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Effects of Daminozide (Alar 85) Treatment on the Stomata Movements of Chrysanthemum (*Dendranthema grandiflorum* (Ramat.) Kitamura) Seedlings Grown in Different Day Length Conditions

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Abstract: In this research, the effects of daminozide (Alar 85) treatment on the stomata movements in the leaves of two spray chrysanthemum cultivars (Yellow Reagan and White Reagan) grown in both short and natural day conditions were studied. In Yellow Reagan, the epidermis cell number, stomata number, stomata index and stomata width in the leaves of control seedlings grown in both short and natural day conditions were more in the lower than the upper surface. In addition, the stomata of control seedlings grown in short day conditions was longer in the lower than in the upper surface. As for the natural day conditions, the stomata of control was statistically in the equal length in both surfaces. In White Reagan, the stomata number, stomata index and stomata width in the leaves of control seedlings grown in both short and natural day conditions were more in the lower than the upper surface. Moreover, the epidermis cell number and stomata length of control seedlings grown in natural day conditions were higher in the upper than in lower surface. As for the short day conditions, the epidermis cell number of control was higher in the lower than upper surface, while the stomata of control was statistically in the equal length in both surfaces. On the other hand, it was observed that daminozide (Alar 85) treatment affected in different degrees on the stomata movements in the leaves of both Chrysanthemum cultivars and this difference was statistically important.

Key words: Alar 85, chrysanthemum, daminozide, photoperiod, stomata movements, Turkey

INTRODUCTION

Chrysanthemums are a genus of about 30 species of perennial flowering plants in the family Asteraceae, native to Asia and Northeastern Europe. The species of Chrysanthemum are herbaceous perennial plants growing to 50-150 cm tall, with deeply lobed leaves and large flower heads, white, yellow or pink in wild species (Huxley, 1992). The flower heads or petals are parboiled and served as a salad with tofu and seasoned with vinegar or soya sauce. An aromatic tea is made from the leaves, flowers or flower petals. Moreover, some species are used as food plants by larvae of Lepidoptera species (Reid, 1977; Facciola, 1990). Chrysanthemum is used for its effects on the eyes. It also treats headaches accompanying infection, carbuncles, furuncles and vertigo. Wild chrysanthemum is though to be more useful than the cultivated variety for treatment of sores abscesses, especially those of the head and back (Duke and Ayensu, 1985; Yeung, 1985; Chevallier, 1996).

Plants are able to modify their growth, development and physiology according to a variable environment. This ability of plants plays a key role in determining their tolerance to stresses and maintains efficient growth (Walters *et al*., 2003). One of the most important environmental factors is photoperiod. Photoperiod regulates a number of developmental events in many plant species. Stem and leaf elongation are usually promoted by long days, whereas bud dormancy, adventitious rooting and the formation of storage organs are initiated by short days. Flowering and sex expression may be promoted either by short or by long days, depending on the species and variety (Carre, 2001). In addition, photoperiod also plays an important role in many events such as seed germination (McCormac *et al*., 1993), transpiration (Krizek and Milthorpe, 1973), photosynthesis (Comstock and Etleringer, 1986) and chlorophyll and carotenoid contents (Schoefs *et al*., 1998). However, no study has been encountered concerning effects of photoperiod on the stomata movements until now, especially on the parameters examined in this study.

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Daminozide, known commercially as Alar or SADH (succinic acid-2, 2-dimethyl hydrazide) was introduced as a plant growth regulator (Riddle *et al*., 1962). Daminozide is not an analogue of any known naturally occurring substance, but it shows competitive interaction with naturally occurring growth substances (Lockhart, 1962). It is known that this chemical plays an important role in many physiological processes such as seed germination (Chase *et al*., 1994), seedling growth (Karlovic *et al*., 2004), enzyme activity (Petkova and Angelova, 1995), somatic embryogenesis (Miroshnichenko *et al*., 2009), sex expression (Chauhan *et al*., 1987), flowering (Krause *et al*., 2003) and fruit ripening (Karam and Murr, 1980). Unfortunately, no study has been encountered concerning effects of daminozide (Alar 85) as in the case of photoperiod on the stomata movements until now.

The aim of this study is to examine the influences of daminozide (Alar 85) treatment on the stomata movements in the leaves of chrysanthemum seedlings grown in different day length conditions.

MATERIALS AND METHODS

Plant materials, growth regulators and growth conditions: The experiments were conducted in plastic covered greenhouse located at the Experimental Research Station of Agriculture Faculty at Suleyman Demirel University (latitude 37°50'N, longitude 30°32'E altitude 1019 m), in Isparta, Turkey.

Two spray type cultivars (Yellow Reagan and White Reagan) belonging to *Dendranthema grandiflorum* (Ramat.) Kitamura species were used as plant materials. Rooted chrysanthemum cuttings were planted in soil beds at 25 plants m^{-2} on 19th May 2005. The experiment was arranged according to randomized block designs with three replicates and 350 plants were used for each replication. Plants were soft pinched above the 7th nodes on 1st June 2005 and pruned to 3 stems/plant on 11th June 2005. Water and nutrient requirements of the plants were supplied through a complete nutrient solution, applied with a drip irrigation system. The plants were subjected to 2 different days length. So, as to ensure short day conditions, the blackout application was started in half of the experimental plots on July 01 2005. The short day conditions were achieved by closing the blackout screens between 17:00-08:00 h (Kofranek, 1980; Adams *et al*., 1998). The blackout was finished when flower buds showed color on 1st September 2005. On the other hand, the other plants in the experimental plots were grown under natural day-length conditions. About 3000 ppm Alar 85 (Daminozide) was applied as a foliar spray as a growth inhibitor in half of the plants grown under short day conditions and natural day-length while only water was sprayed on leaves to the plants in the plots with no Alar-85 application. The first Alar-85 application was carried out on July 02 (when lateral shoots from the pinches 30 cm long), whereas the second application was performed 2 weeks after first application.

Anatomical observations: Superficial sections were taken from the second leave of seedlings by a microtome, in 6-7 μm thickness. Stomata and epidermis cells in a 1 mm^2 unit area were counted to determine the stomata index. The counts were made both in the lower and upper surfaces of each leaf 10 times as 3 replicates and the averages were calculated. After the determination of the number of stomata and epidermis cells in the leaf unit area, the stomata index was estimated according to Meidner and Mansfield's method (1968). Stomata width and length were also determined in micro meter by using ocular micrometer. Statistical evaluation concerning all parameters was realized by using SPSS program according to Duncan's multiple range test.

RESULTS

The findings related with effects of daminozide (Alar 85) treatment on the stomata movements in the leaves of Yellow Reagan and White Reagan seedlings are presented in Table 1 and 2, respectively.

Yellow reagan: The epidermis cell number, stomata number and stomata index in the leaves of control seedlings grown in both short and natural day conditions were higher in the lower than in the upper surface. Alar 85 treatment partly increased these parameters in both surfaces.

The stomata of control seedlings grown in short day conditions was longer in the lower than in the upper surface. As for the natural day conditions, the stomata of control was statistically in the equal length in both the lower and upper surfaces. Alar 85 applying slightly decreased this parameter in the upper surface, while it showed the same values as the control in the lower one.

The stomata of control seedlings grown in both short and natural day conditions was wider in the lower than in the upper surface. In short day conditions, Alar 85 treatment relatively increased the stomata width in the upper surface and in natural day conditions, it significantly reduced this parameter in the lower one (Table 1).

White reagan: The epidermis cell number in the leaves of control seedlings grown in short day conditions was

Table 1: Stomata movements in the leaves of yellow reagan seedlings grown in different day length conditions

Day length	Treatments (ppm)	Epidermis cell number		Stomata number		Stomata index		Stomata length		Stomata width	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
Short day	Control	$14.0\pm1.0^{b*}$	13.1 ± 1.8^{b}	4.0 ± 0.7^{b}	1.0 ± 0.8^{a}	22.2	11.6	19.5 ± 1.4^{a}	18.9 ± 1.3^{b}	9.6 ± 1.4^{a}	8.8 ± 1.2^{a}
	Alar 85	15.9 ± 2.0^{c}	15.0 ± 2.0^{c}	4.4 ± 1.0^{c}	2.1 ± 0.8^{c}	23.3	12.2	19.7 ± 2.4^{a}	18.3 ± 2.1^{a}	9.4 ± 1.0^{a}	9.3 ± 0.8^{b}
Natural day	Control	13.5 ± 1.8^{a}	10.8 ± 1.1^{a}	3.0 ± 1.0^{a}	1.7 ± 0.7^{b}	18.2	11.0	21.4 ± 1.2^{b}	21.5 ± 1.7^{d}	12.0 ± 1.5^{c}	10.2 ± 1.3^{c}
	Alar 85	14.6 ± 3.0^{bc}	13.0 ± 1.2^{b}	4.2 ± 0.9^{bc}	1.8 ± 0.6^{bc}	21.1	11.6	20.3 ± 2.0^{b}	20.8 ± 2.3^{c}	10.7 ± 1.0^{b}	10.7 ± 0.7^{c}

Table 2: Stomata movements in the leaves of white reagan seedlings grown in different day length conditions

Day length	Treatments (ppm)	Epidermis cell number		Stomata number		Stomata index		Stomata length		Stomata width	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
Short day	Control	$13.5\pm1.3^{c*}$	11.5 ± 1.2^{a}	4.3 ± 1.0^{b}	1.5 ± 0.6^{a}	20.7	11.5	19.2 ± 1.4^{a}	19.5 ± 1.6^{a}	10.9 ± 0.7^{a}	10.1 ± 1.4^{a}
	Alar 85	14.5 ± 1.2^{d}	13.5 ± 1.3^{b}	4.3 ± 0.9^{b}	1.6 ± 0.5^{b}	22.7	12.3	19.9 ± 1.7^{b}	20.7 ± 1.2^{a}	10.5 ± 1.1^{a}	10.1 ± 0.9^{a}
Natural day	Control	10.4 ± 1.1^{a}	14.1 ± 0.9^{c}	3.5 ± 0.9^{b}	1.4 ± 0.7^{a}	16.6	10.4	20.7 ± 1.5^{b}	21.8 ± 1.4^{a}	12.1 ± 1.5^{b}	11.2 ± 1.0^{c}
	Alar 85	12.9 ± 1.2^{b}	12.6 ± 1.2^{a}	2.3 ± 0.7^{a}	1.8 ± 0.8^{b}	19.9	11.3	21.4 ± 1.2^{c}	20.2 ± 1.8^{a}	12.0 ± 1.7^{b}	10.4 ± 1.3^{b}

*The difference between values with the same letter in each column is not significant at the level 0.05, Mean±SD

higher in the lower than in the upper surface. Alar 85 applying caused a slight increase on this parameter in both surfaces. As for the natural day conditions, the epidermis cell number of control seedlings was higher in the upper than in the lower surface. Alar 85 treatment partly increased the epidermis cell number in the lower surface, while it relatively decreased this parameter in the upper one.

The stomata number of control seedlings grown in both short and natural day conditions was higher in the lower than in the upper surface. In short day conditions, Alar 85 applying slightly increased this parameter in the upper surface, while it showed the same value as the control in the lower one. As for the natural day conditions. Alar 85 treatment markedly reduced the stomata number in the lower surface, whereas it increased this parameter in the upper one.

The stomata index of control seedlings grown in both short and natural day conditions was higher in the lower than in the upper surface. Alar 85 applying increased this parameter in the varying degrees in both surfaces.

The stomata of control seedlings grown in short day conditions was statistically in the equal length in both the lower and upper surfaces. As for the natural day conditions, the stomata of control was longer in the upper than in the lower surface. Alar 85 treatment partly increased this parameter in the lower surface, while it showed the same values as the control in the upper one.

The stomata of control seedlings grown in both short and natural day conditions was wider in the lower than in the upper surface. In short day conditions, Alar 85 applying did not show a meaningful and statistically significant effect on the stomata width in both surfaces. As for the natural day conditions, Alar 85 treatment relatively decreased this parameter in the upper surface, while it showed the same value as the control in the lower one (Table 2).

DISCUSSION

Plant stomata, the vital gate between plant and atmosphere may play a central role in plant responses to environmental conditions, which have been and are being investigated from molecular and whole plant perspectives, as well as at ecosystem and global levels (Nilson and Assmann, 2007). Many researchers have reported the stomata movements response to various environmental factors, such as CO_2 concentration (Sharkey and Raschke, 1981), high temperature (Beerling and Chaloner, 1993), salinity (Cavusoglu *et al*., 2007a), drought (Xu and Zhou, 2008), light (Kim *et al*., 2004), precipitation change (Yang *et al*., 2007) and plant density (Zhang *et al*., 2003). Besides, the changes in environmental conditions, internal factors such photo hormones were found to play a significant role in regulating of the stomata movements (Cavusoglu *et al*., 2007b, 2008).

In the present study, daminozide (Alar 85) applying relatively increased the epidermis cell number, stomata number and stomata index in both surfaces of the leaves of Yellow Reagan seedlings grown in both short and natural day conditions, according to control, but it partly decreased the stomata length in the upper one in short day conditions. Moreover, daminozide treatment slightly increased the stomata width in the upper surface in short day conditions, while it significantly reduced this parameter in the lower one in natural day conditions (Table 1). As for White Reagan seedlings, daminozide applying relatively increased the epidermis cell number and stomata length in the lower surface of leaves of the seedlings grown in both short and natural day conditions, whereas, it increased the stomata number, especially in the upper one. In addition, daminozide treatment increased the stomata index in both surfaces, while it had no effect on the stomata width in both day conditions, particularly in the lower one (Table 2).

These results indicate that chrysanthemum leaves acquire xeromorphic (for example, on the upper surface the increase in stomata number and on both surfaces the increase in stomata index) properties (Strogonov, 1964) in both short and natural day conditions by daminozide applying. Moreover, daminozide treatment can provide adaptation of the seedlings to different day length conditions by incresing the stomata width in the upper surface in yellow reagan seedlings grown in short day conditions and the stomata length in the lower surface in white reagan seedlings grown in both day conditions. Because, a greater stomata size can facilitate CO_2 diffusion into the leaf (Parkhurst, 1994) and thus, increase photosynthesis rate. In addition, daminozide applying can serve to the same aim by causing an increase of leaf area as a result of increasing the epidermis cell number of both surfaces especially in Yellow Reagan seedlings.

CONCLUSION

There are no study, yet on the effects of photoperiod and daminozide (Alar 85) treatments on the stomata movements. There is a need for more comprehensive and detailed researches for this subject to be made clear. We believe that present study will contribute to future studies.

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