ADVANCES IN BIORESEARCH
Adv. Biores., Vol 8 [3] May 2017: 85-90

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.8.3.8590

Advances in Bioresearch

ORIGINAL ARTICLE

Study of some Biochemical attributes responses to water deficit stress on Sunflower lines

Elnaz Farjzadeh[1*], Mostafa Valizadeh[1], Mohammadreza Shakiba[2], Mehdi Ghaffari[3]
[1]Department of Plant Breeding and Biotechnology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.
[2]Department of Plant Ecophysiology, Faculty of Agriculture, University of Tabriz, Iran.
[3]Seed and Plant Improvement Institute, Karj, Iran.
*Corresponding author: elnazfarjzadeh@gmail.com

ABSTRACT
In order to evaluate the seed yield, Photosynthetic pigment content, total phenolics, proline and glycine betaine responses of eight sunflower lines under control and field water deficit stress in 2014 was carried out. Seed yield significantly decreased under drought stress. The pigment concentrations were substantially declined in all sunflower lines under water deficit stress conditions. Water deficit stress declined the levels of total phenolics in RGK21, AGK30, AGK46 and AGK330, but water deficit stress increased the content of total phenolics in RGK15, RGK25, RGK26 and AGK2 lines. On the other hand, the osmotic stress markedly enhanced the levels of proline and glycine betaine in whole sunflowers lines, but this was more pronounced in AGK330. The present results showed that drought stress retards the yield and some biochemical traits of sunflower lines. Thus, AGK330 could be considered as "drought tolerant" and RGK15 as "drought sensitive" in response to water deficit stress. In conclusion, the amount of proline and glycine betaine in sunflower could improve drought tolerance.
***Key words**: Glycine betaine, pigment content, proline, sunflower, water stress.*

Received 08.03.2017 Revised 20.03.2017 Accepted 30.04.2017

How to cite this article:
E Farjzadeh, M Valizadeh, M Shakiba, M Ghaffari ·Study of some Biochemical attributes responses to water deficit stress on Sunflower lines. Adv. Biores., Vol 8 [3] May 2017.85-90

INTRODUCTION

Drought is undoubtedly one of the most important environmental stresses limiting the productivity of crop plants around the world [1]. Sunflower is one of the major and most important non-conventional oilseed crops in the world due to its excellent oil quality[2]. Although sunflower is known as a drought tolerant crop or grown under dry land conditions, substantial yield increases are achieved with frequent irrigation.

Drought stress damage the thylakoid membrane, disturb its functions, and ultimately decrease photosynthesis and crop yield [3]. Severe drought stress also inhibits the photosynthesis of plants by causing changes in chlorophyll content, by affecting cholorophyll components and by damaging the photosynthetic apparatus [4]. Ommen *et al.* [5] reported that leaf chlorophyll content decreases as a result of drought stress. Drought stress caused a large decline in the chlorophyll *a* content, the chlorophyll *b* content, and the total chlorophyll content in all sunflower varieties investigated [18]. The decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by active oxygen species. Of various secondary metabolites, terpenes and phenolics are more important to abiotic stress tolerance than the others due to their structural properties [23].

Plants can partly protect themselves against mild drought stress by accumulating osmolytes. Proline and glycine betaine (GB) are the most common compatible osmolytes in drought stressed plants. For example, the proline content increased under drought stress in sunflower [7]. Proline accumulation can also be observed with other stresses such as high temperature and under starvation [24]. Proline metabolism in plants, however, has mainly been studied in response to osmotic stress [25]. The accumulation of proline

in plant tissues is also a clear marker for environmental stress, particularly in plants under drought stress. Proline accumulation may also be part of the stress signal influencing adaptive responses. In addition, it has been reported that GB is an effective osmo-protectant which accumulates in a number of plants under drought stress [9] thereby playing a vital role in plant tolerance to drought. A positive effect of GB foliar spray on yield and yield component in plants grown under water limited environment has been reported in different sunflower [14]. However, the aim of this study was to investigate the effect of water deficit stress on proline, glycine betaine, pigment contents, total phenolics and yield of sunflower plant.

MATERIALS AND METHODS

Plant material and experimental conditions

Two separate experiments in randomized complete block designs were carried out, in 2014 growing season for comparing the above mentioned eight sunflowers lines (Table 1) at the Research Station of University of Tabriz, one with common irrigation water control and the other interrupted irrigation during 27 days before flowering. Three replications were used in each experiment. The experimental plots included two rows of 3 m long and 0.75 m wide.

Photosynthetic pigment

Chlorophyll-*a*, chlorophyll-*b*, carotenoids and anthocyanins were determined in leaves. About 200 mg weight of fresh leaf was placed in a mortar half full with liquid nitrogen and it was ground to powder. Then, pigments were extracted from the powdered sample by adding 2.0 ml of the extraction solvent 85% acetone and 15% Tris stock buffer (1% w/v Tris final concentration; adjusted to pH 8 with HCl) previously cooled in ice. The extract was centrifuged at 12,000 g for 3 min. A defined quantity of supernatant (1 ml) was removed and diluted to 3.0 ml. Its absorbance was measured at 537, 663, 647 and 470 nm in a 1-cm path length cell [27].

Total phenolics

Fresh leaf tissue (50 mg) was homogenized with 80% acetone and centrifuged at 10,000 g for 10 min. One-hundred microlitres of the supernatant were diluted with 2 mL of water and 1 mL of Folin–Ciocalteau's phenol reagent and shaken vigorously. Then 5 mL of 20% sodium carbonate solution was added and the volume was made up to 10 mL with distilled water. The contents were mixed thoroughly and the absorbance was read at 750 nm [22]. The results were expressed as mg/g of fresh leaf.

Proline

Proline content was quantified by following the method of Bates *et al*. [10]. Fresh leaf samples (500 mg) were homogenized in 3% (w/v) sulphosalicylic acid, and centrifuged at 4000 g for 10 min at 4°C. The supernatant was added with acid ninhydrin and glacial acetic acid in a test tube. The mixture was heated for 30 min at 98°C in a water bath and then allowed to cool at room temperature. The mixture was extracted with toluene and absorbance was read at 520 nm.

Glycine btaine

For glycine betaine, fully expanded upper most leaves were taken from the plants grown under normal and water stressed conditions, and analysis was carried out according to the method of Grieve and Grattan [13]. Leaf extract was prepared in 20 mL test tubes by chopping 0.5 g leaves in 5 mL of toluene-water mixture (0.05% toluene). All the tubes were mechanically shaken for 24 h at 25°C. After filtration 0.5 mL of extract was mixed with 1 mL of 2 N HCl solution then and 0.1 mL of potassium tri-iodide solution (containing 7.5 g iodine and 10 g potassium iodide in 100 mL of 1 N HCl) was added and shaken in an ice cold water bath for 90 min and then 2 mL of ice-cooled water was added after gentle shaking 10 mL of 1, 2 dichloroethane (chilled at -10°C) was pour in it. By passing continuous stream of air for 1-2 minutes two layers were separated, upper aqueous layer was discarded and optical density of organic layer was recorded at 365 nm.

Statistical analysis

Data was analyzed by using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA). The assumptions of variance analysis were tested by ensuring that the residuals were random and homogenous, with a normal distribution. For treatment showing a main effect, means (± SE) compared by Duncan test. $P \leq 0.05$ was considered as significant differences between treatments.

RESULTS AND DISCUSSION

Seed yield

The analysis of variance for the seed yield showed that, water deficit stress had significant effect on whole lines of sunflower ($P < 0.05$). Differences among lines were also found significant for the seed yield ($P < 0.05$). Water deficit stress × lines interaction was not significant for seed yield. The seed yield of all

sunflower lines decreased under water deficit stress (Fig 1). The highest and lowest seed yield was obtained from AGK330 and RGK15, respectively (Fig 1). Water deficit is one of the major abiotic stresses, which adversely impacts crop growth and yield. The plant responses to water stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of growth [12, 19]. Among the crops, sunflower is deep rooted crop that has been shown to deplete available soil water. This makes sunflower more tolerant to short periods of water stress [6].

Table 1. List of sunflowers analyzed in this study

No	Line	No	Line
1	RGK15	5	AGK2
2	RGK21	6	AGK30
3	RGK25	7	AGK46
4	RGK26	8	AGK330

Photosynthetic pigment

The analysis of variance for the photosynthetic pigment content showed that, water deficit stress had significant effect on all lines of sunflower ($P< 0.05$). Differences among lines were not significant for the photosynthetic pigment content. Water deficit stress × lines interaction was not significant for photosynthetic pigment content. Pigment content of leaves from both stressed and control sunflower lines are presented in Table 2. Drought stress decreased chlorophyll-*a*, chlorophyll-*b*, total chlorophyll, anthocyanins and carotenoids concentration in all sunflower lines. The major role of carotenoid through direct quenching of triplet chlorophyll prevents the generation of singlet oxygen and protects from oxidative damage. As a general rule, anthocyanins are considered light attenuators and antioxidants. In this context, it is believed that under stress situations, their main function is the quenching of the reactive oxygen species generated by stress [21]. The efficacy of light captured to drive photosynthesis is strongly related to the chlorophyll concentration in the leaf. The change in chlorophyll contents was used to evaluate the influence of environmental stress on plant growth and yield. Many studies indicated that high chlorophyll concentrations are associated with improved yield under water-limited conditions [26]. In our study, the observed reduction of chlorophyll in water stressed plants might be due to a reduction in the lamellar content of the light harvesting chlorophyll protein. The decreased chlorophyll-*a*, chlorophyll-*b* and total chlorophyll contents under drought stress are consistent with Nazarli *et al*. [20] who reported the reduced chlorophyll-*a*, chlorophyll-*b* and total chlorophyll contents under progressive drought stress in sunflowers. The decrease in chlorophyll-*a* may be caused by the inhibition of biosynthesis of precursors of chlorophyll-*a* under osmotic stress as reported by Moharramnejad *et al*. [19].

Total phenolics

Addition of varying levels of water deficit to the growth condition caused a consistent decrease/increase in the accumulation of phenolics in sunflower lines. Enhanced synthesis of total phenolics has been directly correlated with salt and heat tolerance of sugarcane. In the present study, drought stress decreased/increased total phenolics of sunflower lines. Although sunflower lines differed in growth performance at varying drought conditions (Fig 2). Total phenolics were decrement under drought stress in maize inbred lines [19].

Proline and glycine betaine

Proline content was significantly increased under drought stress conditions. Increasing in drought stress resulted in increase in proline content, so the highest and the lowest values were obtained for AGK330 and RGK15, respectively (Fig 3 a). The increase in proline content due to drought stress was more severe at flowering stage than at the vegetative stage. The proline content depends on plant age, leaf age, leaf position or leaf part. Under vegetative stage, drought stress increased proline content about tenfold, this increasing roles as an osmotic compatible and adjust osmotic potential which resulted in drought stress avoidance in sunflowers. Prolin accumulation is believed to play adaptive roles in plant stress tolerance [25]. Proline accumulation is responsible for the hydration of biopolymers surviving as areadily utilizable energy source and serving as a nitrogen source compound during periods of inhibited growth. A marked increase in proline content in the leaves of could be an indicator of its high drought tolerance [9]. Under progressive drought stress the glycine betaine (GB) concentration in the leaves of sunflowers enhanced at beginning of drought stress. Drought stress increased glycine betaine content in leaves of the all sunflowers lines. However, constitutive level of glycine betaine was higher in AGK330 (Fig 2 b). GB is thought to play adaptive roles in inducing osmotic adjustment and protecting subcellular structures in stressed plants [8]. GB is abundant mainly in chloroplast where it plays a vital role in adjustment and

protection of thylakoid membrane, thereby maintaining photosynthetic efficiency (Ashraf and Foolad, 2007). Increased proline and glycine betaine accumulation under osmotic stress in AGK330 is in accordance with previous data [18], which is suggested to be associated with drought tolerance [7]). The glycine betaine content increased under drought stress in maize [19] and in higher plants [15]. High levels of proline enabled the plant to maintain low water potentials. By lowering water potentials, the accumulation of compatible osmolytes, involved in osmoregulation allows additional water to be taken up from the environment, thus buffering the immediate effect of water shortages within the organism [17]. Cell membrane integrity undergoes diverse changes such as increase in penetrability and decrease in sustainability under drought stress [11].

Table 2. Effects of water deficit stress on pigment contents in sunflower lines under control and field conditions.

	Line	Chlorophyll-a	Chlorophyll-b	Total chlorophyll	Anthocyanins	Carotenoids
Control	RGK15	0.0202	0.0075	0.0277	0.0058	0.0082
	RGK21	0.0215	0.0076	0.0291	0.0056	0.0095
	RGK25	0.0181	0.0068	0.0267	0.0073	0.0096
	RGK26	0.0223	0.0081	0.0304	0.0067	0.0101
	AGK2	0.0209	0.0074	0.0283	0.0074	0.0083
	AGK30	0.022	0.008	0.03	0.0069	0.0092
	AGK46	0.02	0.0079	0.0279	0.0075	0.01
	AGK330	0.0212	0.0082	0.0294	0.0074	0.0099
Water deficit	RGK15	0.0168	0.0062	0.0231	0.0051	0.0082
	RGK21	0.0198	0.0070	0.0276	0.0064	0.0091
	RGK25	0.0160	0.0067	0.0255	0.0045	0.009
	RGK26	0.0186	0.0072	0.0257	0.0063	0.0083
	AGK2	0.0199	0.0070	0.0276	0.007	0.0099
	AGK30	0.0193	0.0074	0.0267	0.0052	0.009
	AGK46	0.0179	0.0066	0.0245	0.0074	0.0086
	AGK330	0.0172	0.0062	0.0233	0.0062	0.0076

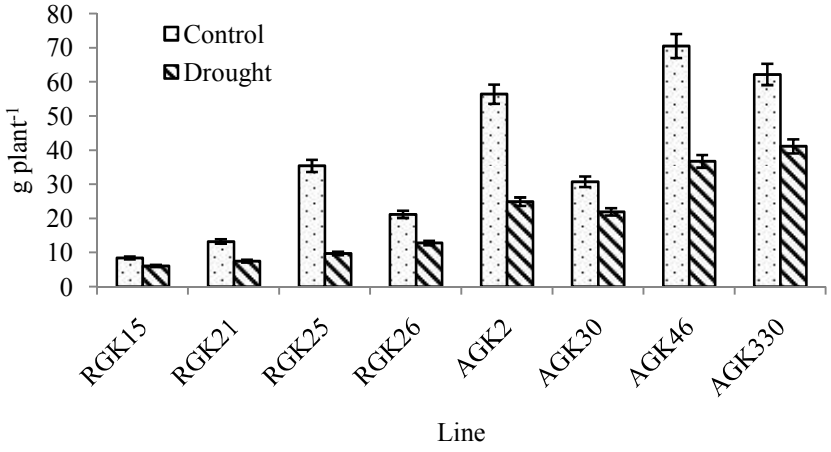

Fig 1. Effect of water deficit stress on seed yield in sunflowers lines.

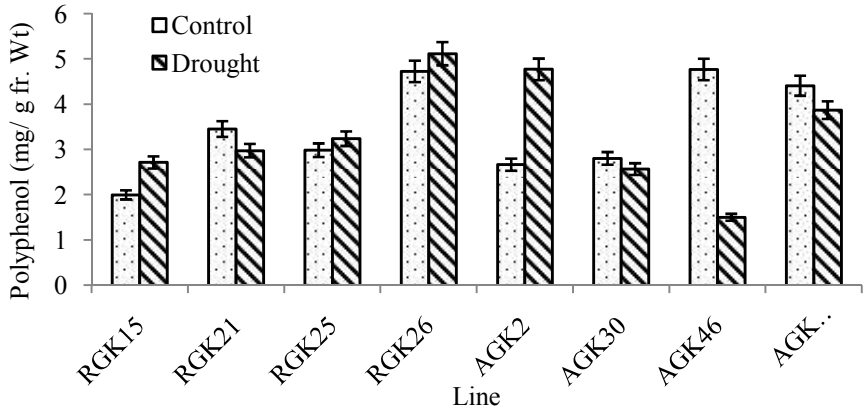

Fig 2. Total phenolics of all sunflower lines under control and water deficit stress conditions.

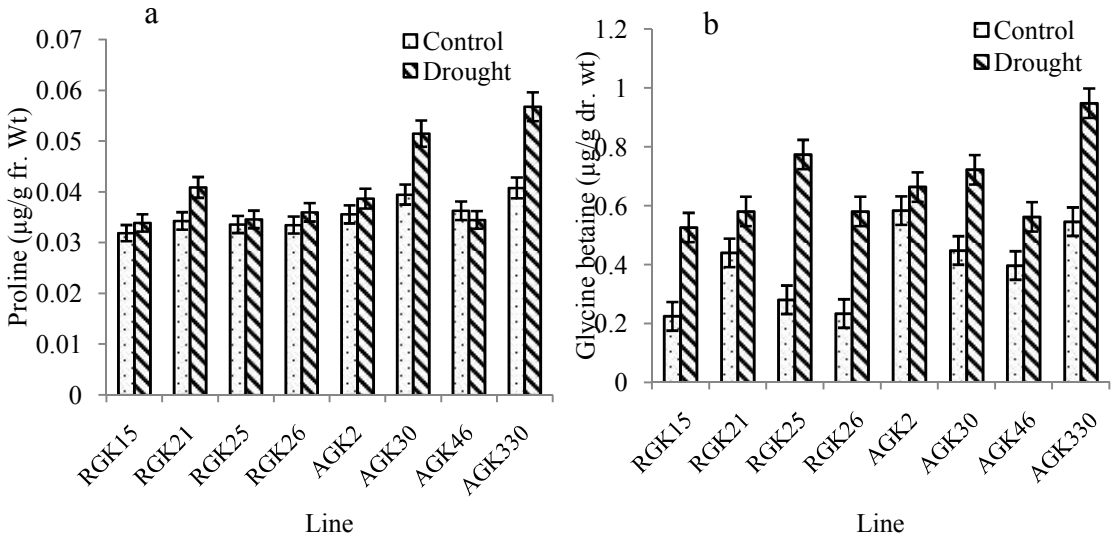

Fig 3. Proline (a) and glycine betaine (b) of all sunflower lines under control and water stress conditions.

REFERENCES

1. Bohnert HJ, Nelson DE, Jensen RG. (1995). Adaptations to environmental stress. Plant Cell 7, 1099-1111.http://www.plantcell.org/content/7/7/1099.full.pdf+html.
2. Baydar H, Erbas S. (2005). Influence of seed development and seed position on oil, fatty acids and total tocopherol contents in sunflower (*Helianthus annus* L.). Turkish Journal of Agronomy 29, 179-186.http://dergipark. ulakbim.gov.t r/ tbtkagriculture/ article/download/5000027492/5000027729.
3. Huseynova IM, Suleymanov SY, Aliyev JA. (2007). Structural functional state of thylakoid membranes of wheat genotypes under water stress. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1767, 869–875. http://dx.doi.org/%2010.1016 /j.bbabio. 2007.01.014.
4. IturbeOrmaetxe I, Escuredo PR, Arrese-Igor C, Becana M. 1998. Oxidative damage in pea plants exposed to water deficit or parquet. Plant Physiology 116, 173–181. http://dx.doi.org/10.1104/pp.116.1.173.
5. Ommen OE, Donnelly A, Vanhoutvin S, Oijen M**,** Manderscheid R. (1999). Chlorophyll content of spring wheat flag leaves grown under elevated CO_2 concentrations and other environmental stresses within the ESPACE-wheat project. European Journal of Agronomy 10, 197-203. http://dx.doi.org/10.1016/S1161-0301(99)00011-8.
6. Alahdadi I, Oraki H, Parhizkar-Khajani F.(2011). Effect of water stress on yield and yield components of sunflower hybrids. African Journal of Biotechnology 10, 6504-6509. http://www.academicjournals.org /journal/AJB/ article-full-text-pdf/9EB6E3934872.
7. Andrade A, Vigliocco A, Alemano S, Llanes A, Abdala G. (2013). Comparative morpho-biochemical responses of sunflower lines sensitive and tolerant to water stress. American Journal of Plan Science 4, 160-167. http://dx.doi.org/10.4236/ajps.2013.412A3018.
8. Ashraf M, Iram A. (2005). Drought stress induced changes in some organic substances in nodules and other plant parts of two potential legumes differing in salt tolerance. Flora 200, 535–546. http://dx.doi.org/ 10.1016/j.flora.2005.06.005.

9. Ashraf M, Foolad MR. (2007). Roles of glycinebetaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany 59, 206–216. http://dx.doi.org/10.1016/j.envexpbot.2005.12.006.
10. Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39, 205–207. http://link.springer.com/article/10.1007%2FBF00018060.
11. Blokhina O, Virolainen E, Fagerstedt KV. (2003). Antioxidants, oxidative damage and oxygen deprivation stress. Annals of Botany 91, 79–194. http://aob.oxfordjournals.org/content/91/2/179.full.pdf+html.
12. Chaves MM, Pereira JS, Rodrgues ML, Ricardo CPP, Osório ML, Carvalho I, Faria T, Pinheiro C. 2002. How plants cope with water stress in the field? Photosynthesis and growth, Annals of Botany 89, 907-916. http://aob.oxfordjournals.org/content/89/7/907.full.pdf+html.
13. Grieve CM, Grattan SR. (1983). Rapid assay for determination of water soluble quaternary ammonium compounds. Plant Soil 70, 303-307. http://link.springer.com/article/10.1007%2FBF02374789.
14. Iqbal N, Ashraf M, Ashraf MY. 2008. Glycinebetaine, an osmolyte of interest to improve water stress tolerance in sunflower (*Helianthus annuus* L.), Water relations and yield. South African Journal of Botany 74, 274-281. http://dx.doi.org/10.1016/j.sajb.2007.11.016.
15. Jun HR, Adam L, Rozwadowski KL, Hammerlineli JL, Keller WA, Selvaraj G. (2000). Genetic engineering of glycinebetaine production towards enhancing stress tolerance in plants. Plant Physiology 122, 747–756. http:// dx.doi.org/10.1104/pp.122.3.747.
16. Kala S, Godara,AK. (2011). Effect of moisture stress on leaf total proteins, proline and free amino acid content in commercial cultivars of *Ziziphus mauritiana.* Journal of Scientific Research 55, 65-69. http://bhu.ac.in/journal /Issues/Journalof ScientifcResearchVol55/7.%20Shashi%20Kala-Effect%20of%20moisture.pdf.
17. Kumar SG, Mattareddy A, Sudhakar C. (2003). NaCl effects on proline metabolism in two high yielding genotypes of mulberry (*Morus alba* L.) with contrasting salt tolerance. Plant Science 165, 1245–1251. http://dx.doi.org /%2010.1016/S0168-9452(03)00332-7.
18. Manivannan P, Abdul Jaleel C, Sankar B, Kishorekumar A, Somasundaram R,. Lakshmanan GMA, Panneerselvam R. 2007. Growth, biochemical modifications and proline metabolism in *Helianthus annuus* L. as induced by drought stress. Colloids and Surfaces B 59, 141–149. http://dx.doi.org/10.1016/j.colsurfb.2007.05.002.
19. Moharramnejad S, Sofalian O, Valizadeh M, Asgri A, Shiri M. 2015. Proline, glycine betaine, total phenolics and pigment contents in response to osmotic stress in maize seedlings. Journal of Bioscience and Biotechnology 4, 313-319. http://www.jbb.uni-plovdiv.bg/documents/27807/1014563/jbb_2015-4(3)-pages_313-319.pdf.
20. Nazarli H, Faraji F, Zarsashti MR. (2011). Effect of drought stress and polymer on osmotic adjustment and photosynthetic pigment of sunflower. Agronomy Research of Moldavia., 1, 35-41. http://dx.doi.org/10.2478/ v10298-012-0022-9.
21. Neill SO, Gould KS. (2003). Anthocyanins in leaves, light attenuators or antioxidants? Functional Plant Biology, 30, 865–873. http://dx.doi.org/10.1071/FP03118.
22. Noreen Z, Ashraf M. (2009). Assessment of variation in antioxidative defense system in salt-treated pea (*Pisum sativum*) cultivars and its putative use as salinity tolerance markers. Journal of Plant Physiology 166, 1764-1774. http://dx.doi.org/10.1016/j.jplph.2009.05.005.
23. Ruiz JM, Romero L. 2001. Bioactivity of the phenolic compounds in higher plants. In, Atta-ur-Rahman, editor. Studies in natural products chemistry. Bioactive natural products, 25, part F. Oxford, Elsevier Science Ltd., pp. 651. http://dx.doi.org/10.1016/S1572-5995(01)80020-X.
24. Sairam RK, Veerabhadra-Rao K, Srivastava GC. (2002). Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Science 163, 1037–1046. http://dx.doi.org/10.1016/S0168-9452(02)00278-9.
25. Verbruggen N, Hermans C. (2008). Proline accumulation in plants, a review. Amino Acids 35, 753 759.http://dx.doi.org/10.1007/s00726-008-0061-6.
26. Verma V, Foullces MJ, Worland AJ, Sylvester-Bradley R, Caligari PDS, Snape JW. (2004). Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. Euphytica 135, 255–263.http://link.springer.com/article /10.1023%2FB%3AEUPH.000001 3255. 31 618.14.
27. Yaryura P, Cordon G, Leon M, Kerber N, Pucheu N, Rubio N, Garc G, Lagorio G. (2009). Effect of phosphorus deficiency on reflectance and chlorophyll fluorescence of cotyledons of oilseed rape (*Brassica napus* L.). Journal of Agronomy and Crop Science 195, 186–196.http://dx.doi.org/10.1111/j.1439-037X.2008.00359.x.