International Research Journal of Applied and Basic Sciences. Vol., 3 (5), 1000-1004, 2012
Available online at http:// www. irjabs.com
ISSN 2251-838X ©2012

Physiological and morphological responses of two almond cultivars to drought stress and cycocel

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Abstract

In order to determine a drought-tolerant cultivar for cultivation in semi-arid areas, the response to drought stress and anti-transpirant (cycocel) of two almond cultivars (Princesse and Tuono) was evaluated by measuring some physiological and morphological parameters including stomatal resistance, chlorophyll fluorescence, chlorophyll content, leaf area, fresh and dry weight of leaf. There were significant differences between the two cultivars. Stomatal resistance, chlorophyll fluorescence, chlorophyll content and leaf dry weight were high for Tuono and Tuono leaves revealed lower leaf area compared to princesse cultivar. So, Tuono is more tolerance to drought stress than cv. Princesse. stomatal resistance, chlorophyll content and leaf fresh weight were increased by foliar application of cycocel, while chlorophyll fluorescence, leaf area and leaf dry weight were reduced. Also, chlorophyll fluorescence, chlorophyll content, leaf area, leaf dry and fresh weight were reduced by increasing the irrigation intervals, while stomatal resistance was increased.

Keywords: drought stress, chlorophyll fluorescence, anti-transpirant, stomatal resistance.

Introduction

Water availability is an important factor affecting plant growth and yield, mainly in arid semi-arid regions, where plants are often subjected to periods of drought. The occurrence of morphological and physiological responses, which lead to some adaptation to drought stress, may vary considerably among species. In general, strategies of drought-avoidance or drought tolerance can be recognized, both involving diverse plant mechanisms that provide the plants the ability to respond and survive drought (Rouhi et al., 2007; Souza et al., 2004).

Almond species, in general are characterized as drought resistant (Romero et al., 2004). They are able to withstand frequent periods of low soil moisture accompanied by high evaporative demand and high air temperature during the growing season (De Herralde et al., 2003). Drought stress has significant effects on plant physiology in general, and thus on almond productivity and growth in particular. Plant physiological, such as photosynthesis and transpiration depend on the rapidity, severity and duration of the drought event (Rouhi et al., 2007). In general, plants are able to control of stomatal aperture and morphology to acclimate to changes in the environment (Camposeo et al., 2011). In particular, fruit tree species contain morphological mechanisms of long-term plant drought acclimation which include variations in leaf area and in size and number of stomata (Rouhi et al., 2007).

Stomatal regulation of photosynthesis during drought stress has been well documented (Souza et al., 2004). Under drought conditions, it has been shown that stomata play the dominant role in controlling the decline of net CO_2 uptake; by leading to decrease in leaf internal CO_2 assimilation imposed by stomatal closure promote an imbalance between photochemical activity at photosystem II (PSII) and electron requirement for photosynthesis (Souza et al., 2004). As a result, net photosynthesis is unavoidably reduced during drought stress (Rouhi et al., 2007). Miyashita et al. (2005) reported that the Fv/Fm of chlorophyll fluorescence, transpiration rate and stomatal conductance decreased with increasing drought stress in kidney bean. Rodriguez et al. (2005) and Pandey et al. (2003) mentioned that chlorophyll content (in cotton), biomass and leaf area (in *Asteriscus maritimus*) declined under drought stress. Guerfel et al. (2009) found that considerable genotypic differences between the two olive cultivars under drought stress and they introduced a drought-tolerant cultivar for semi-arid areas. The use of practical methods to mitigate drought stress practically by chemical treatment is likely to increase productivity under drought stress conditions. Hence application of anti-transpirants (such as paclobutrazole, cycocel and daminozide), which are helpful tools in reducing transpiration losses, is becoming popular (Parkash and Ramachandran, 2000). Memari et al. (2011) reported that application of cycocel (500 mgl^{-1}) increased chlorophyll content in olive cultivars. Probably, positive effect of cycocel on enzyme activity results in increasing of chlorophyll content (Memari et al., 2011). Also, Pandey et al. (2003) showed that plant growth retardant (ABA) decrease stomatal conductance and transpiration rate in cotton. Similar results were reported by other researchers (Nejadsahebi et al., 2010; Singh and usha, 2003).

The selection of the best cultivar is very important to optimize production in dry environments. Therefore, the objective of this study was to evaluate the effects of drought stress and cycocel on several physiological and morphological parameters of the two almond cultivars.

Materials and methods

2.1. Plant material and treatments

The experiment was carried out at Fruit and Nut tree Nursary (chelcheragh Soosan Company of Moghan) in 2011. Two-year-old almond trees (A_1: Princesse and A_2: Tuono) grafted on bitter almond were obtained from mentioned Fruit and Nut tree Nursary. Prior to planting, trees were pruned to obtain uniform planting material for all cultivars. Trees planted in 5L plastic pots containing freely drained light soil and the trees allowed to acclimatize for 2 months in tree nursery (out door) with regular irrigating. After the 2 months acclimation period, cycocel (C_1= 0 ppm and C_2= 500 ppm) was sprayed on the trees (Kofidis et al., 2008) and the same time, drought stress was imposed for a period of 2 months (from early June till early Augoust). Three drought stress levels (ds_1= 3, ds_2= 6 and ds_3= 9 days intervals) were chosen according to the prevailing climatic and soil water conditions in moghan.

2.2. Measurements of stomatal resistance and chlorophyll fluorescence

During the drought stress period, five fully expanded leaves from the middle part of branches of each tree were selected and marked and then the stomatal resistance was measured for each tree weekly (eight times) using a porometer (DELTA-T DEVICES –U-K). At the end of experiment, chlorophyll fluorescence of selected leaves was measured using the plant efficiency analyzer (Hansatech Instruments LTd) as previously described by (Miyashita et al., 2005). Maximal PSII photochemical efficiency Fv/Fm, the ratio of variable fluorescence (Fv) to maximum fluorescence (Fm), was calculated automatically.

Stomatal resistance and chlorophyll fluorescence of leaves were measured from 2 h after sunrise approximately from 9:00 h till 10:00 h (Grant et al., 2010).

2.3. Total chlorophyll chl (a+b) concentration

During the last day of the experiment, five fully expanded leaves of comparable physiological age were selected and chlorophyll content was recorded by means of chlorophyll meter (SPAD 502 plus chlorophyll meter).

2.4. Leaf anatomy

At the end of experiment, all leaves per tree were collected and then leaf area (LA) of those leaves was measured by means of a planimeter (LI-3100c.nebraska USA). Fresh and dry weights of the leaves were also measured according to the method described by Guerfel et al. (2009).

2.5. Experimental design and statistical analysis

The experiment was conducted in a factorial experiment design, completely randomized with three replications. In this study, drought stress (three levels), cycocel (two levels) and cultivar (2 cultivars) were treatments (Ranjbarfordoei et al., 2006). The data were analyzed using GLM producer SAS 9.1 version software package and the means were separated by Duncan's multiple range tests (Jamalzadeh and Shareghi, 2004).

Results and discussion

3.1. Stomatal resistance and chlorophyll fluorescence

The results of ANOVA showed that cultivar, cycocel and drought stress were significant effect on stomatal resistance and chlorophyll fluorescence. The stomatal resistance and chlorophyll fluorescence of Tuono was higher than Princesse cultivar. Tekalign and Hammes (2005) reported the existence of genotype difference regarding stomatal resistance in potato. This may be linked to abscisic acid accumulation which is an important trait to improve yield in a water-limited environment (Tekalign and Hammes, 2005). Foliar application of cycocel increased stomatal resistance which is agreement with the observations of Singh and Usha (2003) in wheat. For that reason, cycocel treatment minimizes the water loss in treated plants (Pandey et al., 2003). Also, drought stress levels had significant effect on stomatal resistance and the highest stomatal resistance was observed in ds_3. Indeed, there is a close relationship between stomatal behavior and plants survival ability under drought conditions. Stomatal closure significantly decreases transpiration rate and results in maintaining positive turgor pressure of the cells (Saei et al., 2006). Similar results were reported by other researchers (Patakas et al., 2003; Souza et al., 2004).

Selection of cultivars with high net photosynthetic rate will result in higher yield if all other factors are equal (Rouhi et al., 2007). The characteristics of chlorophyll fluorescence have the potential of providing valuable information on the efficiency of photosynthesis (Miyashita et al., 2005). In this study, Tuono cultivar showed a higher ratio of Fv/Fm as compared to Princesse cultivar. Genotype differences could be the major factor explaining our finding (Tekalign and Hammes, 2005). Also, application of cycocel indicated a decrease of Fv/Fm ratio. Probably, structural damage to the thylakoid membranes of chloroplasts results in decrease of Fv/Fm ratio (Pereira et al., 2000). Results obtained by Kofidis et al. (2008) supported our observations.
Fv/Fm ratio in this experiment decreased under drought stress. This change possibly reflects a disorder in PSII (Ranjbarfordoei et al., 2006). The effect of drought stress upon the photochemical system was revealed by significant decreases in the maximum quantum yield of PSII accompanied by increases in the levels of minimum fluorescence (Souza et al., 2004)

3.2. Chlorophyll content and leaf area

There was significant difference in chlorophyll content between cultivars. Tuono had higher chlorophyll content than Princesse. Dehnavy et al. (2004) found that high chlorophyll content in drought-tolerant cultivars was due to small leaf area. Also, cycocel enhanced the chlorophyll status of the plants. Similar results of an increase in chlorophyll content with cycocel application were reported by Parkash and Ramachandran (2000) in Brinjal. In general, anti-transpirants could be effective in chlorophyll synthesis by high Rubisco activity (Singh et al., 2003). While, chlorophyll content reduced under drought stress. The decrease in chlorophyll content can be attributed to the sensitivity of this pigment to increasing environmental stresses, especially to drought and salinity (Guerfel et al., 2009), which has been reported by other researchers (Dehnavy et al., 2004; Pandey et al., 2003).

Cultivars had significant effect on leaf area, the largest and smallest leaves attributed to Princesse and Tuono, respectively. Small leaves are a characteristic for growing in dry environments (Rasooli and Golmohamadi, 2009; Rouhi et al., 2007). In supporting to our finding, Camposeo et al. (2011) reported that drought tolerant cultivars have very small leaf area. Foliar application of cycocel reduced leaf area. Generally, growth retardants induced lower transpiration by decreasing growth leaf (Luoranen et al., 2002). Also, reduction of leaf area was observed with drought stress treatment. The reduction in leaf area under drought stress could be considered as an avoidance mechanism which minimizes water losses (Rodriguez et al., 2005). Similar results were reported by other researchers (Cordeiro et al., 2009; Zamani et al., 2002).

3.3. Leaf fresh and dry weight

There was no significant difference in leaf fresh weight between cultivars, while, cycocel increased leaf fresh weight. This observation is in agreement with the findings of Marshall et al. (1991), who reported that paclobutrazol enhanced water retention by reducing transpiration and increased leaf fresh weight. In contrast, drought stress reduced leaf fresh weight. In general, water content of leaf, declined under drought stress (Singh and Usha, 2003).

The increase of leaf dry weight was observed in Tuono cultivar, genotype differences in relation to rates of photosynthesis could be the major factor explaining in total biomass production (Tekalign and Hammes, 2005). There was no significant difference in leaf dry by foliar application of cycocel, whereas drought stress reduced leaf dry weight. This could be related to the decrease in the values of net assimilation rate (NAR) (Rodriguez et al., 2005). Parkash and Ramachandran (2000) found that drought stress caused a significant reduction in photosynthetic rate and this reduced rate of photosynthesis may be mainly due to the reduction in chlorophyll content. For this reason, leaf dry weight reduced under drought stress.

Conclusions

From the results, it can be concluded that there is variation between cultivars in response to drought stress. The results indicate that Tuono withstand drought stress more effectively than Princesse. We believe that this cultivar may be very promising for cultivation in semi-arid areas. Also, these observations suggest that cycocel increases plant adaptation to drought stress by stimulating the process of turgor adjustment, which would help to minimize reduction in yield.

Acknowledgments

The authors are grateful to Moghan agriculture faculty and chelcheragh Soosan Company of Moghan for supporting this work.

Table 1. Mean simple effect of cultivar type, cycocel and drought stress on some of physiological and morphological parameters of almond

		Stomatal resistance ($S.cm^{-1}$)	Chlorophyll fluorescence	Chlorophyll content (mg/g)	Leaf area	Leaf fresh weight (g)	Leaf dry weight (g)
Cultivar type	A1	72.5^{b*}	0.7876^{b}	48.8^{b}	46.4^{a}	8^{a}	3.6^{b}
	A2	83.8^{a}	0.8386^{a}	54.5^{a}	42.1^{b}	7.6^{a}	5.4^{a}
Cycocel	C1	76.1^{b}	0.8246^{a}	49.1^{b}	46.2^{a}	7.45^{b}	4.61^{a}
	C2	80.1^{a}	0.8016^{b}	54.2^{a}	42.3^{b}	8.01^{a}	4.4^{a}
Drought stress	Ds1	72.5^{c}	0.8424^{a}	56.5^{a}	48.3^{a}	8.33^{a}	5.22^{a}
	Ds2	77.4^{b}	0.8120^{b}	51.7^{b}	44.2^{b}	7.73^{b}	4.5^{b}
	Ds3	84.6^{a}	0.7849^{c}	46.7^{c}	40.1^{c}	7.1^{c}	3.8^{c}

*Means followed by the same latter did not differ significantly at p= 0.05

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