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Evaluation of Drought Stress on Relative Water Content and Chlorophyll Content of Sesame (*Sesamum indicum* L.) Genotypes at Early Flowering Stage

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Abstract: In order to evaluate drought stress on relative water content (RWC) and chlorophyll content of 27 Sesame genotypes, a factorial experiment based on randomized complete block design with three replications was conducted in 2006 in Moghan region, Iran. Factors were 27 Sesame genotypes (Karaj 1, Yekta, Oltan, Moghan 17, Naz takshakheh, Naz chandshakheh, Borazjan 2, Borazjan 5, Darab 14, Varamin 37, Varamin 237, Varamin 2822, Zoodrass IS, Hendi, Chini, Yellow white, 5089, Panama, Do-1, TF-3 , TKG-21, J-I, RT-54, Hendi 9, Hendi 12, Hendi 14 and Jiroft) and irrigation levels (full irrigation and irrigation until flowering stage). Results showed that Varamin 2822 and Varamin 237 genotypes had the highest RWC of 84.100 and 81.217%, respectively. The most chlorophyll a content was observed in Hendi 9 genotype of 106.237, the most chlorophyll b in Karaj 1 genotype of 84.665 and the most chlorophyll total in Hendi genotype of 182.395 mg g^{-1} leaf fresh weight. It seems that Varamin 2822 genotype having the highest RWC and Hendi 9 and Hendi genotypes having the most chlorophyll a and chlorophyll total, respectively, are recommended for planting in arid and semi-arid conditions.

Key words: Sesame, drought stress, relative water content, chlorophyll content

INTRODUCTION

Chlorophyll content is one of the major factors affecting photosynthetic capacity. Reduction or no-change in chlorophyll content of plant under drought stress has been observed in different plant species and its intensity depends on stress rate and duration (Rensburg and Kruger, 1994; Kyparissis *et al*., 1995; Jagtap *et al*., 1998). Chlorophyll content in plant is considered as a favorite aspect for plant growth (Farquhar and Richards, 1984). Chlorophyll content of leaf is indicator of photosynthetic capability of plant tissues (Nageswara *et al*., 2001; Wright *et al*., 1994). Flooding irrigation about 1 cm above soil surface led to senescence and decrease in chlorophyll content of leaves. In this study, severe drought stress resulted in increase in chlorophyll content and then, remained constant (Mensah *et al*., 2006). De-Souza *et al*. (1997) reported that soybean plants grown at the greenhouse and were subjected to drought stress from early seed filling stage to maturity stage, rapidly

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lost their leaf chlorophyll content than control plants. Schelmmer *et al*. (2005) stated that drought stress had no significant effect on chlorophyll content of maize leaf and concluded that decrease in turger pressure caused by water deficit, resulted in change in amount of far red radiation passed through the leaf and this reason, read of chlorophyll meter device was changed. In other words, light reflection from leaf was increased with increasing drought stress. Barry *et al*. (1992) reported chlorophyll destruction in barley as affected by water deficit. Xian-He *et al*. (1995) stated the same results in wheat. Also, Fotovat *et al*. (2007) found that by exerting severe drought stress on wheat, chlorophyll content of leaf significantly decreased.

In mid 80s, RWC was introduced as a best criterion for plant water status which, afterwards was used instead of plant water potential as RWC referring to its relation with cell volume, accurately can indicate the balance between absorbed water by plant and consumed through transpiration. Schonfeld *et al*. (1988) showed that wheat cultivars having high RWC, are more resistant against drought stress. Generally, it seems that osmoregulation is one of the main mechanisms preserving turger pressure in most plant species against water loss from plant so, it causes plant to continue water absorption and retain metabolic activities (Gunasekera and Berkowitz, 1992). Zlatko Stoyanov (2005) found that by exerting drought stress for 14 days and reaching soil water potential to -0.9 MPa, osmotic potential and turger pressure in first leaf of bean strongly was decreased. Ramos *et al*. (2003) stated that RWC of bean leaves under drought stress significantly was lesser than control. Lazacano-Ferrat and Lovat (1999) subjected bean plant to drought stress and after 10, 14 and 18 days after irrigation was withholded, they evaluated RWC of stem and found that RWC was significantly lower comparing with control plants. Gaballah *et al*. (2007) applied antitranspirant maters on two Sesame cultivars named Gize 32 and Shanavil 3 and observed that this matters by preventing water transpiration from leaves, led to in crease in RWC in these cultivars.

The objective of this research was to determine RWC and chlorophyll content of Sesame leaves under drought stress in Moghan region, Iran.

MATERIALS AND METHODS

In order to evaluate drought stress on relative water content (RWC) and chlorophyll content of 27 Sesame genotypes, a factorial experiment based on randomized complete block design with three replications was conducted in 2006 in Moghan region, Iran. Factors were 27 Sesame genotypes (Karaj 1, Yekta, Oltan, Moghan 17, Naz takshakheh, Naz chandshakheh, Borazjan 2, Borazjan 5, Darab 14, Varamin 37, Varamin 237, Varamin 2822, Zoodrass IS, Hendi, Chini, Yellow white, 5089, Panama, Do-1, TF-3, TKG-21, J-I, RT-54, Hendi 9, Hendi 12, Hendi 14 and Jiroft) and irrigation levels (full irrigation and irrigation until flowering stage). In order to calculate leaf chlorophyll content, 1 g punched fresh leaf sample was grinded along with 40 mL acetone 80% (v/v) until it was well smoothed.

Resulted green liquid was transferred through Whatman paper No. 2. Eventually, final liquid volume using acetone 80% reached to 100 mL. Thereafter, chlorophyll extract light densities were read using Spectrophotometer at 645, 663 and 652 mn wavelengths. Chlorophyll a, b and total as mg g^{-1} leaf fresh weight were calculated according to Dhopte and Manuel (2002):

$$\text{Mg chlorophyll a} = [12.7(D_{663}) - 2.69\ (D_{645})]\times V/1000\ w$$

$$\text{Mg chlorophyll b} = [22.9(D_{645}) - 4.68(D_{663})]\times V/1000\ w$$

$$\text{Mg chlorophyll total} = [20.2(D_{645}) + 8.02(D_{663})]\times V/1000\ w$$

where, D is the chlorophyll extract light density, V is final volume of chlorophyll extract in acetone 80% and w is leaf fresh weight as gram. Also, in order to calculate RWC, leaf fresh weight samples were weighed, then were submerged in distilled water and finally were dried at 70°C for 48 h and were weighed again. RWC was calculated according to Dhopte and Manuel (2002):

$$RWC = (FW- DW/TW-DW)\times 100$$

where, FW is fresh weight, DW is dry weight and TW is turger weight of leaf samples.

RESULTS AND DISCUSSION

Change of Leaf Chlorophyll

Effects of irrigation was significant ($p<0.01$) on leaf chlorophyll content. Chlorophyll a content significantly was higher (Table 1) in irrigation treatment than stress. Its value in irrigation and stress conditions was 80.039 and 77.239 mg g^{-1} leaf fresh weigh. Based on the results, it was revealed that genotypes with lowest yields, had the highest chlorophyll a content so that, Hendi 9 genotype which is classified in low yielding genotypes, significantly had the highest chlorophyll a. Chlorophyll b content under irrigation and stress treatments was obtained of 74.626 and 85.862 mg g^{-1} leaf fresh weight. The highest chlorophyll b was belonged to Karaj 1 genotype of 100.513 mg g^{-1} leaf fresh weight. Unlike the chlorophyll a, it is clear that the genotypes with the lowest yields had the lowest chlorophyll b contents. Also, chlorophyll total like the chlorophyll b content, significantly was higher ($p<0.01$) in irrigation treatment than stress. The highest chlorophyll total was observed in Hendi genotype of 182.395 mg g^{-1} leaf fresh weight.

Table 1: Mean comparisons of main effects of genotypes and irrigation levels on measured traits

Treatments	RWC	Chlorophyll a	Chlorophyll b	Chlorophyll total
Irrigation levels				
Irrigation	81.696a	80.039a	74.626b	154.665b
Stress	73.90b	77.239b	85.892a	163.132a
High yielding genotypes				
Karaj 1	81.500abc	73.040ghij	100.513a	173.555abcd
Yekta	86.367ab	73.000ghij	90.287bcde	163.287cdef
Oltan	69.900fg	82.732bcdef	81.055efgh	163.787bcdef
Zoodrass IS	75.167cdefg	59.565k	77.743fghij	137.308k
Naz takshakheh	82.000abc	81.033cdefg	78.772fghi	159.807defgh
Naz chandshakheh	70.167efg	75.770fghij	57.638l	133.410k
Varamin 237	88.483a	84.328bcdef	83.997cdefg	168.325abcdef
Varamin 2822	77.367bcdefg	84.890bcdef	93.868ab	178.758ab
Medium yielding genotypes				
Moghan 17	86.567a	73.412ghij	69.560ijk	142.972jk
Varamin 37	77.950bcdefg	85.350bcde	85.173bcdef	170.523abcde
Borazjan 2	80.200abcde	68.597j	77.335fghij	145.932hijk
Borazjan 5	69.383fg	83.002bcdef	63.075kl	146.077hijk
Darab 14	88.450a	78.365fghi	91.027bcd	169.392abcdef
Hendi	81.200abc	88.388bcd	94.008ab	182.395a
Chini	80.417abcd	85.047bcdef	80.072fgh	165.118bcdef
Jiroft	75.783cdefg	69.668ij	92.623abc	162.292cdefg
5089	69.483fg	90.990b	85.140bcdef	176.132abc
Panama	73.450cdefg	78.215efghi	82.800defg	161.015cdefg
DO-1	79.250abcdef	75.728fghij	78.625fghi	154.353fghij
RT-54	69.433fg	79.148defgh	80.290fgh	159.438defghi
TKG- 21	79.417abcdef	72.747ghij	75.308fghij	148.055ghijk
J-1	70.900defg	89.373bc	68.608jk	157.982fghi
Low yielding genotypes				
TF-3	83.200abc	69.932hij	74.925ghij	144.857.ijk
Hendi 9	76.733bcdefg	106.273a	68.278jk	174.552abcd
Hendi 12	68.017g	54.465k	79.553fgh	134.020k
Hendi 14	79.533abcdef	90.380b	85.052bcdef	175.432abc
Yellow white	80.233abcde	69.825hij	71.678hijk	141.503jk

Numbers with the same letters, have no significant difference to each other

Results showed that the genotypes with the lowest yield had the lowest chlorophyll total and vice versa. Water deficit can destroy the chlorophyll and prevent making it (Lessani and Mojtahedi, 2002). Also, some researchers have reported damages to leaf pigments as a result of water deficit (Montagu and Woo, 1990; Nilsen and Orcutt, 1996). Mensah *et al*. (2006) found that subjecting Sesame to drought stress caused leaf chlorophyll was increased and then remained unchanged. Beeflink *et al*. (1985) reported increase in chlorophyll in onion under drought stress. Mentioned results are in accordance with this results confirming increase in chlorophyll b and total under drought stress. Ramalho *et al*. (2000) stated that ratio of chlorophyll a/b in late summer is less than spring of 2.6 units which may be attributed to more precipitations in spring. A reason for decrease in chlorophyll content as affected by water deficit is that drought or heat stress by producing reactive oxygen species (ROS) such as O_2^- and H_2O_2, can lead to lipid peroxidation and consequently, chlorophyll destruction (Mirnoff, 1993; Foyer *et al*., 1994). Also, with decreasing chlorophyll content due to the changing green color of the leaf into yellow, the reflectance of the incident radiation is increased (Schelmmer *et al*., 2005). It seems that this mechanism can protect photosynthetic system against stress. According to the Lawlor and Cornic (2002) reduction of carbon assimilation confronting water deficit, is due to limitation of Rubisco synthesis and ATP storage. Studies done as *in vivo*, showed that water deficit resulted in destruction of D1 protein of photosystem 2 (Xian-He *et al*., 1995) but the reason have not been known, yet.

Change of Relative Water Content

Irrigation and genotypes significantly ($p<0.01$) affected RWC (Table 1). In full irrigation, RWC was 81.69% and in stress, it was 73.90%. Moghan 17, Darab 14 and Varamin 237 genotypes had the highest values of 86.56, 88.45 and 88.48%, respectively. Hendi 12 genotype had the lowest RWC of 68.02%. Mensah *et al*. (2006) reported that with decreasing irrigation, RWC in Sesame decreased from 79.8 to 66.5%. Leaf RWC is of the best growth/biochemical indices revealing the stress intensity (Alizadeh, 2002). The rate of RWC in plants with high resistance against drought is higher than others. In other words, plant having higher yields under drought stress should have high RWC. So, based on the results, mentioned genotypes which are classified as high and medium yielding genotypes, should be of high-content RWC. Decrease in RWC in plants under drought stress may depend on plant vigor reduction and have been observed in many plants (Liu *et al*., 2002). Under water deficit, cell membrane subjects to changes such as increase in penetrability and decrease in sustainability (Blokhina *et al*., 2003). Microscopic investigations of dehydrated cells, revealed damages including cleavage in the membrane and sedimentation of cytoplasm content (Blackman *et al*., 1995). Probably, in these conditions, ability to osmotic adjustment is reduced (Meyer and Boyer, 1981). It seems that concentration of appropriate solutes to preserve membrane is not sufficient in this case.

Relations Between RWC and Chlorophyll Content

As shown in Table 2, RWC had the positive and significant correlations with chlorophyll b and total but non-significant and negative correlation with chlorophyll a. Also chlorophyll a had the negative and significant correlation with chlorophyll b and had non-significant correlation with chlorophyll total. In addition, chlorophyll b had non-significant correlation with chlorophyll total, as well.

Table 2: Correlations between measured traits

Measured traits	RWC	Chlorophyll a	Chlorophyll b	Chlorophyll total
Chlorophyll a	-0.003^{ns}	1.00		
Chlorophyll b	0.64**	-0.32**	1.00	
Chlorophyll total	0.59**	-0.08^{ns}	0.13^{ns}	1.00

ns, * and ** are non significant, significant at $p<0.01$ and significant at $p<0.05$, respectively

CONCLUSIONS

As shown, drought stress making mechanisms inside the plant, leads to decrease in chlorophyll a but increase in chlorophyll b and total. Also leaf RWC was decreased as affected by drought. The highest chlorophyll a, b and total was belonged to Hendi 9, Karaj 1 and Hendi genotypes, respectively which two latter ones were of high and medium yielding genotypes. So, this indicates that Sesame genotypes with high yields include high chlorophyll b and total under drought conditions. Moreover, Moghan 17, Darab 14 and Varamin 237 genotypes which were among the high and medium yielding genotypes, had the highest RWC. So, mentioned genotypes (because of retaining chlorophyll and RWC against drought along with the high yields) in order to planting as dry-farming, are recommended.

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