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EFFECT OF NITROGEN AND FOLIAR APPLICATION OF PHENOLIC COMPOUNDS ON FLAG LEAF PIGMENTS AND GRAIN YIELD OF SPRING WHEAT (*Triticum aestivum* L.) GENOTYPES

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Abstract

Application of phenolic compounds such as proline, glycinebetaine, silicon, cytokinins, methanol etc. are getting important consideration in modern agricultural research for managing plants to get maximum yield under different environmental conditions. However there is a lack of knowledge about the effects of these phenolic compounds on grain yield, yield components and physiological properties such as leaf photosynthetic pigments. In the context the the present study was conducted during 2015-2016 growing season at the experimental field in the faculty of agriculture, University of Cukurova, Adana, Turkey to evaluated the effects of some phenolic compounds such as proline, glycinebetaine, silicon, cytokinins, methanol etc on chlorophyll (Chl) as well as total carotenoid contents of four bread wheat genotypes ('Adana-99', 'FSD-2008', 'Basribey-98' and 'Seher-06') under two nitrogen levels *viz.,* low nitrogen level, LN: 60 kg N ha^{-1} and high nitrogen level, HN: 180 kg N ha^{-1} under well-watered condition. The results of the LN showed that the grain yield was only significantly correlated with $Chl_{a/b}$ (r=-0.751**) of wheat genotypes. While, under HN, Chl_a, Chl_b, carotenoid and $Chl_{a/b}$ were strongly correlated with grain yield (r=0.422*, r=0.635**, r=0.444* and r=-0.590**) respectively. Among the phenolic compounds, proline highly sitimulated the relationships between grain yield and Chl_b, carotenoids and $Chl_{a/b}$ (r=0.795*, r=0.795* and r=-0.811*), respectively. Similary, methanol effects were strongly correlated with grain yield and Chl_b and $Chl_{a/b}$ (r=0.844** and r=-0.772*), respectively. Therefore it is concluded that foliar application of methanol and proline were significantly increased the flag leaf Chl_b concentration which ultimately lead to increase the grain yield of wheat genotypes.

Keywords: wheat, nitrogen, phenolic compounds, pigments.

Introduction

Wheat (*Triticum aestivum* L.) is the most important cereal crops in Turkey. The arable agricultural land of Turkey is only 24 million hectares, where wheat is cultivated between 7.5-9.8 million ha land (TUIK, 2016). Among them, only 5 million hectares of agricultural land can be irrigated in Turkey, almost all of wheat cultivated depends on rainfall. For this reason annual wheat production fluctuates range from 17 to 19 million tonnes. The annual wheat requirement of the country with a population of 80 million is about 19 million tons. While annual population growth rate in Turkey is 1.35% (TUIK, 2016), the average rate of yield increase only 0.64% per year (Kuşçu, 2002). It is anticipated that the situation will be continue, due to the growing population and declining farmland that ultimately threat to food security of increasing population of the country. In addition earlier studies on genetic improvement of wheat in Turkey is still insufficient (Sener et al. 2009; Kuşçu, 2006; Avcin et al. 1997). Therefore it is very essential to increase the research on genetic improvement of wheat in combination with modern agricultural management practices and technological for sustainable wheat production under stressful environments. Recently, phenolic compounds application of different phenolic compounds such as proline, glycinebetaine, silicon, cytokinins, methanol etc. are getting important consideration in modern agricultural research (Bruulsema et al. 2008; Chien et al. 2009). But knowledge about the effects of these phenolic compounds on physiological properties such as leaf photosynthetic pigments is still in intial stage of the country. These pigments mainly consist of chlorophyll a, chlorophyll b and carotenoids. Chl_a can act as a light harvesting pigment and the reaction centre for leaf photosynthesis (Raven et al 2005). Chl_b can act as an accessory light-harvesting pigments and helps Chl_a to perform leaf photosynthesis (Ishikita et al 2006). Carotenoids serve two major recognized functions in photosynthesis i.e., first, as an accessory light-harvesting pigment and, the other, as a triplet quencher to provide protection against photo-oxidative damage (Young 1991). Photosynthetic pigments and their ratio to each other can shows wide variation due to the variation of plant species, genetic progress (Siddique et al. 1989), stress factors (XinWen et al. 2008; Manivannan et al. 2007), growth stage (Kura-Hotta et al. 1987), plant nutrient deficiency etc. For instance Kitajima and Hogan. (2003) reported that $Chl_{a/b}$ ratio increased while Chlorophyll content decreased in response to N limitation in photosynthetic cotyledons and leaves of seedlings of four tropical woody species. Considering the above issues the present study was under taken to evaluated the effects of some phenolic compounds such as proline, glycinebetaine, silicon, cytokinins, methanol etc on chlorophyll (Chl) as well as total carotenoid contents of four bread wheat genotypes under two nitrogen levels *viz.,* low nitrogen level, LN: 60 kg N ha^{-1} and high nitrogen level, HN: 180 kg N ha^{-1}under well-watered condition.

Material and methods

An experiment was conducted during the 2015-16 wheat growing seasons at the experimental field of the faculty of agriculture, University of Cukurova, Adana (37°00′ N, 35°21′ E, 29 m above sea level), Turkey. The soil type is a fine loamy, montmorillonitic typic xerofluvent, low in organic matter and slightly alkaline (pH 7.1–7.6). Treatments were with a similar developmental pattern of four spring bread wheat genotypes *viz.,* 'Adana-99', 'FSD-2008', 'Basribey-95' and 'Seher-06' ; two nitrogen levels *viz.,* low nitrogen level, LN: 60 kg N ha^{-1} and high nitrogen level, HN: 180 kg N ha^{-1} and foliar application of five phenolic compounds *viz.,* Cytokinins, 40µM,; Silicon, 6 mM; Glycinebetaine, 100mM; Proline, 100mM; Methanol, 20% (v/v)). The experiment was conducted with split-split plot design with three replications. Nitrogen levels were assigned in main plots, phenolic compounds were in sub plots and genotypes were arranged in sub-sub plots. Phenolic compounds tratments were applied in three times at Zadok's growth stages (ZGS) 20, 30 and 60 (Zadoks et al. 1974). The sowing density was 500 viable seeds m^{-2}. Every plots consisted of 6 m long 8 rows with a row spacing of 0.15 m.

Extraction Processes

Chlorophylls (Chla and Chlb) and carotenoid was determined in two leaves disks of 8 mm diameter taken from an identical position of the same leaves (flag leaves collected at antesis period). After the disks were weighed, pigments were thoroughly extracted in 80% (v/v) acetone using a glass mortar, and the homogenate was then filtered. Absorbance was measured at the relevant wavelength with a spectrophotometer (Shimadzu, UV-1208, Kyoto, Japan). Chlorophylls and carotenoids as mg/l was calculated by using the following formulae by Arnon‟s (1949) method. Carotenoid mg/g = 7.6 (OD 480) – 1.49 (OD 510) x (v/1000) x (w). Chlorophyll a (mg/gm) = 12.7(OD 663) – 2.69(OD 645) x (v/1000) x (w).Chlorophyll b (mg/gm) = 22.9 (OD 645) – 4.68 (OD 663) x (v/1000) x (w). Total chlorophyll (mg/gm) = OD 652 x (1000/34.5) x (v/1000) x (w). Carotenoid mg/g = 7.6 (OD 480) – 1.49 (OD 510) x (v/1000) x (w).Where OD = Optical density, V = Final vol. of 80% acetone (25ml), W = Wt. of sample taken (0.25g).

Statistical analysis

Data were subjected to analysis of variance (ANOVA), and means were compared using Least Significant Difference (LSD) values were calculated at the 0.05 level of probability. All calculations were performed with MSTAT-C and SPSS statistical software.

Results and discussion

Effects of nitrogen on grain yield of wheat genotypes

Increased nitrogen level was significantly increased the average grain yield of cultivars from 688 kg da^{-1} to 730 kg da^{-1} (Fig. 1). At the non drought area, over the low limits of nitrogen application was generally positively affected the grain yield (Khan et al. 2000; Jan et al. 2003; Amjed et al. 2008). In this study, genotypes had shown Nitrogen x Genotype interaction, except, Adana-99 and Baribey-97, increases of nitrogen amount was increased grain yield in FSD-2008 and Seher cultivars (Fig.1).

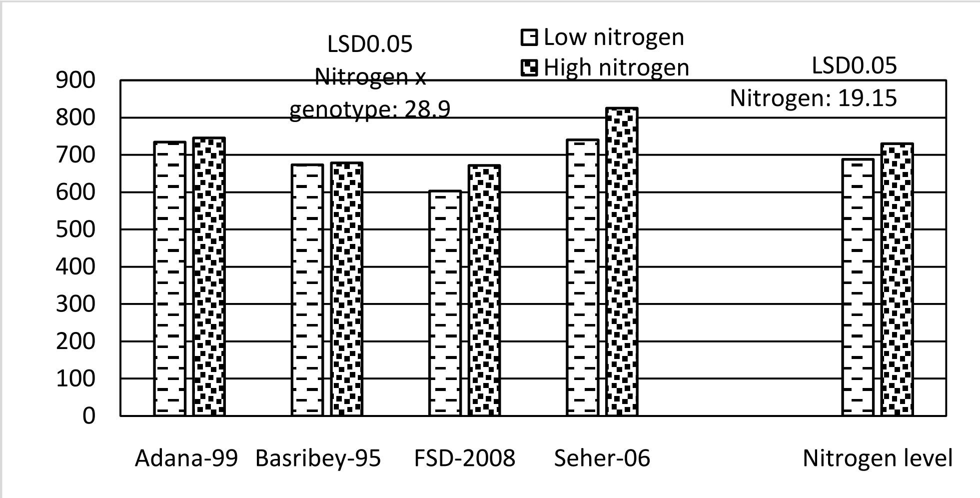

Figure 1. Effect of nitrogen levels on grain yield.

Effects of phenolic compounds on grain yield of wheat genotypes

Exogenous application was affected grain yield (*P*= 0.102) only at the low nitrogen treatment (Fig. 2). Methanol and cytokinin were improved the grain yield (8%). Under non-stress condition (High level of nitrogen), exogenous application didn't affect on grain yield.

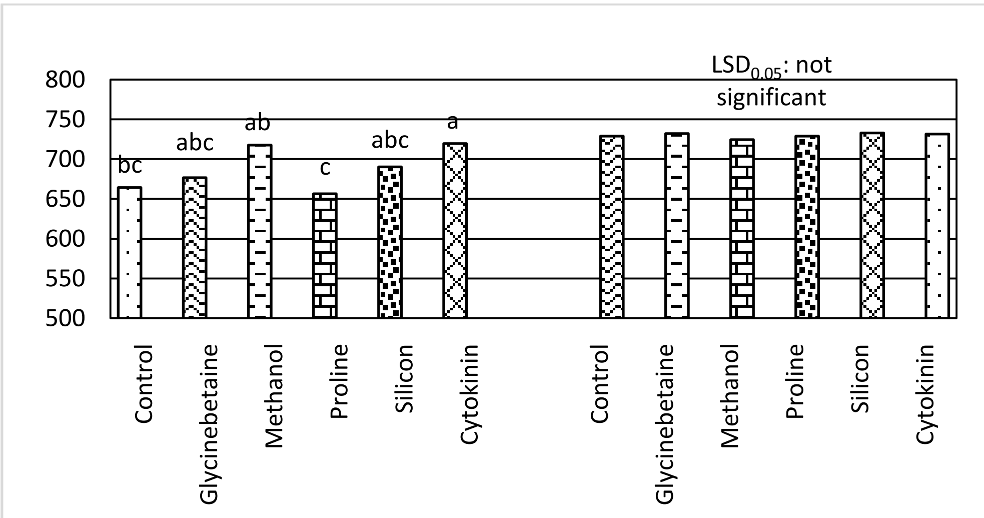

Figure 2. Effects of phenolic compounds on grain yield at the level of low and high nitrogen.

Relationships between grain yield and flag leaf pigments

If, both nitrogen levels and $Chl_{a/b}$ ratio were negatively correlated with grain yield, but all of the pigment content showed the positive correlation. However, Chl_b, Chl_{a+b} and $Chl_{a/b}$ were most effective for grain yield than of Chl_a and carotenoid. In a similar study Kitajima and Hogan, (2003) reported that when $Chl_{a/b}$ ratio was increased, chlorophyll content was decreased due to nitrogen limitation in photosynthetic cotyledons and seedlings leaves of tropical woody species.

Table 1. Correlation coefficient between grain yield and flag leaf pigments under low and high levels of nitrogen.

Pigment	Low level of nitrogen	High level of nitrogen
Chl_a	-0.393	0.422*
Chl_b	0.256	0.634**
Chl_{a+b}	-0.230	0.533**
$Chl_{a/b}$	-0.751**	-0.590**
Carotene	0.098	0.445*

Relationships between grain yield and flag leaf pigments under application of different phenolic compounds

In the present study, there was no significant relationship was found between grain yield and flag leaf pigment contain under control conditions, but application of different phenolic compounds and pigment types were significantly correlated with grain yield (Table 2). In the study, proline was most effective phenolic compound to improve the relationships between grain yield and Chl_a, Chl_b and carotene. However, silicon and cytokinins were not influenced that grain yield of all wheat genotypes. Earlier study reported by Maghsoudi et al (2016) noticed that silicon improved Chl_a and Chl_b in all cultivars under water deficit conditions.

Table 2. Correlation coefficient between grain yield and flag leaf pigments under application of different phenolic compounds

	Control	Glisin betaine	Methanol	Proline	Silicon	Cytokinins
Chl_a	0.213	0.410	0.536	0.662	0.003	0.049
Chl_b	0.536	0.600	0.845**	0.794*	0.371	0.347
Chl_{a+b}	0.325	0.501	0.664	0.722*	0.147	0.158
$Chl_{a/b}$	-0.608	-0.762*	-0.773*	-0.811*	-0.669	-0.626
Carotene	0.294	0.519	0.636	0.795*	0.159	0.467

Conclusions

The results and discussion of the present study it can be concluded that grain yield and $Chl_{a/b}$ were negatively correlated. Application of low level of nitrogen was significantly correlated with grain yield, but under high level of nitrogen, Chl_a, Chl_b, carotenoid and $Chl_{a/b}$ were strongly correlated with grain yield. Among the phenolic compounds, proline highly sitimulated the relationships between grain yield and Chl_b, carotenoids and $Chl_{a/b}$, respectively. Similary, methanol effects were also strongly correlated with grain yield and Chl_b and $Chl_{a/b}$, respectively. Therefore it is concluded that foliar application of methanol and proline were significantly increased the flag leaf Chl_b concentration which ultimately help to increased the grain yield of wheat genotypes.

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