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Growth and Photosynthetic Characteristics of Two Strawberry Cultivars in Response to Furostanol Glycosides Treatments

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Abstract

Furostanol glycosides represent a large group of steroid compounds of plant origin with a broad spectrum of biological activities (anabolic, antioxidant, anti-fungal and nematicidal). Most of the research exhibits this effect in stress induced response on different pathogen attacks and only a few studies show the effect of glycoside on plants growth and development. In order to investigate the effects of furostanol glycoside treatment on rooting, growth performance and photosynthetic system efficiency, young unrooted strawberry plants (cv. 'Real' and 'Magic') were immersed in different concentrations (0.03 mM, 0.3 mM, 3 mM) of G1 solution (glycoside extracted from *Lycopersicon* sp.) and G2 (extracted from *Digitalis* sp.) and morphometric parameters were determined. The results showed that immersion in 0.3 mM glycoside solution improved the quality of strawberry planting material by increasing the number and length of roots, as well as by stimulating formation of new leaves. Moreover, the influence of foliar spraying with G1 and G2 on plants growth, assimilator pigments content and photosynthesis was determined. Foliar spraying with both glycosides solutions improved radicular growth and development, but dimensions of foliar apparatus increased only in G1 treated variants. Although both glycoside treatments induced an increase in assimilator pigments content, photosynthetic rate decreased as a consequence of stomatal limitations associated with better efficiency of water use and of internal CO_2, which suggests that these chemicals may have an antitranspirant action.

Keywords: antitranspirant, assimilator pigments, leaves, photosynthesis, roots

Introduction

Strawberry (*Fragaria* × *ananasa* Duch.) cultivation has an important economic and nutritional interest due to its high production potential and fruits quality i.e., rich in vitamin C, manganese, potassium, folic acid, anthocyanins, flavonols and flavonoids which, due to its antioxidant action, plays an important role in preventing neuronal and cardiovascular diseases, even diabetes or cancer.

The economic profitability of this species resides in its ecological plasticity, productivity and precocity that allows extra-seasonal crops, according to the culture technology utilized.

More and more in the last decade, ecological farms have been using environmentally friendly production methods to improve plants yield and fruit quality. A real challenge in ecological fruit production is the reduction of chemical fertilization and some other chemical treatments for pests and disease control. In this direction, scientists actively search for compounds of plant origin that are natural adaptors and do not disturb plants ecological balance. It is supposed that the adaptive effect of some natural compounds is associated with their influence on the state of the lipid components of cell membranes. In light of this, the most promising compounds are steroid glycosides of the furostanol series (Vasil'eva *et al.*, 2005).

Furostanol glycosides represent a large group of steroid compounds of plant origin with a broad spectrum of biological activity. There are many papers related to a great number of compounds belonging to furostanol glycosides which make use of methods of isolation and characterisation (Arthan *et al.*, 2006; Kirmizibekmez *et al.*, 2002; Napolitano *et al.*, 2011; Peng *et al.*, 1995; Yahara *et al.*, 1994) but only few of them mention their specific effect on different organisms. Several studies show that furostanol glycosides may have anabolic (Aswar *et al.*, 2010) antioxidant (Volkova *et al.*, 2007), anti-fungal (Liu *et al.*, 2003) and nematicidal activities (Vasil'eva *et al.*, 2005).

The adaptive effect of furostanol glycosides in biotic stress was first discovered in tomatoes and cucumber plants infested with gull nematode. Activating the general nonspecific systems of stress response, furostanol glycosides extracted from *Dioscorea deltoidea* facilitated the activation of specialized mechanisms of long-term adaptation, which allowed plants to have an increased resistance to biotic stress conditions for an extended period of time (Vasil'eva *et al.*, 2005).

Most of the research related to the role of furostanol glycoside in plants states its effect in stress induced response on different pathogens attack and only a few studies show the effect of glycoside on plants growth and development. According to Munteanu *et al.* (2008), furostanol glycoside

extracted from seeds of *Lycopersicon* sp. and *Capsicum* sp., applied by foliar spraying, has stimulative effect on vegetative growth and increase photosynthetic pigment content in grapevine and apple trees. The hypothesis that glycoside treatments may improve photosynthetic efficiency was also put forward.

Therefore the purpose of this study was to determine the influence of furostanol glycoside treatments on both young and mature strawberry plants growth and to evaluate the possible changes in photosynthetic activity and chlorophylls content.

Materials and methods

Experiments were conducted in the greenhouse of Horticulture Faculty of Iasi, Romania. The two glycosides, provided by the Biological Research Institute from Iasi, were extracted and purified from seeds of *Lycopesicon* sp.- G1 (M=1082) and leaves of *Digitalis* sp. G2 (M=1230). Their influence on strawberry growth and development was studied in two separate experiments.

The first experiment was made in order to determine the optimum glycoside concentration from the two treatment solutions and to evaluate the effect of glycoside treatment on planting material quality of two strawberry cultivars ('Real' and 'Magic'). Therefore, unrooted daughter plants (central bud and 1-2 leaves) were immersed in 3 mM, 0.3 mM and 0.03 mM glycoside solution for one hour and planted in pots, in a mixture of soil: sand: litter in (1:1:1, v:v:v).

The experiments were carried out in the greenhouse and for each variant three replications (10 plants/replication) were used. Photosynthetic active radiation (PAR) was 400-500 μmol m^2s^{-1}, relative humidity 52% temperature 22-23°C and CO_2 concentration 380 μmol mol^{-1}. Plant material quality was evaluated by morphometric measurements: rooting percentage, number and length of roots and number of new leaves.

The second experiment was focused on determining the influence of glycoside treatments (G1 and G2) on growth, photosynthesis and assimilator pigments accumulation in mature strawberry plants. Biologic material was represented by mature plants, (4-5 leaves) from 'Real' and 'Magic' varieties, previously acclimated at greenhouse conditions (similar with those described in the above experiment).

Glycoside treatments were applied by foliar spraying twice a week with 0.3 mM solution; control plants were sprayed with distilled water. After one month the effect of furostanol glycosides on photosynthesis and chlorophyll pigments accumulation was evaluated.

Photosynthetic rate (A), stomatal conductance (g_s), transpiration rate (E) and other parameters were measured using infrared gas analyser (LCi 600, ADC BioScientific Ltd., England) on fully uppermost expanded leaves at saturation light intensity on six plants from each replicate.

For determinations of the assimilator pigments, the youngest fully expanded leaf was collected the same day when gas exchange measurements were made. The total chlorophylls (Chl) and carotenoids (Car) were extracted with 80% acetone. The absorbance was measured on the T70 UV/VIS spectrophotometer and the amount of pigments was calculated according to the equations of Lichtenthaler (1987) method, while results were reported to dry weight.

At the end of experiment total leaf area, root length, and newly developed leaves and roots were determined. For leaf area measurements Image J software for Windows was used.

For each variant 5 plants were used and results are reported as the mean ± standard error (SE) values of three independent experiments, measuring at least three different replicates (plants) in each experiment, followed by Duncan's t-test at $p < 0.05$ for separation of differences between means. Tests assume equal variances and are adjusted for all pairwise comparisons within a row of each innermost suitable using the Bonferroni correction.

Results and discussion

Plants growth stimulators are frequently used in vegetative propagation to promote rooting and thereby to enhance plants growth and development. In our experiments rooting percentage was about 90-92% in all of the variants, however, a significant influence of glycoside treatments has nevertheless been observed.

After 4 weeks of growth, both furostanol glycoside treatments induced a better growth of the radicular system and leaves compared to the control (Fig. 1). The results showed that glycoside treatments improved new roots formation (Fig. 1A) up to 37% (at 'Real' cultivar treated with G1 0.3 mM) and their elongation (Fig. 1B) by 16-18% and increased the number of new leaves per plant up to 26% (Fig. 1C).

Glycoside concentration significantly ($p<0.05$) influenced all morphometric traits (Fig. 1). Irrespective of glycoside type, maximum increase in roots number (by 37% at 'Real' and by 27% at 'Magic') has been recorded at plants sprayed with 0.3 mM glycoside solution, while both 3 mM and 0.03 mM concentrations produced statistically similar results to those obtained in the control.

Interdependency of treatments action and concentrations has been demonstrated for strawberry by many researchers (Botta *et al.,* 2009; Laugale *et al.,* 2006; Sas-Paszt *et al.,* 2008; Sarli *et al.,* 2009). Results of other experiments have shown that the effect of the treatment increases with concentration until it reaches a maximum, after which it starts to decline (Rosier and Frampton, 2004). The 0.3 mM has been shown to be the optimal concentration in both glycosides treatments; therefore this concentration was used in further experiments.

Likewise, the growth parameters were differentially affected by the glycoside type (Fig. 1A). Irrespective of cultivar our results pointed out that the use of G1 treatments induced a stronger increase in number of roots per plant (37% G1 *vs* 29% G2 at 'Real') (and 27% G1 compared to 20% G2 at 'Magic'), and this effect has also been observed on root length (Fig. 1B). Although not directly comparable, the present results are highly similar to those of Bañón *et al.* (2003) and Górnik *et al.* (2008), who reported increases in root system growth in their experiments with different biostimulants.

The new leaves development has been slightly influenced by the glycoside type used with the 'Magic' cultivar, (which recorded 4% more leaves in G1 treated plants than in G2 treated ones), but no effect has been recorded with 'Real', (Fig. 1A). An increase in leaves number/plant has been also observed with strawberry (Neri *et al.*, 2002) and vegetables (Boehme *et al.*, 2005) treated with seaweed extracts. Therefore we suppose that our furostanol glycosides may have stimulative effects in plants growth and development.

The two varieties reacted similarly to glycoside treatments by increasing their roots number per plant as well as their length and number of new leaves. Irrespective of glycoside type, a better growth of the radicular system has been observed with Real, which recorded a 10% higher increase in roots' number/plant than 'Magic'. However, the latter recorded a better development of foliar apparatus than 'Real', by increasing the mean new leaves number by 10% in G1 treated plants and by 5% in G2 treated ones.

In the second experiment, furostanol glycosides were applied by foliar spraying on mature plants cultivated in pots. It is well known that plant response to different treatments varies from a different vegetation stage to another (Aminifard *et al.*, 2010). The results obtained showed that the stimulating effect on radicular system growth was also maintained in adult plants, although increases in roots number and length were 30% smaller than those recorded in young treated plants.

Glycoside treatments increased significantly the number of new roots per plant (up to 25%) (Fig. 2A), but did not induce any modification in root length. It has been observed that in both cultivars G1 treated plants had 5% more roots than G2 treated ones (Fig. 2B).

The effect of furostanol glycosides treatments on foliar apparatus growth and development was different and dependent on glycoside type. Treatments with G1 (extracted from *Lycopersicon* sp.) enhanced new leaves formation (up to 23%) (Fig. 2C) and increased leaf area (Fig. 2E) (up to 30%) but reduced the leaf mean length up to 10% (Fig. 2D).

Treatments with G2 (extracted from *Digitalis* sp.) had no influence on new leaves number (Fig. 2C), but reduced leaf length more than G1 (by 15%) (Fig. 2D) and decreased leaf area by 10-20% (Fig. 2E). It seems that G2 effect depends on vegetation stage and application on mature plants may induce some changes in hormonal balance which determine root growth and diminish leaves growth and leaf area. Similar results have been obtained by Deyton *et al.* (1991) for strawberry and Christov *et al.* (1995) for grapevine treated with paclobutrazol.

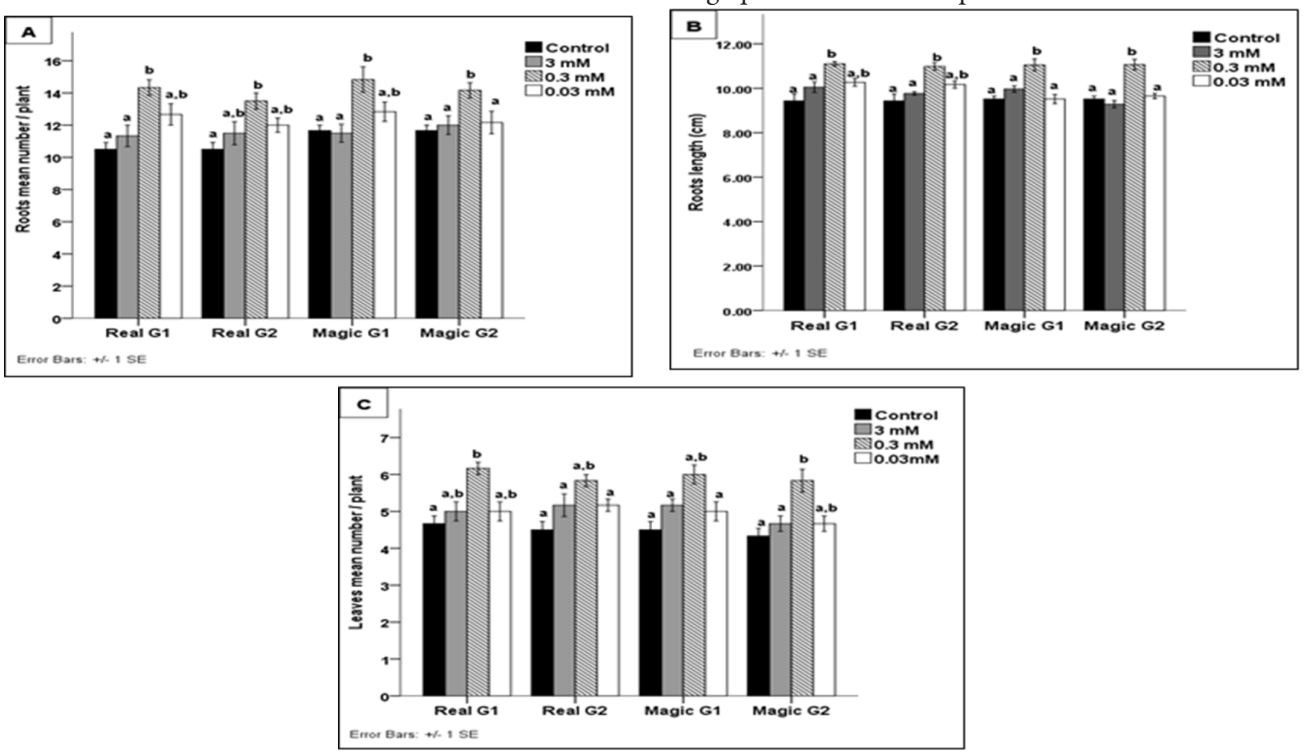

Fig. 1. Glycoside treatment influence on young strawberry plants

A-Roots mean number/plant, B-roots mean length, C-New leaves mean number / plant

The induced changes in plants growth depend on studied genotype. With 'Real' cultivar, both G1 and G2 treatments determined a 6% increase in roots number per plant compared to 'Magic'. Moreover, in G1 treated variants 'Real' developed more leaves (up to 15%), with a larger leaf area (up to 10%), suggesting that 'Real' responded better than 'Magic' to glycoside treatments.

Moreover, glycoside treated plants exhibited a darker green colour than untreated ones due to 6-10% increase in total chlorophyll content. An increase in chlorophyll content has also been reported in experiments with biosimulators (Blunden *et al.*, 1997) and may be attributed to an enhanced chlorophyll synthesis (Sebastian *et al.*, 2002) and/or more densely packed chloroplasts per unit leaf area (Khalil, 1995).

Chlorophyll a and b levels were not equally sensitive to glycoside treatment. Chlorophyll b increased by 15 % comparing with control, while chlorophyll a increased

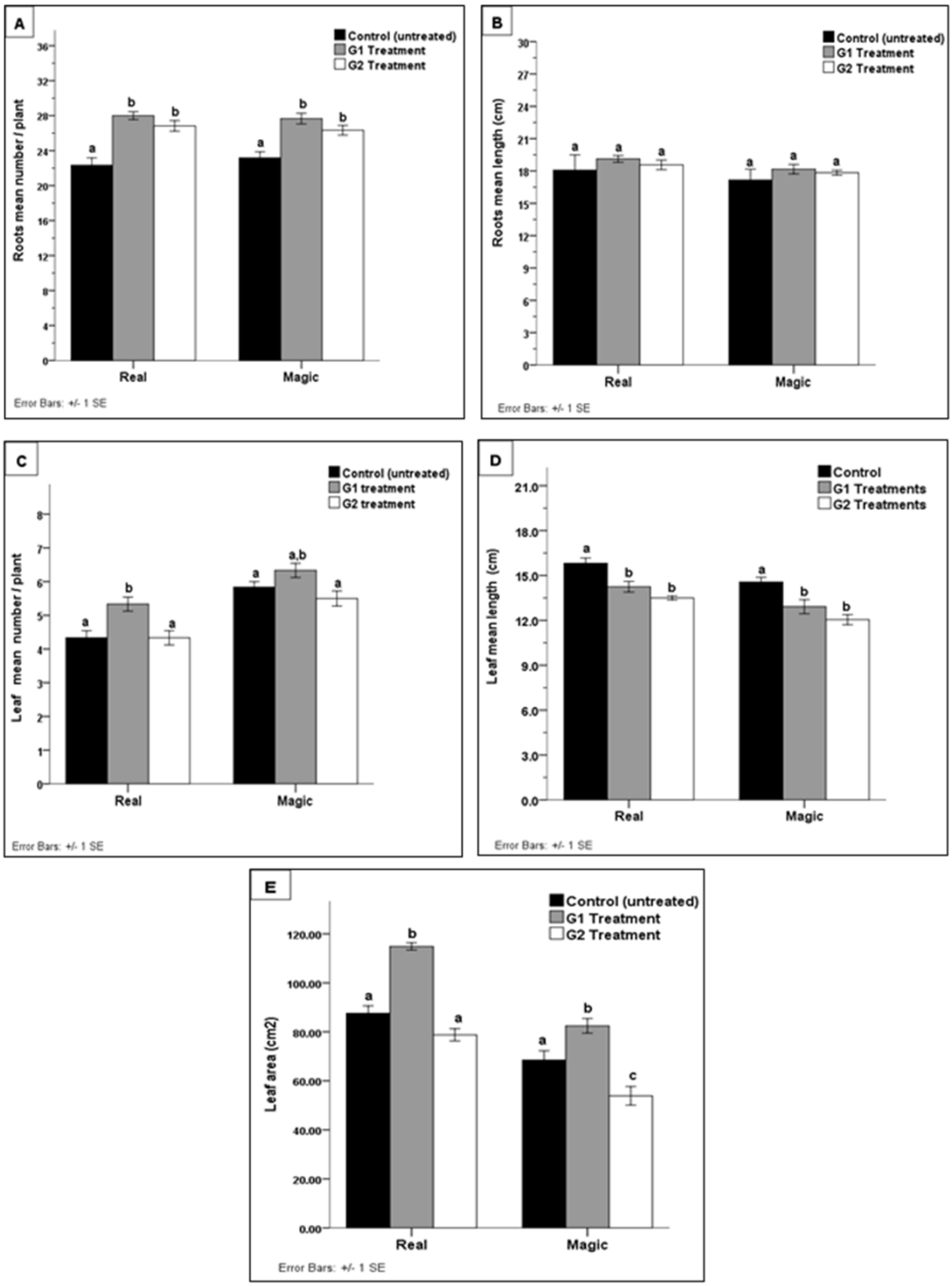

Fig. 2. Influence of glycoside treatments on mature strawberry plants growth

A-Roots` mean number/plant, B-Roots` mean length, C-New leaves` mean number / plant, D-Leaves` mean length, E-Leaf area

only by 9%. Consequently, chlorophyll a/chlorophyll b ratio (Tab. 1) decreased by 5-7% in G1 treated variants and by 8% in G2 treated plants.

Carotenoid pigments increased by 23-30% in G1 treated plants, and by 12-16% in G2 treated ones. Several other studies also report increases in carotenoid pigments content in plants treated with seaweed extracts (Pise and Sabale, 2010; Thirumaran *et al.*, 2009).

Significant differences were recorded among glycosides treatments. In both cultivars, G1 had a better effect than G2. We recorded an increase of 8-9% in chlorophyll a amount, while in G2 treated plants, this parameter was only 4% higher. Smaller differences were also recorded in chlorophyll b content (Tab. 1).

High concentration of chlorophyll b and carotenoids pigments in treated leaves could be due to the protector

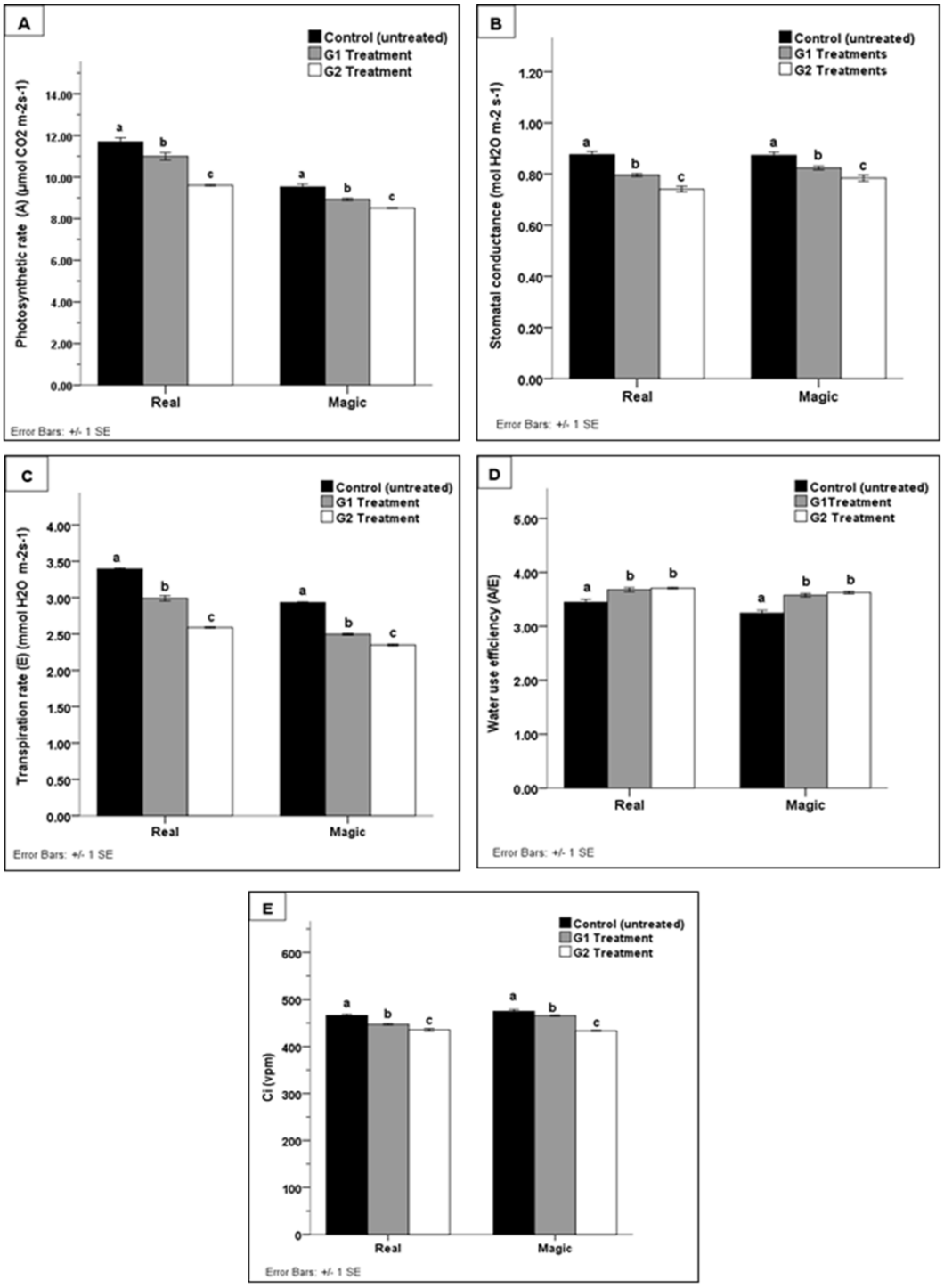

Fig. 3. Glycoside treatment influence on photosynthesis

A-Photosynthetic rate, B-Stomatal conductance, C-Transpiration rate, D-Water use efficiency (A/E), E-internal CO_2 concentration

Tab. 1. The influence of furostanol glycoside treatment on chlorophylls and carotenoids content at two strawberry cultivars

Cultivar	Variant	Chlorophyll a mg/g d.w.	Chlorophyll b mg/g d.w.	Carotenoids mg/g d.w.	Chl. a/ Chl. b	Total Chlorophyll
'Real'	Control	$2.74^{a} \pm 0.06$	$1.18^{a} \pm 0.01$	$0.85^{a} \pm 0.01$	$2.32^{a} \pm 0.03$	$3.92^{a} \pm 0.07$
	G1 treatment	$2.95^{b} \pm 0.02$	$1.36^{b} \pm 0.02$	$1.05^{b} \pm 0.01$	$2.18^{b} \pm 0.03$	$4.30^{b} \pm 0.02$
	G2 treatment	$2.84^{a,b} \pm 0.01$	$1.33^{b} \pm 0.01$	$0.98^{c} \pm 0.02$	$2.13^{b} \pm 0.02$	$4.17^{b} \pm 0.01$
'Magic'	Control	$2.73^{a} \pm 0.01$	$1.10^{a} \pm 0.01$	$0.85^{a} \pm 0.02$	$2.48^{a} \pm 0.03$	$3.84^{a} \pm 0.01$
	G1 treatment	$2.95^{b} \pm 0.01$	$1.25^{b} \pm 0.01$	$1.11^{b} \pm 0.02$	$2.37^{b} \pm 0.01$	$4.20^{b} \pm 0.01$
	G2 treatment	$2.84^{c} \pm 0.01$	$1.19^{c} \pm 0.01$	$0.72^{c} \pm 0.03$	$2.39^{b} \pm 0.01$	$4.02^{c} \pm 0.01$

Note: Values in the same row and subtable not sharing the same subscript are significantly different at $p < ,05$ in the two-sided test of equality for column means. Cells with no subscript are not included in the test. Tests assume equal variances

effect of glycosides against chlorophyll molecules degradation by improving water use efficiency, as a consequence of reduction in leaf transpiration rate caused by stomata closure (Shanan and Shalaby, 2011). Enhancing of chlorophyll b and carotenoids is usually associated with an increase in photosynthetic antenna size, which leads to a better energy transfer as an adaptive reaction to the different environmental conditions (Chartzoulakis *et al.*, 1993; Pirzad *et al.*, 2011). This may suggest that foliar spraying with G2 may induce a suite of physiological adaptations related to stomatal limitation, which may confer a degree of stress tolerance and aid in the recovery from stress-induced damage.

It is known that photosynthetic efficiency depends on chlorophyll content as well as stomatal response. Previous results on furostanol glycoside effects report only that foliar spraying enhances biosynthesis of assimilator pigment content (Muntenau *et al.*, 2008; Vasil'eva *et al.*, 2003), without any concrete statements about their implications in photosynthesis. Our results showed that glycoside treatments induced a decrease in photosynthetic rate (A) by 6% in G1 treated plants and by 15-20% in G2 treated ones (Fig. 3A). These reductions (especially of G2 treated plants) are associated with those of stomatal conductance, which decreased by 6-9% in G1 treated plants and by 10-15% in G2 treated ones (Fig. 3B). Stomatal conductance reduction depleted diffusion of CO_2 through mesophyll cell walls, membranes, cytoplasm, and chloroplast wall, leading to decreases in internal CO_2 concentration (Ci) by 4% in G1 treated plants and 8% in G2 treated ones (Fig. 3E).

On the other hand, glycoside treatment determined a decrease in evapotranspiration rate (E) by 12-15% in G1 treated plants and by 20-25% in G2 treated ones (Fig. 3C) which led to increase of water use efficiency (A/E) by 7-11% (Fig. 3D). The similar effect on improving of A/E by decreases in photosynthetic rate as a consequence of stomatal limitation was shown in experiments with white spruce (Fuchs *et al.*, 1999) and pepper (del Amor *et al.*, 2011) treated with antitranspirant substances like ABA and ABA analogues. Therefore, we presume that our furostanol glycoside treatments may have antitranspirant effect by stomatal limitation of photosynthesis in both 'Real' and 'Magic' cultivar.

However, it has been observed that stomatal limitation was stronger in 'Real', which recorded a g_s decrease by 9-15%, than 'Magic' for which g_s was only 6-10% lower. This led to a higher limitation of CO_2 availability for the mesophyll of leaves and to 5% higher decrease in photosynthetic rate with 'Real' than in 'Magic'. This hypothesis will need further investigations in experiments with drought and classic antitranspirants.

Conclusions

The present study showed a significant positive effect of furostanol glycosides on rooting processes and root system growth and development of young daughter strawberry plants when it was applied in 0.3 mM concentration. Although the foliar application of both products seemed to exhibit bioactive properties as they were able to significantly stimulate the root system development, G1 was often more effective than G2 in improving the new leaves formation, growth and photosynthetic pigments content. The decline in assimilation rates was observed in both glycoside treated plants, smaller in G1 compared with G2. However, this decrease was associated with better water use efficiency and better use of internal carbon dioxide, which suggests an antitranspirant action. Work is in progress to investigate the effect that furostanol glycosides have in allowing tissues to dehydrate and then recover.

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