A and J_M, were significantly higher by 59.6 and 55.3% compared with control plants. RFW of the J_A and J_M was also higher (136.4 and 128.2% respectively) compared to non-transgenic plants. The same trend was observed for TFW, which increased by 74.1 and 86.2% for J_A and J_M, respectively, compared to control plants (Tab. 1).

Shoot dry weight (SDW) of J_A and J_M were higher by 50 and 62.5%, respectively, compared to control plants. Root dry weight (RDW) of J_A and J_M also increased by 200 and 400%, respectively, compared to non-transgenic control plants. Total dry weight of transgenic, J_A and J_M, plants increased by 68 and 100%, respectively, compared to control plants (Tab. 1).

Tab. 1: Mean (± STD) *in vitro* shoot (SFW), root (RFW) and total fresh weight (TFW), and shoot (SDW), root (RDW) and total (TDW) dry weight data of transgenic and non-transgenic plants (g/plant).

Treatments	Fresh weight (g/plant)		
	Shoot	Root	Total
Transgenic J_A	0.75±0.19 [a]	0.26±0.12 [a]	1.01±0.29 [a]
Transgenic J_M	0.73±0.12 [a]	0.35±0.13 [a]	1.08± 0.20 [a]
Non-transgenic	0.47±0.12 [b]	0.11±0.05 [b]	0.58±0.15 [b]
	Dry weight (g/plant)		
Transgenic J_A	0.12±0.03 [a]	0.03±0.01 [a]	0.15±0.04 [a]
Transgenic J_M	0.13±0.02 [a]	0.05±0.02 [a]	0.18±0.04 [a]
Non-transgenic	0.08±0.02 [b]	0.01±0.01 [b]	0.09±0.02 [b]

Means in each column that carry the same letter are not significantly different ($p<0.05$).

Pot experiment

The effects of strawberry transgenic lines and soil water content on overall performance of examined plants were highly significant ($p<0.001$). Leaf and root dry matters (LDM and RDM, respectively) of transgenic plants were significantly higher than those of control plants ($p<0.0001$). In well-watered condition, J_A and J_M transgenic plants produced almost 40% more leaf dry matter compared with control plants. When LDM of moderate watered plants were compared, transgenic plants (J_A and J_M) performance were similar to those of control plants (Tab. 2). While in water stressed plants leaf dry matter of transgenic plants J_A and J_M was higher by 51.0 and 41.8%, respectively, compared to that of control plants (Tab. 2). Mean RDM of transgenic plants was significantly higher (41.6%) than that of non-transgenic plants. Total dry matter (TDM) of

transgenic plants also was significantly ($p<0.001$) higher than that of control plants. Well-watered transgenic J_A and J_M plants overgrew non-transgenic control plants by 40 and 44.8%, respectively. Under moderate water condition TDM of transgenic and control plants were not statistically different, while transgenic plants (J_A and J_M) for stressed-water condition outperformed those of control plants by 53.4 and 43.8%, respectively (Tab. 2).

Plant leaf area (LA) had the same trend as dry matter components; there was an overall significant difference ($p<0.0001$) between LA of transgenic plants and that of control plants. For well-watered transgenic J_A and J_M plants leaf area increased by 27.5 and 29.3%, respectively, in comparison to non-transgenic control plants. Again, when moderate watered plants were considered no differences were found between transgenic and non-transgenic plants, while severely stressed transgenic plants (J_A and J_M) leaf area increased by 46.4 and 32.4%, respectively, compared with control plants (Tab. 2 and Fig. 1). Soil water contents also significantly ($p<0.001$) affected LA (Fig. 1).

The expression of chitinase gene in strawberry had no effect on specific leaf area (SLA) or root to shoot ratio (Tab. 3). Water use (WU) during plant growth and development was highly affected by transgenic strawberry and soil water content ($p<0.001$). Mean water consumption of transgenic plants increased by 10% relative to control plants. Well watered and water-stressed transgenic plants on average consumed 16% and 21% more water, respectively, relative to those of control plants, while moderate watered transgenic plants and corresponding control plants were not different (Tab. 3). When water use per leaf area (WU LA^{-1}) was considered, non-transgenic plants on average consumed more water (14%) compared to transgenic plants (Tab. 3). In all three lines both WU and WU LA^{-1} increased as soil water content increased (Tab. 3 and Fig. 1b).

Transgenic lines and soil water content had significant ($p\leq0.001$) effects on water use efficiency (WUE). Mean WUE of transgenic J_A and J_M plants was significantly higher (24% and 15%, respectively) than that of control plants (Tab. 3). Transgenic well, moderate and stress watered plants WUEs increased (23%, 16% and 19%, respectively) relative to those of control plants (Tab. 3 and Fig. 1c). The effect of soil water content on WUE was also highly significant and stressed plants on average produced higher dry matter for unit of consumed water (5.32 g/kg) relative to moderate and well watered plants (4.71 and 3.59 g/kg , respectively) (Fig. 1c).

Mean instantaneous photosynthetic rates of different lines under different soil water conditions were not statistically different when watered to 100% or 60% of SWC (Tab. 4). While soil water content significantly ($p<0.001$) affected mean photosynthetic rates, when soil water content was kept at 100% or 60% (Tab. 4). Mean photosynthetic rates across different lines under 60% SWC significantly dropped for both well and moderate soil contents, while it did not changed under longterm stressed plants (Tab. 4).

Discussion

The main finding of this study is that the constitutive and high level expression of chitinase gene in strawberry (Chalavi et al., 2003) enhanced growth of transgenic plants. To our knowledge, this study is the first study to evaluate the effects of a chitinase gene expression and soil water content on growth of strawberry plants. Chitinase enzymes have been suggested to be implicated in a number of plant normal metabolic functions such as hypocotyle, leaf, root and seed developments (Robinson et al., 1997). Although chitinase activity has been correlated with growth, the mechanism of such involvement is not well known. Stress factors, mainly pathogen infections and abiotic stresses are responsible for chitinases induction (Kasprzewska, 2003). The role of these enzymes is usully considered as a defence mechanism against pathogens (Chalavi et al., 2003; Regalado et al., 2000). Besides defence mechanism of chitinases against pathogen attack (Patil et al., 2000), it is speculated that, they may get involved in stress response and also in growth and development processes (Regalado et al., 2000) by

Tab. 2: The effect of soil water content and strawberry lines on mean leaf area (LA, cm^2), leaf (LDM), petiole (PDM), root (RDM) and total (TDM) dry matter (g/plant) of transgenic and non-transgenic strawberry plants (mean ± SD).

Treatments	LA	LDM	PDM	RDM	TDM
Transgenic J_A					
Well-water	1759.9±106.2	9.69±0.77	2.99±0.14	2.31±0.42	14.96±1.08
Moderate	1419.4±79.6	7.24±0.45	2.37±0.30	1.74±0.27	11.34±0.97
Stress	1086.7±77.6	4.96±0.42	1.77±0.47	1.30±0.22	8.04±0.67
Maen*	1422.0±298.9[a]	7.29±2.07[a]	2.37±0.56[a]	1.78±0.54[a]	11.45±3.08[a]
Transgenic J_M					
Well-water	1783.9±108.6	9.80±0.98	3.14±0.28	2.55±0.82	15.48±10.08
Moderate	1335.1±216.8	6.41±1.38	2.21±0.41	1.40±0.43	10.02±2.20
Stress	982.5±102.2	4.65±0.71	1.62±0.13	1.26±0.15	7.54±0.93
Mean*	1367.2±374.1[a]	6.95±2.45[a]	2.32±0.71[a]	1.73±0.82[a]	11.01±3.90[a]
Non-transgenic					
Well-water	1379.8±73.8	6.98±0.74	2.27±0.22	1.44±0.28	10.69±1.22
Moderate	1227.4±93.5	6.41±0.92	1.97±0.25	1.46±0.20	9.84±1.27
Stress	742.2±95.6	3.28±0.48	1.08±0.19	0.88±0.21	5.24±0.78
Mean*	1116.5±295.8[b]	5.56±1.84[b]	1.77±0.57[b]	1.26±0.37[b]	8.59±2.73[b]

*Means across different soil water contents for each strawberry line. Means in each column that carry the same letters are not significantly different ($p<0.05$).

Tab. 3: The effect of soil water contents and strawberry lines on mean specific leaf area (SLA, $cm^2\ g^{-1}$), shoot to root ratio, water use (WU, kg/plant), water-use efficiency (WUE, $g\ kg^{-1}$) and water use per leaf area (WU LA^{-1}, $g\ cm^{-2}$) of transgenic and non-transgenic strawberry plants (mean ± SD).

Treatments	SLA	Shoot/ Root	Water use	WUE	WU LA^{-1}
Transgenic J_A					
Well-water	5.49±0.14	0.181±0.013	4.07±0.07	3.62±0.11	2.32±0.03
Moderate	5.09±0.05	0.180±0.010	2.15±0.05	5.28±0.11	1.51±0.01
Stress	4.56±0.09	0.194±0.017	1.36±0.08	6.00±0.43	1.25±0.07
Mean*	5.05±0.19 [a]	0.185±0.008[a]	2.525±0.53[a]	4.98±0.51[a]	1.67±0.29 [b]
Transgenic J_M					
Well-water	5.48±0.14	0.193±0.012	3.92±0.12	3.97±0.31	2.21±0.11
Moderate	4.76±0.17	0.158±0.012	2.15±0.15	4.61±0.25	1.62±0.04
Stress	4.72±0.17	0.202±0.008	1.44±0.08	5.26±0.25	1.47±0.07
Mean*	4.99± 0.22 [a]	0.184±0.016[a]	2.504±0.49[a]	4.61±0.34[a]	1.77±0.26 [b]
Non-transgenic					
Well-water	5.05±0.19	0.155±0.007	3.43±0.08	3.15±0.15	2.49±0.05
Moderate	5.20±0.19	0.175±0.008	2.32±0.07	4.24±0.20	1.90±0.06
Stress	4.42±0.11	0.202±0.020	1.12±0.08	4.68±0.24	1.52±0.10
Mean*	4.89± 0.21[a]	0.177±0.015[a]	2.290±0.44[b]	4.02±0.36 [b]	1.96±0.20[a]

*Means (± STD) across different soil water contents for each strawberry lines. Means in each column that carry the same letters are not significantly different across strawberry lines ($p< 0.05$).

Tab. 4: Mean (± STD) photosynthetic rates ($\mu mol\ m^{-2}\ s^{-1}$) of transgenic and non-transgenic strawberry plants across different soil water contents.

Water treatments	Strawberry lines			Mean[1¥]
	Line J_A	Line J_M	Control	
Well	10.84±0.39	10.18±1.44	10.45±0.55	10.49±0.96 [b]
Moderate	10.87±0.98	10.95±1.51	10.58±1.40	10.80±1.33 [b]
Stress	11.46±0.69	12.12±0.68	11.61±0.47	11.72±0.69 [a]
Mean*[2]	11.05±0.81	11.08±1.52	10.86±1.06	
Well	3.78±1.29	4.80±2.50	4.11±1.17	4.44±1.90[b]
Moderate	5.06±1.37	6.39±3.32	7.5.0±2.28	5.90±2.62[b]
Stress	9.38±3.16	10.07±2.47	11.07±1.59	10.04±2.72[a]
Mean**[2]	6.19±3.25	7.08±3.82	7.33±3.19	

* During photosynthesis measurements SWC kept at 100% FC. ** During measurements SWC kept at 60%. [1] and [2], means across different soil water contents and different strawberry lines, repectively, when watered to 100 and 60% SWC. ¥Means in the last column for each separate row that carry the same letter are not significantly different ($p<0.05$).

generating or degrading signal molecules (VAN-DER HOLST et al., 2001). It is assumed that overproduction of ethylene, a plant growth regulator, by enhanced chitinase activity might be involved in enhanced plant growth and development (ZHONG et al., 2002; KASPRZEWSKA, 2003). PATIL et al. (1997) have suggested a role for chitinase in cell wall disruption, presumably in the cell division cycle. They found some correlation between growth and chitinase activity in the seedlings and suspension cultures of transgenic and wild type tobacco plants. In our study in both *in vitro* and pot experiments, strawberry plants overexpressing chitinase gene showed significant growth enhancement compared to non-transgenic plants. The J_A line had a high level of chitinase gene expression showed superiority to J_M plants (CHALAVI et al., 2003), which had a lower expression of the gene, and to non-transformed control plants.

Soil grown transgenic plants produced 25 and 30% more dry weight and leaf area compared to control plants. Likewise, the growth enhancement of the *in vitro* transgenic plants was much higher and transgenic plants dry weight increased more than 83% compared to non-transgenic plants. When plants were exposed to different soil water contents, the growth of both well and stress watered transgenic plants enhanced significantly as compared with non-transformed plants. Interestingly, moderate watered transgenic plants showed no advantage to corresponding control plants. These results could happen if strawberry endogenous chitinase genes were induced only at optimal and stress water conditions, but not at moderate soil water condition. The same circumstance was reported by ALVIN et al., (2001). When they subjected their transgenic and control tobacco plants to progressive water stress, the endogenous binding protein (BiP) was induced in tobacco control plants after withholding water for 1 week, and it was declined after 3 weeks of water deficit The binding protein levels in transgenic plants was steady and high before and during period of progressive water stress.

Contrary to our data and PATIL et al. (1997) findings, BOLAR et al. (2001) have reported a negative correlation between chitinase activity and growth in transgenic apple plants. They produced transgenic apple lines expressing endochitinase gene from *Trichoderma harzianum*. Expression of endochitinase had a negative effect on apple growth; those plants with highest level of endochitinase activity did not grow when transferred to soil. The same authors (BOLAR et al., 2001) also produced transgenic apple plants simultaneously expressing two chitinase genes, an endochitinase and an exochitinase. Growth of these transgenic plants was negatively affected by the level of endochitinase activity and irrespective to the level of exochitinase.

Therefore, they concluded that only the level of endochitinase activity had the negative effect on growth of apple plants.

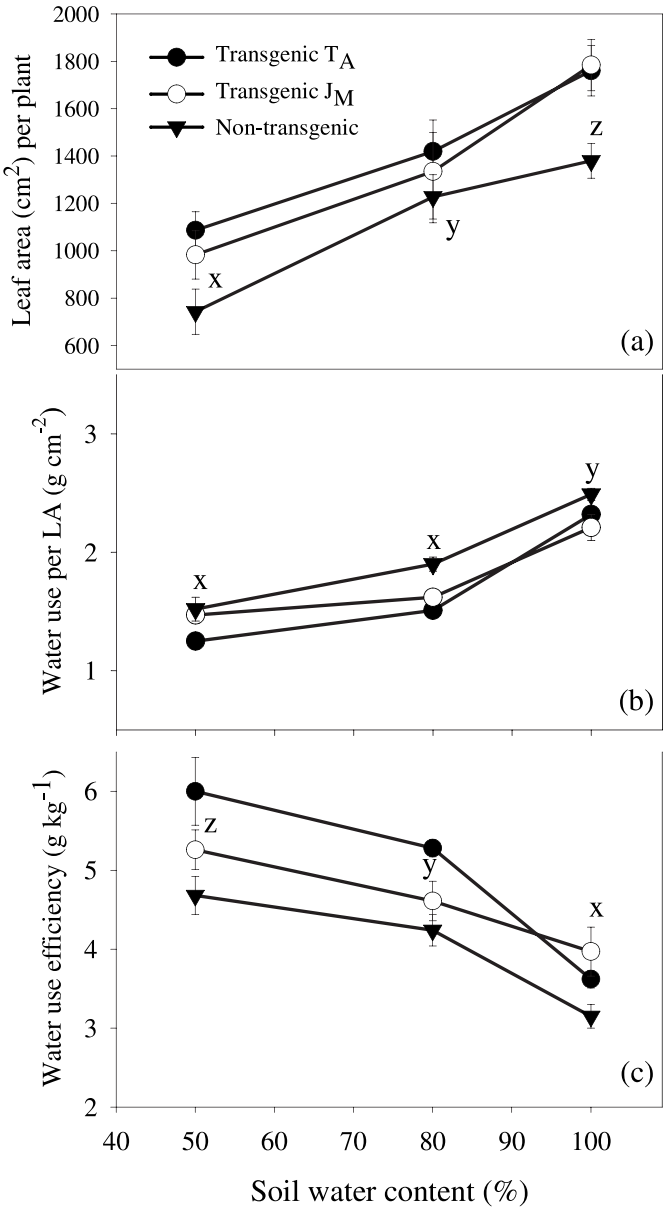

Fig. 1: (a), (b) and (c) respectively show mean leaf area per plant, water use per leaf area, and water use efficiency along different soil water contents for different strawberry lines. different letters of ^{x}, ^{y} and ^{z} on the graph show differences ($p<0.05$) between different soil water contents.

Leaf instantaneous photosynthesic rate (P_i) in transgenic strawberry plants with high level of chitinase expression was not different from control plants. The increased P_i in plants has been shown to be correlated to leaf thickness, or specific leaf weight (THOMPSON et al., 1996). In our study neither SLW nor P_i of plants did change due to chitinase gene. From these results it could be assumed that high level expression of chitinase had no effect on leaf photosynthesic rate. But enhanced growth of transgenic strawberry lines relative to control could be related to other physiological parameters. Therefore, it is speculated that chitinase expression had significant effect on long-term photosynthesic rate through higher stomatal conductance. This assumption is supported by reduction of ^{13}C enrichment in leaf tissues of transgenic lines, which could be an indirect marker for longterm higher stomatal conductance (RAEINI-SARJAZ and CHALAVI, 2011). It has been shown that carbon isotope discrimination against $^{13}CO_2$ in leaf tissues is correlated to dry matter production (ZACHARISEN et al., 1999) and leaf stomatal conductance (OSÓRIO et al., 1998; FARQUHAR et al., 1989). Carbon isotope discrimination due to genotype or growth environment is considered as an indicator of higher long-term stomatal conductance (FARQUHAR et al., 1989). Therefore, higher dry matter production in transgenic plants could be due to larger leaf area and higher long-term stomatal conductance. The ^{13}C enrichment of leaf tissues significantly reduced in transgenic plants compared to control plants (RAEINI-SARJAZ and CHALAVI, 2011), while dry matter increased. This could indicate a long-term higher stomatal conductance to ambient CO_2 relative to non-transgenic plants. The vigorous growth enhancement of transgenic plants for both well and stress watered plants could be related to this long-term stomatal conductance increase. Therefore, the expression of chitinase in these lines kept the stomata open for longer time relative to non-transgenic plants, perhaps via direct or indirect effects on mechanisms which control stomatal conductance. However, this higher performance was not seen for moderate watered transgenic plants compared to corresponding treatment of control plants. The same trend was reported for ^{13}C enrichment of moderate watered transgenic plants (RAEINI-SARJAZ and CHALAVI, 2011). It seems under this experimental condition and probably due to the induction of strawberry endogenous chitinase gene, the moderate watered control plants were able to compete with their transgenic counterpart plants.

The high level of expression of chitinase (CHALAVI et al., 2003) in transgenic lines not only enhanced plant growth, it also increased plants' tolerance to a fungal disease (CHALAVI et al., 2003) and abiotic stresses such as water stress. Water consumption of transgenic plants reduced per leaf area compared to non-transgenic ones. Transgenic lines also demonstrated enhanced WUE and produced more dry matter per unit of consumed water in comparison to non-transgenic ones, which is in agreement with dry matter production and water used per leaf area of these lines. Water stress also enhanced WUE in both transgenic and control plants, in agreement with findings of GRANT et al. (2010) and LIU et al. (2007).

In conclusion although our results demonstrates that the chitinase gene is possibly involved in the growth enhancement of transgenic lines relative to non-transgenic ones under well water and water stressed conditions, further investigation at the protein level would provide more information. Such study might help to define the correlation between the transgene transcript levels, the corresponding protein content and the growth of transgenic plants.

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