

# **RESEARCH PAPER**

OPEN ACCESS

# Effect of polyethylene glycol on the amount of chlorophyll a, chlorophyll b and total leaf of sugar beet genotypes

Mojtaba Ghasemi Fahim, Bahram Mirzamasoumzadeh\*, Babak Ahadzadeh

Department of Agronomy and Plant Breeding, Ardabil branch, Islamic Azad University, Ardabil, Iran

Article published on January 29, 2014

# Key words: Chlorophyll, drought stress, sugar beet, greenhouse.

# Abstract

Photosynthesis was one of important physiological processes in the plant. Water shortages would reduce its intensity. Photosynthesis persistency and maintaining leaf chlorophyll under stress conditions were physiological features of stress resistance. So, this research was performed on 2012 at greenhouse in order to investigate the effect of polyethylene glycol on chlorophyll a, b and Total on three sugar beet genotypes. Experiment was done as Two-factor factorial in form of randomized complete block design with three replications. Factor a (stress level: 1 normal irrigation, 2: Polyethylene glycol 6000 with 30% concentration) and factor b (genotypes) was performed. Results showed that the factors a and b were not significant in All traits. But interaction between factors level a × b showed significant differences at the 5% level the two characters of chlorophyll a and total chlorophyll. Bilateral comparison showed that the combination (normal × genotype 7233-P29) with an average of 6.36 had highest levels of chlorophyll a and combination (PEG 6000 × genotype-7233-P29) with an average of 3.79 had lowest level. The composition of the total chlorophyll (PEG 6000 × genotype Jolge and normal × genotype 7233-P29), respectively, with a mean of 7.83 and 7.93 had highest Total chlorophyll and combination (PEG 6000 × genotype-7233-P29) with an average of 5.44 had lowest amount. Rate of chlorophyll a and b are increased with stress intensity, but this issue was not true about total chlorophyll and with increasing stress intensity, total chlorophyll was decreased.

\*Corresponding Author: Bahram Mirzamasoumzadeh 🖂 bm\_masoumzadeh@yahoo.com

# ji bioi a hivi be

# Introduction

Sugar beet progenitor was emerged in seashore lands, so sugar beet are resistant to drought and salinity condition compared to other crops plants. It has shown that just cotton and grain are more resistant to drought and salinity condition compared to sugar beet (Cook and Scott, 1998). Sugar beet is a two years old plant, during first year this plant is laid in the earth without any root and sugar is congregate. Producing natal structure is done in sugar beet during third year and then produce blossom root (AbdollahianNoghabi, 2001). Sugar beet is a plant in moderate regions and is consistent to extent area of continental situation and is resistant to environmental stress (Cook and Scott, 1993). Stress concept in plants includes negative and intensive effect of alive or not alive factors on natural mechanism of plants in the environment which caused to disorders dry material production and decreasing performance (Fisher and Wood, 1989). Environmental stress is most factors to decrease corps performance in the world. In most of corps, mean performance in plants is less than 10-20 percent of performance potential. In specific areas of the earth, stress factors have more negative effect on corps due to geographical situation and agricultural actions are expensive with low productivity (Kafi and Mahdavi-Damghani, 2002 and Wittenmayer and Merbach, 2005).

Knowledge about plant physiological aspects at environmental stresses guides researchers to attain productivity potential and avoiding undesirable situation (Madhaj and Fathi, 2008). they believe that drought stress is increased when evaporative demand of leaves (evapotranspiration) is exceeded from roots capacity to water absorption (Kafi and Mahdavi-Damghani, 2002). Polyethylene glycol (PEG-6000) with Large-molecular-weight is more appropriate to develop drought stress compared to smaller molecules such as (PEG-4000), because the percentage of seed germination is approximately same at polyethylene glycol 600 solution and at soil condition (Kaufman and Eckard, 1971 Hardgree and

Emmerich, 1990). Photosynthesis was one of important physiological processes in the plant. Water shortages would reduce its intensity (Gusegnova et al., 2006). Photosynthesis persistency and maintaining leaf chlorophyll under stress conditions were physiological features of stress resistance. Drought stress caused to produce reactive oxygen and decrease chlorophyll degradation. During stress condition, chlorophylls will disappear at chloroplast structure (Sairam et al., 1998). Results have shown that mild drought stress doesn't effect on chlorophyll content in two cold plants namely Festuca and Poa pratensist, but severe drought will be reduced chlorophyll content in two cold plants (Hauny, 2001). Stability of Chlorophyll is an indicator of plant resistance to drought stress. The purpose of this study was to investigate the effect of polyethylene glycol on the content of chlorophyll a, chlorophyll b and total leaf genotype of sugar beet.

#### Material and methods

#### Location of test implementation

in order to prepare seeds, modification institute of seeds at Karaj was visited and after receiving seeds Table 1) Bracteole was done. This study was done in 2011 at greenhouse as two-factor factorial experiments. Factor a (drought level: 1 normal irrigation, 2: Polyethylene glycol 6000 with 30% concentration) and factor b (genotype) was performed. Experiment was done as Factorial in completely randomized design frame with three replications in this study; traits of chlorophyll a, chlorophyll b and total chlorophyll were evaluated.

#### Table 1. Genotypes used in this study.

Number	Name of genotype
1	30906
2	30908
3	30915-88

### Mode of test implementation

Experiment dry osmotic first treatment (normal water) and a second treatment of dry osmotic using

polyethylene glycol 6000 concentration was 30% and in pots with a diameter of 30 cm and a height of 40 cm that have drainage was 20 seeds each digit in depth 2.5 cm using forceps straight perlite medium diameter of 4 mm were grown. Varieties that were less than 30 seeds were planted viability. Immediately after planting, the pots were irrigated with water under each potwa spliced in containers with a capacity of 500 cc. And every 3 days by municipal water volume was 500 cc. In the first month according to the needs of low concentrations of plant nutrients in half Hoagland solution (Table 1), the experimentally and a detailed comparison table was properly used and the subsequent months of full concentration Hoagland solution was used. After 30 days of sowing (stage 3 or 4 true leaves), so meperlite were added to the pots. To help establish appropriate plants and after 60 days of implantation stage (5 to 6 leaf stage) plants in each pot were thinned to 8 plants remained low after 70 days of treatment was begun planting treatments using Overall solution were carried out under the pots. Hoagland solution was used in all solutions to environmental elements

Table 3	<b>3.</b> Analy	ysis of	variance.
---------	-----------------	---------	-----------

required for plant growth and lack of any tension or toxic elements into the plant will be, and the results affect.



Fig. 1. Measurement of chlorophyll.

**Table 2.** Compounds and their levels in Hoagland solution.

Chemical name	Stock solution	Amount
	amount(g/1lit)	of 100
		liters(ml)
NH4H2PO4	115	100
KNO3	101	600
Ca(NO3)24H2O	236	400
MgSO47H2O	246	200
Fe-EDTA	5	150
H3BO3	0.38	150
ZnSO47H2O	0.22	150
MnSO44H2O	1.02	1000
CUSO45H2O	0.08	100
(NH4)6MO7O244H2O	0.02	100

S.O.V	df	Mean Square		
	-	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Replication	2	0.24	0.013	0.141
Stress level	1	0.64	0.149	0.17
Genotype	2	0.009	0.243	0.308
Stress level × Genotype	2	0.862*	0.028	10.698*
Error	10	1.389	0.163	2.457
Coefficient of Variation (%)		23.09%	26.59%	23.67%
* and ** Significantly at p < 0.05 and	l < 0.01, 1	respectively.		

Traits of chlorophyll a, chlorophyll b and total chlorophyll were sampled through four to seven leaves of three remained plants in order to examine chlorophyll a, b and their ratios (koumari, 2007). Sampling was done from four to server leaves under drought stress situation at evaluated genotypes during two month after treatment, leaves were cut and have been covered in a paper, after putting in nitrogen solution, liquid were transferred to Frazer. 0.5 gram of each leaf were pashed separately in a plate for ten seconds and 10 milliliter of 80 percent acetone were added in two steps, then were covered in aluminum papers. Chlorophyll examination was done in low light and temperature. Samples were centrifuged in four degree centigrade with 3000 rpm circuit and in were centrifuged in centrifuges device during five minutes (KUBOTA 6900 model) which was made in japan, then one milliliter of centrifuged green solution were mixed with nine milliliter of 80 percent acetone, chlorophyll a and b amount were respectively 645 nanometer and 663 nanometer. In order to do this, spectrophotometer were changed to zero with acetone. Mentioned numbers were estimated to microgram /milliliter of chlorophyll a and b concentration according to below quotation (koumari, 2007) and finally in term of milligram to gram by spectrophotometric device.

1-3 relation	Chl a= 13.19 A663 – 2.57 A645
2-3 relation	Chlb= 22.10 A645 - 5.26 A663
3-3 relation	Tot chl= 7.93 A663 + 19.53

A645

Chla Chlorophyll a concentration in term of microgram/milliliter

Chlb Chlorophyll b concentration in term of microgram/milliliter

Tot chl total Chlorophyll concentration in term of microgram/milliliter

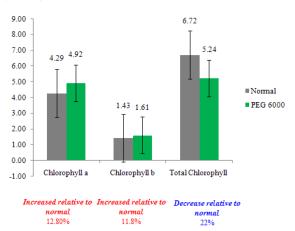
A spectrophotometric device reading in wavelength 663 and 645 nanometer

#### Statistical analysis

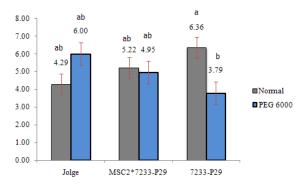
Before data analysis, establish the assumption of normal distribution of deviations, homogeneity of variance was examined. The mean yield using Duncan test at 5% probability level by SPSS-18 software and graph drawing was done by Excel.

# **Results and discussion**

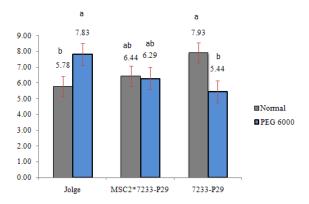
Chlorophyll a of PEG 6000 level with 4.92 averages had highest value and Chlorophyll a of leaf in stress situation compared to normal situation increase 8.12%. Chlorophyll b of PEC 6000 level with 1.61 averages had highest value and Chlorophyll b of leaf in stress situation compared to normal situation increase 8.11%. Also, total Chlorophyll in normal level with 6.72 averages had highest value and total Chlorophyll in stress situation compared to normal situation increase 22% (Fig. 2). Results showed that the factors a and b were not significant in All traits But interaction between factors level a × b showed significant differences at the 5% level (Table 3). Comparison showed that the combination (normal  $\times$ genotype 7233-P29) with an average of 6.36 had highest levels of chlorophyll a and combination (PEG 6000 × genotype- 7233-P29) with an average of 3.79 had lowest level and placed in b statistical group. The composition of the total chlorophyll (PEG 6000  $\times$  genotype Jolge and normal  $\times$  genotype 7233-P29), respectively, with a mean of 7.83 and 7.93 had highest Total chlorophyll and combination (PEG 6000  $\times$  genotype- 7233-P29) with an average of 5.44 had lowest amount and placed in b statistical group (Fig. 3 and 4).



**Fig. 2.** stress level mean in chlorophyll a, b and total and percent of decrease and increase these traits.



**Fig. 3.** drought stress level interaction × genotype of chlorophyll a traits.



**Fig. 4.** drought stress level interaction × genotype of total chlorophyll traits.

#### References

**AbdollahianNoghabi M.** 1999.Ecophysiology of sugar beet cultivars and Weed species subjected to water deficiency stress. Ph.D. Thsis, University of Reading.

**Cook DR, Scott C.** 1998. Sugar from Science to Practice. Translation: Faculty Improvement Institute Beet Seed. Publications Produced Sugar Beet Seed Improvement Institute, p. **731**.

**Cooke DA, Scott RK.** 1993 The sugar Beet crop science into practice. London, new York chapmon and Hall. 675:456 -469.

**Fischr RA, Wood JT.** 1989. Drought resistance in spring wheat cultivar, yield associations with morpho-phisiological traits. Australian Journal Agricultural **30**, 1001-1020.

**Gusegnova IM, Suleymanov S, Aliyev JA.** 2006. Protein composition and native state of pigments of thylakoid membrane of Wheat genotypes differently tolerant to water stress. Biochemistry **71.** 223-228.

Hardgree SP, Emmerich WE. 1990. The effect of polyethylene glycol exclusion on the water potential of solution-saturate filter paper. Plant Physiology **92**, 462-466.

**Hauny B.** 2001. Involvement of antioxidants and lipid peroxidantion in the adaptation two season grasses to localized drought stress. Environmental and Experimental Botany **45**,105-114.

**Kafi M, Mahdavi-Damghani A.** 2002. Resistance mechanisms of plants to environmental stresses (translation). University of Mashhad.

**Kaufman MR, Eckard AN,** 1971. Evaluation of stress control by polyethylene glycol byanalysis of guttation. Plant Physiological **47**, 453-456.

Madhaj A, Fathi Gh. 2008. Crop Physiology. Islamic Azad University Press. p. **128**.

Sairam RK, Deshmukh PS, Saxna DC. 1998. Role of antioxidant systems in Wheat genotype tolerance to water stress. Biologia Plantrum **41(3)**, 387-394.

Wittenmayer L, Merbach W. 2005. Plant responses to drought and phosphorus deficiency: Contribution of phytohormones in root- related processes. Journal of Plant Nutrition and Soil Science **168**, 531-540.