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Investigation of Proline, Chlorophyll and Carotenoids Changes Under Drought Stress in Some Onion (*Allium Cepa* L.) Cultivars

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Abstract

Drought is one of the major limitations for vegetable growth and productivity all over the World. In this study the effects of drought stress on proline, chlorophyll a/b, total chlorophyll and carotenoids were investigated in some onion (*Allium cepa* L.) cultivars (Kantartopu-3, Akgun-12 and Texas Early Grano) under the early plant growth phase. Seeds were germinated in peat material and transferred to plastic pots after 21 days of sowing. The plants have been grown in vermiculite by "substrate culture" technique. Three different irrigation applications (pot/field capacity (PC) PC-90 (90% of PC), PC-70 (70% of PC), PC-40 (40% of PC) were tested under greenhouse conditions. After six weeks of transferring, leaf parts were isolated and studied for various indices. Irrigation rates affected significantly the all parameters of onion. The results indicated that drought increased accumulation of proline in onion seedlings, while decreased the content of chlorophyll and carotenoid.

Keywords: Onion, Abiotic Stress, Drought, Proline, Chlorophyll

Kuraklık Stersi Altında, Bazı Soğan (*Allium cepa* L.) Çeşitlerinde, Prolin, Klorofil ve Karotenoid Değişimlerinin İncelenmesi

Özet

Kuraklık, Dünya genelinde, sebze yetiştiriciliğini ve üretimini sınırlayan önemli bir faktördür. Bu çalışmada, bazı soğan (*Allium cepa* L.) çeşitlerinde (Kantartopu-3, Akgun-12 and Texas Early Grano), erken fide dönemindeki kuraklığın, prolin, toplam klorofil, klorofil a, klorofil b ve karotenoid miktarlarına etkileri araştırılmıştır. Torf içerisinde çimlenen soğan fideleri, 21. günde plastik saksılara şaşırtılmıştır. Fideler burada "substrat" kültürü ile vermukilit içerisinde yetiştirilmiştir. Sera koşullarında üç farklı sulama uygulaması (saksı/tarla kapasitesi, FC (%90, %70, %40) gerçekleştirilmiştir. Şaşırtmadan altı hafta sonra, yapraklardan kesitler alınarak analizler yapılmıştır. Sulama oranları, soğandaki incelenen tüm parametreleri önemli düzeyde etkilemiştir. Sonuçlar, kuraklığın soğan fidelerinde prolin birikimini artırırken, klorofil ve karotenoid miktarını düşürdüğünü göstermiştir.

Anahtar Kelimeler: Soğan, Abiotic stress, Kuraklık, Prolin, Klorofil

Introduction

Onion (*Allium cepa* L.) is an important crop that is now cultivated globally. According to the most recent data of the United Nations Food and Agriculture Organization (FAO), worldwide onion production was approximately 83 million tonnes in 2012 from 4.20 million hectares. According to the FAO, Turkey produces 1.81 million tonnes of onions annually, which is 2.1% of world onion production, and it ranks as the 7th largest onion producer (FAO, 2012).

Drought is generally defined as an extended period - a season, a year, or several years - of deficient precipitation compared to the statistical multi-year average for a region that results in water shortage for some activity, group, or environmental sector (FAO, 2013).

Although the maximum root penetration of onion was at 0.76 m, most of the roots were in the top 0.18 m of soil and only a few roots were found deeper than 0.31 m. This trait limits the amount of soil water available to the onion, especially when grown on coarse-textured soils. Most likely, irrigation water that moves below 0.76m is not available to the onion crop. (Drinkwater and Janes, 1955). Because of this root system, onion is sensitive to water stress and requires frequent and light irrigation to avoid water deficiency and to adequately recharge the plant root zone (Koriem et al., 1994). Few research studies have been conducted to characterize of response to drought stress tolerance in onion (Pelter et al, 2004). In a trial with several vegetable crops, Singh and Alderfer (1966) observed that soil-water stress at any growth stage leads to reduction in quality characters of onion. They further observed that with regard to yield reduction, onions are more sensitive to water stress during bulb formation and enlargement than during the vegetative stage. Dragland (1974) reported that, when compared to an unstressed control treatment, an imposed 3-week-long drought early in the season reduced onion yield more than when the 3-week drought was imposed near the end of the growing season. Van Eeden and Myburgh (1971) found that water stress imposed late in the season (at 84–103 days after transplanting) reduced onion total yield by 15% when compared to the yield with no water stress. The objective of this study was to determine the effect of imposing soil-water stress at early growth stages of onion on amount of proline, chlorophyll a/b, total chlorophyll and carotenoids.

Materials and Methods

The study was conducted with a local onion cultivars Akgun-12, Kantartopu-3 and a common cultivar: Texas Early Grano. Seeds of onion were sown in viols which filled with perlite/peat (1:1) mixture. Four weeks old onion seedlings were then transplanted into 1.6 liter pots. These pots were filled with 1.5 liter of vermiculite. The transplanted onion seedlings were watered a two-days period with Hoagland's solution (Hoagland and Arnon, 1950) before initiating water treatments in order to improve root development. Six pots with one seedling each were randomly assigned to each of the three levels of water until end of experiment. The pots were put on top of a black plastic paper to avoid direct contact with the soil surface. The amount of water to be added was determined based on the percentage of pot water capacity.

Pot (included vermiculite) water content by gravimetric test was observed in the end of application drought stress (S0 was 90% of field capacity, S1 was 70% of field capacity, and S2 treatment was 40% of field capacity). The 70% field (pot) capacity was chosen as drought starting point because Sanders (1997) reported that the this soil moisture rate is minimum point for onion. Application of drought stress did according to Djekoun and Planchon (1991) method with little modification. Djekoun and Planchon applied stress by stopping watering for 4, 8 and 10 days, whereas we formed stress of drought by stop watering for five days. S0= control (90% FC), the plant watering at the two-days period. S1: (70%) begining of drought stress carried by stop watering to plants during five days. S2: (40%) drought stress, carried by stop watering to plants during ten days.

Assessments of chlorophyll, proline and carotenoids content were performed after six weeks of transferring. Chlorophyll and carotenoids content was determined in 80% acetone extract. After centrifugation (14.000 rpm, 20 min) the absorbance was read spectrophotometrically at 663, 652, 646 and 470 nm. The concentrations were calculated according to Lichtenthaler and Welburn (1983) method. Equations used for calculation are presented below:

Chlorophyll a (μg/ml)= 12.21 A_{663} – 2.81 A_{645}
Chlorophyll b (μg/ml) = 20.13 A_{645} – 5.03 A_{663}
Total Chlorophyll (μg/ml)=A_{652} x 27,8
Carotene (μg/ml)= (1000 A_{470} – 3.27 Chl-a – 104Chl-b)/227

Proline was extracted from a sample of 0.5 g fresh leaf material samples in 3% (w/v) aqueous sulphosalycylic acid and estimated using the ninhydrin reagent according to the method of Bates et al. (1973). The absorbance of fraction with toluene aspired from liquid phase was read at a wave length of 520 nm. Proline concentration was determined using a calibration curve and expressed as μmol proline g^{-1} fresh weight.

The experiment was conducted in the laboratories of the Department of Vegetable Breeding and Tissue Culture, and Greenhouse at the Atatürk Central Horticultural Research Institute. For comparison of multiple means, one-way ANOVA and the LSMeans Differences Student’s t test were used. Significant difference in statistical tests was set at $P < 0.01$.

Results

The results of variance analyses for the measured characters were presented in Table 1. The total chlorophyll (C_T), chlorophyll a (C_a) and proline (P) amount were significant for three factors (cultivar, drought and their interaction). *(P <* 0.01) Chlorophyll b (C_b) was significant for only interaction of cultivar x drought while carotenoids (Crt) was only for drought. (Table 1). Results were summarized on the Table-2.

Table 1. A two-way analysis of variance

		F Ratios					
Source	DF	C_a	C_b	C_T	C_a/C_b	Crt	P
Cultivar	2	5.7786*	0.8702	4.3491*	21.8379*	2.2633	34.4553*
Drought	2	18.0938*	2.2959	5.7172*	51.2740*	7.5408*	4613.281*
Cultivar x Drought	4	4.9117*	6.1131*	3.0671*	28.9475*	2.0865	506.0319*
C. Total	35						
Error	27						

*Significant ($p < 0.01$) F: Freedom, DF: Degree of freedom, C. Total: Corrected Total, C_a: Chlorophyll a, C_b: Chlorophyll b C_T: Total Chlorophyll, Crt: Carotenoids, P: Proline

The change of proline (P) amount was significant for three factors (cultivar, drought and their interaction) *(P < 0.01)*. Proline increased significantly under drought stress in comparison with control in all cultivars (Fig. I). Akgun-12 and Texas Early Grano cultivars exhibited low free proline content under normal water supply except for Kantartopu-3, which contain a high level of proline content compared to other genotypes (Figure 1). The maximum mean proline content was recorded at S3 drought treatment in cultivar Texas Early Grano (8.80 µmol/g) which was 4.51 fold higher than control. It was followed by 7.70 µmol/g in Akgun-12 that exhibited 4.10 fold increase proline accumulation.

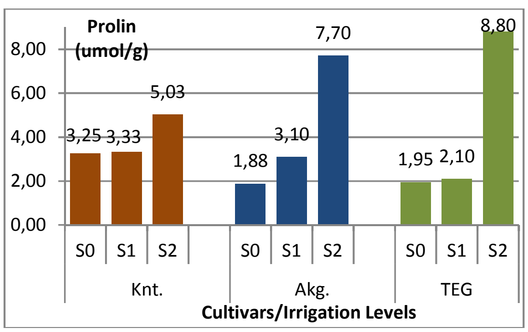

Fig. 1. Effect of drought on Proline content of Onion *cultivars*

The total chlorophyll (CT) change was significant for three factors (cultivar, drought and their interaction) ($P < 0.01$). Compared to control, total chlorophyll contents decreased by drought levels (Fig. 2). But this decreasing was not as sharpe as in proline content. Maximum decrease in total chlorophyll content was shown at S2 drought treatment (68%) in Akgun-12 and this was followed by same treatment in Kantartopu-3 (4%). In contrast to Akgun-12 and Kantartopu-3, total chlorophyll contents of Texas Early Grano cultivar increased at S1 treatment.

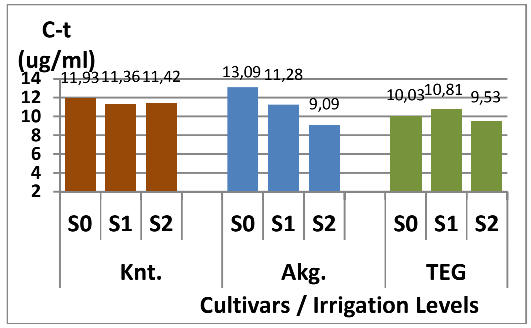

Fig. 2. Effect of drought on total chlorophyll content of Onion cultivars

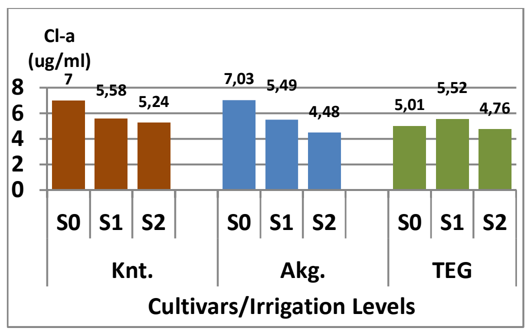

Fig. 3. Effect of drought on chlorophyll a of Onion cultivars

The change of chlorophyll-a (C_a) amount was significant for three factors (cultivar, drought and their interaction) *(P* < 0.01). Application of drought caused a significant decrease in Chlorophyll-a content of all cultivars ($p < 0.01$). Maximum decrease of Chl-a contents were determined as 37% at S2 irrigation level in Akgun-12 and 25% at same treatment in Kantartopu-3 (Fig.4).

The chlorophyll-b (C_b) change was significant for only interaction of main factors (Cultivars x drought interaction) *(P < 0.01)*. Chlorophyll-b increased significantly under drought stress in comparison with control in Kantartopu-3 (Fig. 4). In contrast to this cultivar, chlorophyll-b contents of Texas Early Grano and Akgun-12 cultivar decreased at S2 treatment. Compared to chlorophyll-a all cultivars exhibited low chlorophyll-b content under normal water supply.

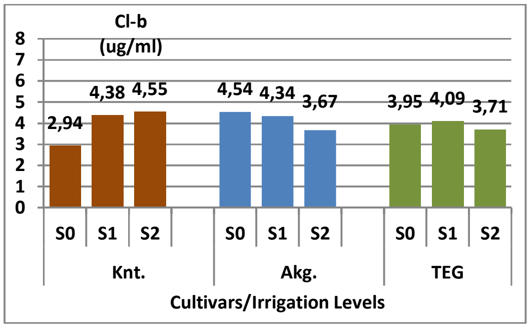

Fig. 4. Effect of drought on chlorophyll-b content of Onion cultivars.

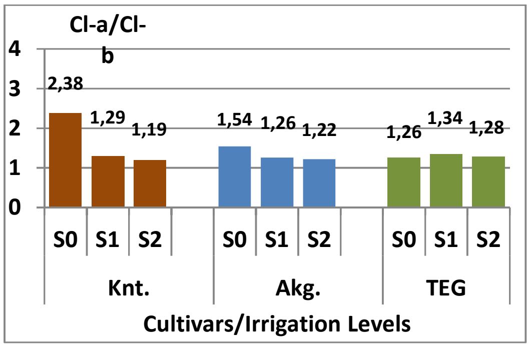

Fig. 5. Effect of drought on chlorophyll-a/b content of Onion cultivars

The carotenoids change was significant only for drought *(P < 0.01)*. Compared to control, contents decreased by drought levels in comparison with control in Kantartopu-3 and Akgun-12 cultivars (Fig. 6). In contrast to these cultivars, carotenoids content of Texas Early Grano did not affected by drought. Maximum decrease in carotenoids content was shown at S2 drought treatment (53%) in Akgun-12 and this was followed by same treatment (44%) in Kantartopu-3.

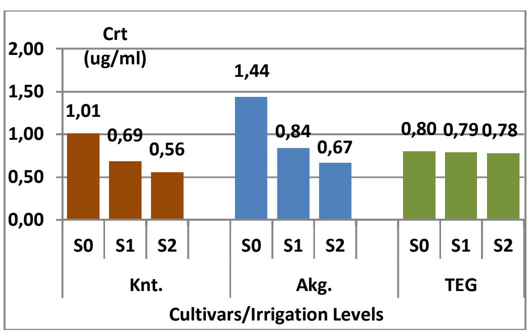

Fig. 6. Effect of drought on carotenoids content of Onion cultivars

Discussion

Plants may be affected by drought at any time of life, but certain stage such as germination and seedling growth are critical (Pesarakli, 1999). Biochemical and physiological changes occur in response to low water condition in different plants. Plants generally accumulate some kinds of compatible solutes such as proline, betaine to raise osmotic pressure and thereby to maintain both turgor and driving gradient for water uptake (Rhodes and Samaras 1994). The increase of proline occurs in decrease in water supply (Zhang et al., 2006). The synthesis of proline in plants extensively protects cell membrane and protein content in plant leaves. The synthesis and storage of osmolites differs in various plants. The accumulation of proline is considered by many authors as an indicator of abiotic stress such as drought stress, heat, salt or even the stress caused by pathogens. Under water deficit conditions, an increase of proline content in other plants was also reported by Zgallaiel et al, (2005), Vendruscoloet et al, (2007), Tatar and Gevrek (2008), Johari-Pireivatlou (2010). The results of our study are in agreement with other investigations.

Drought stress imposed at the vegetative stage, significantly decreased chlorophyll a content, chlorophyll b content and total chlorophyll content. The lack of effects on the chlorophyll a/b ratio indicates that chlorophyll-a is more sensitive to drought

than chlorophyll-b (Mafakheri et al. 2010) (Table 2, Fig.4-5). The results are agreement with other reports. Decreased or unchanged chlorophyll level during drought stress has been reported in other species, depending on the duration and severity of drought (Kpyoarissis et al., 1995; Basu and Chaturvedi, 2004).

Carotenoids have essential functions in photosynthesis and photoprotection (Xiao et al., 2008). Besides their structural roles, they are well known for their antioxidant activity by quenching 3Chl and 1O2, inhibiting lipid peroxidation, and stabilizing membranes (Demmig- Adams and Adams 1992). They also play a critical role in the assembly of the light-harvesting complex and in the radiationless dissipation of excess energy (Streb et al. 1998). In our study, at the drought conditions, carotenoids content in the two cultivars (Akgun-12 and Kantartopu-3) decreased. The significant decrease in content of carotenoids in the these cultivars under severe drought suggested that drought caused considerable oxidative stress by accumulation of ROS. The differences in chlorophyll and carotenoid contents between the three cultivars indicated that the some genotypes provided stronger photoprotective system against drought stress compared with the other genotypes.

Table 1. The effects of drought on some parameters of onion cultivars

Cultivars	Irrigation. Level	P	C_a	C_b	C_T	C_a/C_b
Knt.	S0	3.25 de	7.00 a*	2.94 c	11.93 ab	2.38 a*
	S1	3.33 d	5.58 b	4.38 ab	11.36 abc	1.29 c
	S2	5.03 c	5.24 bc	4.55 a*	11.42 abc	1.19 c
Akg.	S0	1.88 g	7.03 a	4.54 a	13.09 a*	1.54 b
	S1	3.10 e	5.49 b	4.34 ab	11.28 bcd	1.26 c
	S2	7.70 b	4.48 c	3.67 bc	9.09 e	1.22 c
TEG	S0	1.95 fg	5.01 bc	3.95 ab	10.03 cde	1.26 c
	S1	2.10 f	5.52 b	4.09 ab	10.81 bcde	1.34 c
	S2	8.80 a*	4.76 bc	3.71 bc	9.53 de	1.28 c
LSD		*0.195*	*0.897*	*0.773*	*1795*	*0.189*

*Means within a group (P, C_a, C_b, C_T, C_a/C_b) that have a different small letter are significantly different from each other. $P < 0.01$. C_a: Chlorophyll-a (µg/ml), C_b: Chlorophyll b (µg/ml) C_T: Total Chlorophyll (µg/ml), Crt: Carotenoids (µg/ml), P: Proline µmol/g. Knt= 'Kantartopu-3', Akg='Akgün-12', TEG= Texas Early Grano

Table 3. Effects of drought on carotenoids anount of onion cultivars

Irrigation Levels	Cultivars	Carotenodis (µg/ml)
S0	Knt.	1,04
	Akg.	1,45
	TEG	0,81
	Mean	1,10 a*
S1	Knt.	0,7
	Akg.	0,85
	TEG	0,8
	Mean	0,78 b
S2	Knt.	0,57
	Akg.	0,68
	TEG	0,79
	Mean	0,68 b
LSD (for Irr. Lev. Means)		*0,231*

**Means within a column that have a different small letter are significantly different from each other. (p>0,01) Knt= 'Kantartopu-3', Akg='Akgün-12', TEG= Texas Early Grano

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