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Changes of the lipid peroxidation and chlorophyll amount of green bean genotypes under drought stress

Fikret Yasar, Ozlem Uzal* and Taylan Ozpay

Department of Horticulture, Faculty of Agriculture, University of Yuzuncu Yil, Zeve Campus, 65080. Van. Turkey.

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One of the environmental stresses, and maybe one of these important drought stresses, cause deterioration of oxidative on the plant cell. Beans is one of the vegetables influenced by high level of drought. Drought stress deterioration effects on beans and its aim determines these effects either as been different or not caused by drought stress dependent on 10 item beans (*Phaseolus vulgaris* L.) variety. To get the hydroponic condition ready in time within 15 days, plant with 10% ratio of polyethyleen glycol (PEG 6000) were used to create drought effects. Bean leaves were harvested 6 days later after PEG implementation and were measured. The amount of lipid peroxidation production and malondyhalheit were directed towards determining the oxidative deterioration and in addition the chlorophyll amount. As a result of drought implementation, Samsun 96 (S96) and Sırık Barbunya (SB) beans genotypes have the most decreased chlorophyll amount than Gevaş Sırık 57(GS57) and Oturak Barbunya (OB) genotypes. At the same time, S96 and SB genotypes have the most increased malondyhalheit (MDA) content than GS57 and OB. Also, of the total ten item of beans variety, Gevaş Sırık 57 and Oturak Barbunya were least affected by drought stress, while S96 and SB genotypes showed more sensitivity to drought stress.

Key words: Chyloropyll, drought, lipid peroxidation, *Phaseolus vulgaris* L.

INTRODUCTION

Stress on vegetable production may be defined as one or more environmental factors that affect the plant and restrict its growing causing a decrease in production. Plants develop certain defense mechanisms against all kinds of stress factors caused by biotic and abiotic resources in nature and they endeavor to continue to grow and develop by adapting themselves to negative circumstances. The plants, which grow under stress factors, produce reactions according to their genotypic characteristics. While some species and varieties are affected by the stress at very low levels, others are fatally damaged. It is known that factors such as different growing stages of a plant, type and magnitude of the stress, application time besides such different adaptation skills based on genetic characteristics are effective as defense mechanisms developed by plants.

It is known that free oxygen radicals, which are released under stress, cause cellular damages in plants. These free oxygen radicals cause lipid per oxidation on cellular membranes and this causes damages on the cellular membrane. The fact that toxic oxygen radicals are synthesized more rapidly under stress and the fact that light density in the ambient is high make them more effective. They cause photo-oxidative damages on chlorophylls and cellular membranes. Free oxygen radicals break phospholipids of cellular membranes (especially non-saturated fat acids) (Fridovic, 1986; Shalata and Tal, 1998; Sreenivasulu et al., 1999), proteins (Davies, 1987), nucleic acids (Fridovic, 1986; Imlay and Linn, 1988) and chlorophyll into their compo-nents and these effects increase under high light density (Foyer et al., 1994; Cakmak et al., 1995; Eker, 2002). Many studies found that necroses seen in plants grown under drought stress are caused by lipid destructions on cellular membranes caused by oxygen radicals. Also, chlorosis occurs as a result of broken chlorophylls by oxygen radicals (Heath and Parker, 1968; Salin, 1987; Gepstein, 1988; Gossett et al., 1994; Streb and Feierabend, 1996). Beans are significant vegetables grown especially

*Corresponding author. E-mail: uzalcan@hotmail.com. Tel: +90-432 2251056. Fax: +90-432 2251104.

in tropic and sub-tropic fields like other vegetables from leguminase family. However, demands from societies for this vegetable cannot be satisfied in recent years because drought and salinity has been increasing especially in the fields on which this vegetable is grown. Most especially, drought experienced in recent years as a result of global heating seriously affects vegetable production. Therefore, bean's mechanisms for resisting drought should be discovered, draught tolerant species of other types should be developed and agronomic performances should be improved (Subbaro et al., 1995). As seen in other species, variations in resistance against drought and salinity also exist in bean germplasm (Lazcano and Lovatt, 1999). *P. vulgaris* generally is more precious against drought and salt than in other leguminase species (Subbaro et al., 1995; Pimentel, 1998; Salinas et al., 1996).

On the other hand, *P. acutifolius*, ancestor of bean, has been produced for centuries. Furthermore, it is grown especially in drought and warm regions (Frederici et al., 1990). Consequently, it is possible that a genotype exist in this species which also reacts very well against drought. Thus, tolerance mechanisms of bean against drought could be discovered by employing biochemical and physical methods and selection and treatment strategies specific to them should be developed. Significant selective criteria include: growing performance of the plant under the stress caused by physiologic and biochemical variations occurrence in beans; MDA amounts produced by oxidative deterioration and a product of lipid per oxidation and variations in chlorophyll accumulation in the leaves of the plant in previous studies (Turkan et al., 2005).

Selection studies were implemented in a climatic room under control and by employing Poly-ethylene glycol (PEG-6000) due to easy and short time implementation. PEG is a chemical matter used for creating drought stress in plants (Murillo-Amadaor et al., 2002). Bean is grown in our country and around the world in various ecologies and it shows quite genotypic variation. It has populations suitable for fresh and dry consumption. The subject of the present study is to deter-mine reactions of the plants under stress by employing certain biochemical methods after stress is applied on bean genotypes. Oxidative deterioration on membrane lipids and also chlorophyll amounts were examined. Then, the reactions from bean genotypes against drought stress and variations between them were determined.

MATERIALS AND METHODS

Material

A total of 10 bean (*Faseolus vulgaris* L) genotypes from various regions of Anatolia were employed in the present study: Gevaş Bodur 64 (GB64), Kırkgünlük (KG) and Oturak Barbunya (OB) in oturak types, Sazova 1946 (Sazova), Samsun 95 (S95), Samsun 96 (S96), 4F-89 Fransiz (4F-89), Gevaş Sirik 57 (GS57), Gevaş Sirik 26 (GS26) and Sirik Barbunya (SB) (Table 1).

Growing the plants

Bean seeds were planted in pots made of foam with a hole on the pots' bottom and filled with pumice. They were placed in a climatic room at a temperature of 25±2°C and with a relative humidity of 50% under a light density of 500-µmol.$m^{-1}s^{-1}$. Then, they were left waiting for a photo-period of 16/8-hour daylight/dark. After their first real leaves had been seen, the saplings were watered with Hoagland nutrient solution (Hoagland and Arnon, 1938). And after the saplings had produced their second real leaves in perlite medium, they were taken to water culture. The plastic dishes in sizes of 25 x 25 x 18 cm filled with Hoagland nutrient solution were used for water medium. Nutrient solutions were refreshed once a week and dishes were re-positioned to ensure that all plants benefit equally from lightening conditions.

Implementing drought application

The samplings were grown in the water medium for a week and then, drought application was started. It was seen that the saplings had 3 - 4 real leaves at this stage. 15 plants from each genotype were assigned for the test with three repetitions. Polyethylene Glycol (PEG 6000) at a concentration of 10% was added to Hoagland nutrient solution (Turkan et al., 2005; Kalefetoğlu, 2006).

Measuring weights of green parts of the plants

Six plants from each variety were selected randomly on the seventh day of the stress and their roots, shoots and leaves were divided. Their fresh weight was measured with the help of digital balance precious to 1/10000.

MDA analysis

The method defined by Lutts et al. (1996) was employed for measuring the amount of malondialdehyde (MDA), which is produced as a result of lipid per oxidation causing damage to cellular membrane. MDA concentration was determined by using "extinction" coefficient, which is 155 mM^{-1} cm^{-1}, in µmol/g Fresh Weight. The following equation was used in the calculation:

MDA= (A 523 - A 600) x volume of the extract (ml) / (155 mM/cm x sample amount)

Chlorophyll analysis

200 mg sample was collected by taking three leaves from the end of offshoot of the plants backward. They were placed in ethanol with a concentration of 80% and were left in a water bath at 50°C for 20 min. The absorbance values were read at 654 nm in spectrophotometric way (Luna et al., 2000). As a result of these measurements, total chlorophyll amount in the fresh leaf sample was calculated by employing the following equation in µg/mg fresh weight:

Total chlorophyll amount: A 654 x 1000/39.8 x sample amount

RESULTS

Considering leaf weights of the genotypes, leaf weights of

Table 1. Test number, code, variety or name from where it is supplied, location and type of the bean genotypes used in the study.

No	Code	Genotype name	Location	Supplier	Type
1	GB64	Gevaş bodur 64	Van/Gevaş	Farmer	Brush type
2	S100	Samsun 100	Samsun/Kavak	Farmer	Climbing type
3	S95	Samsun 95	Samsun/Kavak	Farmer	Climbing type
4	4F-89F	4F-89 furans	Eskişehir	Anatolian agricultural research	Climbing type
5	GS57	Gevaş sırık 57	Van/Gevaş	Farmer	Climbing type
6	GS26	Gevaş sırık 26	Van/Gevaş	Farmer	Climbing type
7	S96	Samsun 96	Samsun/Kavak	Farmer	Climbing type
8	SB	Sırık barbunya	Antalya/Korkuteli	Farmer	Climbing type
9	KG	Kırkgünlük	Eskişehir	Anatolian agricultural research	Brush type
10	OB	Oturak barbunya	Antalya/Korkuteli	Farmer	Brush type

the plants of the control group varied. The highest values were found in GB64, OB and SB numbered genotypes and the lowest values were found in S96, KG and 4F-89F numbered genotypes. Leaf weights of the plants, which experienced drought stress, also varied. The highest values were found in OB, S100 and GS57 numbered genotypes and the lowest values were found in S96, S95 and GS26 numbered genotypes. Leaf weights of the plants grown under drought stress were low compared with the control group in some genotypes while that for others were the same with the control group. Leaf weights of GB64, S100, S95, GS57, GS24, SB and OB numbered genotypes were lower. However, drought stress did not affect leaf weights of 4F-89F, S96 and KG numbered genotypes.

Considering MDA accumulation in the leaves of the genotypes, there is no statistically significant variation between the plants of the control group. MDA accumulation in the leaves of the plants grown under drought stress increased in all genotypes. The highest MDA accumulation was seen in S96, SB and GS26 numbered genotypes while the lowest values were found in OB, GS57 and S95 numbered genotypes (Table 2).

Total chlorophyll amount

Considering chlorophyll amounts in the bean leaf, which is the organ experiencing the most rapid damage under stress, chlorophyll amounts of the plants of the control group, which did not go under drought stress, did not change according to genotypes. However, chlorophyll amount of the leaves of the genotypes grown under drought stress generally decreased. Only OB genotype's chlorophyll amount stayed within the same interval with the control group. The highest chlorophyll accumulation was found in OB, Sazova and GS57 numbered genotypes and the lowest chlorophyll accumulation was found in S96, S95 and SB numbered genotypes (Table 2).

DISCUSSION AND CONCLUSION

In the present study, drought stress was produced by applying PEG-6000 at a concentration of 10% on 10 different bean genotypes including standard varieties. The first significant symptomatic effect of the drought stress was seen in the leaf weights of the plants. Genotypes produced different responses in the manner of leaf weight. The highest values were found in OB, Sazova and GS57 numbered genotypes and the lowest values were found in S96, S95 and GS26 numbered genotypes. Leaf weights of the plants, which experienced drought stress, also varied. The highest values were found in OB, S100 and GS57 numbered genotypes and the lowest values were found in S96, S95 and GS26 numbered genotypes. Many researchers evidenced that the highest oxidative deterioration was generally seen on leaves during their studies done on various plants. (Costa França et al., 2000; Lazcano-Ferrat and Lovatt, 1999; Turkan et al., 2005).

The lowest oxidative deterioration and consequently, the lowest MDA accumulation occurred under drought stress in the leaves of GS57 and OB numbered genotypes. However, the highest MDA accumulation was seen in Samsun 95 (S95) and Sırık barbunya (SB) genotypes. In other words, the highest cellular membrane damages occurred in them. It is seen that GS57 genotype, which had been found salt tolerant in the study done by Yasar (2007), accumulated less MDA in this study compared with other genotypes and accordingly, it is more tolerant. Similarly, MDA values of two grass varieties grown under drought stress had been examined and it had been found that MDA amount increases depending on the increase of drought and MDA value of the more tolerant variety is less by 35% compared with the precious type (Fu and Huang, 2001). It was reported that the genotypes with higher stress tolerance have less MDA and less lipid per oxidation, and those with higher lipid per oxidation are more precious to salt by Karanlik (2001) on wheat, Fu and Huang (2001) on grass, Aktas

Table 2. Leaf weights (g), Chlorophyll accumulation and MDA amounts (µmol/g YA) of the bean genotypes grown under drought stress.

Genotip	Leaf weights		Chlorophyll		MDA	
	Control	Application	Control	Application	Control	Application
GB64	4.70aA	1.92bcB	1.75b A	0.50b B	0.68Ab	2.03bcA
S100	3.26bA	2.13abB	1.44cd A	0.77a B	0.76aB	1.67cdA
S95	2.74cA	1.59dB	0.83ef A	0.44b B	0.67aB	1.45deA
4F-89 F	2.07dA	1.85b-dA	1.56bc A	0.51b B	0.66aB	1.70cdA
GS57	2.77cA	2.12abB	1.30d A	0.73a B	0.73aB	1.42deA
GS26	2.20dA	1.70cdB	0.90e A	0.69a B	0.65aB	2.15abA
S96	1.83dA	1.58dA	1.65b A	0.50b B	0.66aB	2.43aA
SB	3.41bA	1.81cdB	0.97e A	0.50b B	0.66aB	2.23abA
KG	2.03dA	1.75cdA	2.00a A	0.68a B	0.71aB	1.73cdA
OB	3.49bA	2.36aB	0.66f A	0.78a B	0.65aB	1.19eA

The difference between the means of genotypes with the same small letter is not significant. The difference between the means of application with same capital letter is not significant

(2002) on pepper, Turkan et al. (2005) on bean, Yasar et al. (2006) on eggplant, Yasar (2007) on bean, Kusvuran et al. (2007) on melon and Yasar et al. (2008) on bean. Thus, it was seen that MDA amount may be considered as a parameter for drought tolerance characteristic. Chlorophyll amount decreased in the leaves of the all genotypes grown under drought stress compared with the control group. However, the highest chlorophyll amount was found in GS57 and OB genotypes while S95 and SB genotypes experienced the highest chlorophyll loss and showed the lowest chlorophyll values compared with the control group. As seen in the study of Yasar et al. (2008) done on salt stress on bean and also in our study on drought stress, we may say that chlorophyll density in the leaves of bean is an important criterion in determining their responses against stress and especially in determining drought stress degree because they stand in a line with MDA accumulations. Increase in lipid per oxidation of the plants under stress may cause chlorophyll losses (Yaşar, 2003; Kusvuran et al., 2008; Yasar et al., 2008). Chlorophyll loss caused by drought stress depends on photo-oxidation produced as a result of oxidative stress (Kato and Shimizu, 1985; Fu and Huang, 2001). Fu and Huang (2001) applied drought to two grass varieties, a tolerant and a precious type. Then, they observed their growing performances and chlorophyll amounts and reported that draught tolerant type did not lose chlorophyll under drought stress. In another study (Sairam et al., 1998), it was seen that chlorophyll amounts decreased in the wheat plant grown under drought stress. We obtained similar results in our study on beans under drought stress.

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