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STUDY OF NITROGEN FERTILIZER AND CYCOCEL ON Fv/Fm AND DRY MATTER MOBILIZATION TO GRAIN YIELD OF WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT. In order to study of effects of nitrogen fertilizer and cycocel on yield, yield component and dry matter mobilization of wheat (*Triticum aestivum* L.) a factorial experiment was conducted based on randomized complete block design with three replications during 2014. Treatments were included nitrogen rates in four levels (without nitrogen application as control (N_0) and application 80 (N_1), 160 (N_2) and 240 (N_3) kg ha^{-1} urea) and four cycocel levels (without cycocel as control (C_0), application of 500 (C_1), 1000 (C_2), 1500 (C_3) ppm). Results showed that cycocel application increased chlorophyll index, photochemical efficiency of PSII (Fv/Fm) and dry matter mobilization from shoots and stem. Application of nitrogen and cycocel reduced dry matter mobilization from shoots and stem, contribution of remobilization from shoots to grain and stem reserve contribution in grain yield. Application of nitrogen and cycocel as N_3C_3 had 58.5% and 46.26% more dry matter mobilization from shoots and stem in comparison with N_3C_0. The highest 1000-grain weight by 28.90 and 28.54 g, respectively, belonged to application of cycocel as C_2 and C_3 and the lowest 1000-grain weight by 26.93 g belonged to the C_0. The highest grain yield (1.068 g per plant), number of grains per ear (37.36) and 1000-grain weight (28.77 g) were obtained in application of 240 kg ha^{-1} urea. It seems that the increase of Fv/Fm ratio due to current photosynthesis in plants that were grown under cycocel and nitrogen treatments decreased mobilization of dry matter and stem reserves to grain yield. Generally, it was concluded that nitrogen and cycocel can be as a proper tool for increasing wheat yield.

Keywords: CCC; grain weight; photochemical efficiency.

INTRODUCTION

Nutrient management is one of the critical inputs in achieving high productivity of plants (Golzafer *et al.*, 2012). Nitrogen fertilizer rate and

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timing are the major tools available after planting for manipulating wheat growth and development to produce a greater grain yield per unit area (Alley *et al.*, 1999). Wheat is the most important crop plant grown in the semi-arid regions, which usually experiences water stress during grain filling period (Yang *et al.,* 2003). Nitrogen is the major macronutrient determining the rate and grain filling period of wheat. Final grain weight was related to grain filling rate, grain filling duration, and their interaction (Sadras and Egli, 2008). N fertilizer is known to affect the plant height, number of grains per plant, thousand kernel weight, grain yield and biological yield of plants (El-Habbasha and Taha 2011; Rehman *et al.,* 2013). Dordas *et al.* (2008) reported that higher rates of N increase photosynthetic processes, leaf area production and leaf area duration as well as grain filling period.

It is generally accepted that plants that are able to sustain photosynthesis in the flag leaf for a more time tend to lead higher yield. Leaf expansion, plant growth, and carbon metabolism of many plants could be negatively affected by nutritional imbalance, osmotic stress, water deficiency, and/or oxidative stress (Bashan and de-Bashan, 2010). In cereals, such as wheat and barley, grain filling depends on current photosynthesis of the upper parts of the plant, i.e. the flag and the ear (Tambussi *et al.,* 2007) and by redistribution of assimilates stored in the stem (Ehdaie *et al.,* 2008). Most of the assimilates used for grain growth is produced by the upper canopy, which derives mainly from the spike, the flag leaf, and its sheath (Loss and Siddique, 1994). Lack of assimilate supply during the grain filling duration could result in a dramatic decline in grain weight (Borrás *et al.,* 2004). Ehdaie *et al.* (2006) suggested that wheat plants during grain filling may depend more on stem reserves for grain filling than on current photosynthesis. There are two components involved in the extent of contribution of stored reserves to grain yield in wheat. The first is the ability to store assimilates in the stem and the second is the efficiency of the crop to mobilize and translocate the reserved materials to the grains. The second component is a function of sink strength in a genotype, which depends on the number of grains per spike and mean grain weight (Ehdaie and Waines, 1996).

Although nitrogen is the key element in increasing of productivity and could increase wheat plants, but large rates of N fertilizer loss to the environment could cause a serious environmental problem, such as groundwater contamination. In such a situation, reduction of applied N fertilizer rate to an optimized level or application of bio fertilizers can reduce the need for chemical fertilizers and decrease adverse environmental effects, increase of soil organic matter, improvement of soil properties and increase of crop yield (Adesemoye *et al.,* 2009). They have suggested that long-term applications of chemical or organic fertilizers prevent the development of soil structure. Therefore, in development and implementation of sustainable agriculture techniques, bio fertilization is important in alleviating environmental pollution and deterioration of nature (Werner and Newton, 2005).

It is well known that cycocel treatment could induce changes in the physiological traits of wheat (Meera and Poonam, 2010) and may increase wheat yield and quality. Cycocel is an essential growth regulator for plants that reduced concentration of gibberellins and interfere with the concentration of other plant hormones, such as cytokinins, ethylene, and abscisic acid, which can affect physiological processes (Rademacher, 2000). Wang *et al.* (2009) have recently reported that cycocel treatment increased photosynthetic capacity and photoassimilates partitioning into plants. Hoque and Haque (2002) reported that CCC prevents ent-kaurene synthesis in GA_3 biosynthetic cycle leading to GA_3 deficiency and the subsequent reduced vegetative growth potential. Wang and Xiao (2009) imply that treatment of plants with cycocel may increase the number of chloroplasts, elevate the concentration of chlorophyll and carotenoids, accelerate the process of photophosphorylation, and stimulate the photosynthetic rate. Cycocel have the ability to delay senescence of leaf, arresting chlorophyll degradation and promoting the synthesis of soluble proteins and enzymes resulting in more assimilation surface area (Osman, 2014). Foliar application of cycocel was increased traits of number of grains per spike, 1000-grain weight, grain yield, biological yield, harvest index and wheat protein of wheat significantly (Fathi and Jiriaie, 2014). Therefore, foliar application of cycocel might be a promising practice for improving plant yield by environmental stresses, such as high or low N supply.

Considering the above facts, the present study was undertaken to know that how do application of cycocel and nitrogen affecting mobilization of reserve accumulation to grain yield.

MATERIAL AND METHODS

A factorial experiment based on randomized complete block design with three replications was conducted under greenhouse condition in 2015**.** Treatments factors included nitrogen rates in four levels (without nitrogen application as control (N_0) and application 80 (N_1), 160 (N_2) and 240 (N_3) kg ha^{-1} urea) and four cycocel levels (without cycocel as control (C_0), application of 500 (C_1), 1000 (C_2), 1500 (C_3) ppm). The soil was silty loam, with pH about 6.9. Air temperature ranged from 22-27 °C during the day and 18-21°C during the night. Humidity ranged from 60-65%. The area is located at 38°15' N latitude and 48°15' E longitude; altitude, 1350 m). Climatically, the area placed in the semi-arid temperate zone with cold winter and hot summer. The wheat cultivar "Attila 4" was used in the experiment. Optimal density of it is 400 seeds m^{-2}, so forty seeds were sown in each pot with 4 cm deep, filled approximately with 20 kg of above mention soil. The pots were immediately irrigated after planting. Foliar application cycocel was done in two stage of period growth (4-6 leaf stage and before of booting stage). Cycocel is registered trademark of BASF Company. There is 2-chlorethyl) trimethylamonium chloride as active substance. This substance is also in different growth regulator.

The chlorophyll content of leaves was determined with a SPAD-502 (Konica Minolta Sensing, Osaka, Japan) (Jifon *et al.,* 2005).

The quantum yield was measured by the uppermost fool expanded leaf using a fluorometer (chlorophyll fluorometer; Optic Science-OS-30 USA). For this purpose, the plants adapted to darkness for 20 minutes by using one special clamp then the fluorescence amounts were measured in 1000 (μM photon m^2s), and calculation was performed using following formula (Arnon, 1949): ØPSII = (Fm-F0)/Fm, where ØPSII = quantum yield amount of photosystem II, Fm = maximum fluorescence after a saturated light pulse on plants adapted to darkness and F_0 = the minimal fluorescence in the light adapted, which was determined by illumination with far-red light.

Various parameters describing the dry matter and stem reserves mobilization within the plant were evaluated as follows (Inoue *et al.,* 2004):

- Dry matter remobilization to grain (g per plant) = maximum shoot dry matter content after anthesis (g per plant) – shoot dry matter maturity (except grains) (g per plant)
- Dry matter contribution of pre-anthesis assimilates to grain [%] = (remobilization/ grain yield) ×100.
- Stem reserves remobilization to grain yield (g per plant) = maximum stem dry matter content after anthesis (g per plant) – stem dry matter in maturity (g per plant)
- Stem reserve contribution to grain yield (%) = [(Amount of stem dry matter remobilization) / (grain yield)] × 100.

At plant maturity, in order to measure number of grains per ear, 1000-grain weight and grain yield per plant, 10 plants of each pot randomly were harvested. Analysis of variance and mean comparisons were performed using SAS computer software packages. The main effects and interactions were tested using the least significant difference (LSD) test.

RESULTS AND DISCUSSION

Analysis of variance showed a significant effect for nitrogen rates on the number of grains per ear, 1000-grain weight and grain yield (*Table 1*). The chlorophyll index, number of grains per ear, 1000-grain weight and grain yield were affected by the cycocel application. We found the significant interaction effect between nitrogen rates and cycocel on maximal quantum yield (Fv/Fm ratio) and dry matter mobilization from shoots and stem (*Table 1*).

Maximal quantum yield (Fv/Fm ratio) increased with increasing N rates and cycocel levels (*Table 2*). In addition, no N application (N_0) and high level of cycocel (C_3) leads to increased dry matter mobilization from the shoots and stem to the grains, leading to a dramatic reduction in photosynthetic capacity of the plant via the reduction of Fv/Fm ratio per unit leaf area (*Table 1*).

Table 1- Means comparison and variance analysis of nitrogen rates and cycocel on chlorophyll index, Fv/ Fm, number of grains per ear, 1000-grain weight and growth parameters of wheat

	Chlorophyll index	Fv/Fm	Number of grains per ear	1000-grain weight (g)	Dry matter remobilization from shoots (g per plant)	Dry matter remobilization from stem (g per plant)	Contribution of remobilization from shoots to grain (%)	Stem reserve contribution in grain yield (%)	Grain yield (g per plant)
Nitrogen rates (kg urea ha^{-1})									
N_0 = 0	42.14a	0.635c	28.17c	27.23c	0.360a	0.281a	39.77a	21.36a	0.767c
N_1 = 80	42.12a	0.718b	30.71bc	27.43bc	0.312b	0.257b	31.41b	17.00b	0.841bc
N_2 = 160	41.87a	0.738b	33.77ab	28.48ab	0.270c	0.221c	24.88c	13.50c	0.974ab
N_3 = 240	40.79a	0.761a	37.36a	28.77a	0.211d	0.169d	18.57d	9.45d	1.068a
LSD 5%	1.38	0.035	4.7	1.1	0.0019	0.0022	0.0019	0.019	0.14
Cycocel (ppm)									
C_0 = without cycocel	40.16c	0.611c	28.89b	26.93c	0.272d	0.216d	24.96d	14.09d	0.778c
C_1 = 500	41.15bc	0.720b	31.31b	27.53bc	0.278c	0.224c	27.20c	14.94c	0.868bc
C_2 =1000	41.80b	0.746ab	33.22ab	28.90a	0.291b	0.239b	29.04b	16.08b	0.959ab
C_3 =1500	43.82a	0.776a	36.59a	28.54ab	0.310a	0.249a	33.41a	16.19a	1.045a
LSD ($p < 0.05$)	1.38	0.035	4.7	1.1	0.0019	0.0022	0.0019	0.0194	0.14
N	ns	**	**	*	**	**	**	**	**
C	**	**	*	**	**	**	**	**	**
N * C	ns	**	ns	ns	**	**	**	**	ns
C.V.	3.96	5.91	15.09	4.74	8.00	1.00	9.00	15.00	18.69

$^{ns, *}$ ^{ns} and $^{*, **}$ show no significant and significant differences at 0.05, 0.01 probability level, respectively.

Table 2 - Means comparison of salinity and bio fertilizers treatments on some physiological traits of wheat

Treatment		Dry matter remobilization (g per plant)	Dry matter remobilization from stem (g per plant)	Contribution of remobilization from shoots to grain (%)	Stem reserve contribution to grain yield (%)	Fv/Fm
Nitrogen rates (kg ha^{-1} urea)	Cycocel (ppm)					
N_0	C_0	0.382a	0.293a	48.05a	27.19a	0.408c
	C_1	0.352c	0.268c	31.44d	16.47d	0.675b
	C_2	0.358b	0.293a	38.25c	19.39c	0.697ab
	C_3	0.349c	0.273b	41.35b	22.40b	0.759a
N_1	C_0	0.285d	0.229d	25.29f	16.42d	0.682b
	C_1	0.301c	0.256c	28.02de	16.85d	0.726ab
	C_2	0.326cd	0.272b	33.95d	17.24cd	0.764a
	C_3	0.338c	0.274b	38.39c	17.52cd	0.782a
N_2	C_0	0.261de	0.210e	22.35fg	12.64e	0.629b
	C_1	0.264de	0.211e	23.24fg	13.42e	0.720a
	C_2	0.270d	0.230d	24.28f	13.45e	0.754a
	C_3	0.287d	0.236d	29.65de	14.49de	0.771a
N_3	C_0	0.164f	0.134fg	13.14gh	8.51f	0.723b
	C_1	0.202e	0.164f	17.16g	9.65f	0.758a
	C_2	0.219e	0.183ef	19.68fg	9.72f	0.768a
	C_3	0.260de	0.196e	24.28f	9.94f	0.794a

Means with similar letters in each column are not significantly different.
N_0, N_1, N_2 and N_3 indicative no application, application of 80, 160 and 240 kg/ha urea, respectively
C_0, C_1, C_2 and C_3 indicative no application, application of 500, 1000 and 1500 ppm cycocel, respectively

The results of this study about maximal quantum yield agree well with the reports of Olszewski *et al.* (2014) and Grant *et al.* (2011). Olszewski *et al.* (2014) suggest that higher nitrogen fertilization levels contributed to an increase in net photosynthesis rates in spring wheat flag leaves and ears. There have been some researches about effect of nitrogen application rate on the flag leaf photosynthesis (Jun-Ye and Zhen-Wen, 2006; Zhang *et al.,* 2007; Mei-Ling *et al.,* 2009) and the nitrogen application rate on wheat yield (Wei-Wei *et al.,* 2012). Although, the maximum of Fv/Fm ratio was obtained in wheat plants with N_3C_3, but plants treated with all three rate of nitrogen and cycocel had the same value of Fv/Fm ratio (*Table 2*). There was an increase about 95% in Fv/Fm ratio in N_3C_3 application, in comparison with N_0C_0 (*Table 2*). In supporting to our finding, Basra and Basra (1997) reported that reduction of chlorophyll and other pigments finally resulted in decrease in the efficiency of photosynthesis. The highest chlorophyll index (43.82 SPAD) was observed in plants with the highest level cycocel (C_3). SPAD is a reliable method to assess the changes in the function of maximum efficiency of PSII photochemistry (Fv/Fm) (Broetto *et al.,* 2007). On the other hand, reduction of chlorophyll and other pigments finally resulted in decrease in the efficiency of photosynthesis. The increased level of total chlorophyll concentration in the cycocel treated plants might be due to the influence of growth retardant on delaying leaf senescence, arresting chlorophyll degradation, chlorophyll synthesis by high Rubisco activity, promoting the synthesis of soluble proteins and enzymes (Osman, 2014).

Number of grains per ear is an important grain yield in wheat (Wei-Wei *et al.,* 2012). As shown in *Table 1*, the highest mean value of number of grains per ear (37.36) obtained in 240 kg ha^{-1} urea application and the lowest (28.17) belonging to the plants with no N application. Application of 240 kg ha^{-1} urea (N_3) increased the number of grains per ear about 37.2%, compared to control. This is supported by a previous report showing that 180 kg ha^{-1} urea increased significantly the number of grains per head in safflower (Seyed Sharifi, 2012; Abbadi and Gerendas, 2009). This researcher also showed that the number of grains per plant about 36.2 %, compared to control in each plant. It has been suggested that improvement number of grains in wheat plants might be due to the positive effect of N fertilization on endosperm cell number (Lemcoff and Loomis, 1994). Application of cycocel as C_3 increased about 26.65, 16.86 and 10.14 % the number of grains per ear compared to C_0, C_1 and C_2, respectively (*Table 1*). Seyed Sharifi (2016) suggested that increasing the number of grains may be due to delay the duration of the vegetative and reproductive period and lengthening of grain filling duration. Thus, there is a possibility that higher number of grains per ear in chlormequat-treated plants were attributed to the slow plant development, which may increase productive of florets (Wang *et al.,* 2009). This speculation is supported by the results of the present study, because cycocel delayed the heading and anthesis time (Toyota *et al.,* 2010).

Grain yield in wheat is the result of number of grains per ear and grain weight. The highest (28.48 g) and lowest (27.23 g) 1000-grain weight was observed in N_3 (240 kg ha^{-1} urea) and N_0 (no nitrogen application), respectively (*Table 2*). The possible reason of increased grain weight with adequate nitrogen supply may be the result of delayed leaf senescence, sustained leaf photosynthesis during the grain filling period and extended duration of grain fill (Fredrick and Camberato, 1995). Some reporters have reported that the increase of 1000-grain weight is resulted from the increase of photosynthesis (Akinirinde, 2006). In the present study, photosynthates and Fv/Fm during grain filling, which is closely correlated with grain yield (*Table 1*). Malek-Mohammadi *et al.* (2013) stated that reduction of crop yield is related to reduction of speed and photosynthesis amount and after that reduction of 1000-seeds weight. Application of cycocel as C_3 showed about 6% more grain weight than C_0. On the other hands, in measuring the mean comparison of cycocel treatments the highest rate of 1000-grain weight by 28.90 and 28.54 g, respectively, belonged to application of cycocel as C_2 and C_3 and the lowest rate of 1000-grain weight by 26.93 g belonged to the C_0 (*Table 1*). The increase in grain weight is the possible effect of remobilization and transfer of the stored assimilates in vegetative tissue to the grain with the initiation of plant senescence (Nooden *et al*., 1997). Researchers stated that the weight of grain would increase due to the treatment with cycocel application and explained that it would be caused by the increase of target power before flowering stage (Waddington and Cartwright, 1988). N deficiency leads to increased dry matter mobilization from the shoot and stem to the grains leading to a dramatic reduction in photosynthetic capacity of the plant via the reduction photosynthetic rate per unit leaf area (Muchow and Sinclair, 1994).

The results showed that N fertilization increased the Fv/Fm ratio and led to low mobilization of dry matter and stem reserves assimilates to grain (*Table 2*). Furthermore, N fertilization increases assimilate partitioning to the generative plant organs and thus may also enhance carbohydrate supply to the grain in the short-term. This is support by the finding of Muchow and Sinclair (1994), who reported that N deficiency leads to increased dry matter mobilization from the shoot and stem to the grains leading to a dramatic reduction in photosynthetic capacity of the plant *via* the reduction photosynthetic rate per unit leaf area. It can be assumed that low cycocel levels decreases dry matter due to low supply of the shoots with carbohydrates and amino compounds during the lag period in which the number of storage cells and starch granules is determined in the endosperm of plant kernels. In the same line, cycocel foliar application increased chlorophyll content and accelerated such mobilization (*Table 2*). The highest dry matter (0.38 g per plant) and stem reserves remobilization to grains (0.29 g per plant) was obtained in N_0C_0 (*Table 2*). But the minimum of the mentioned traits observed in N_3C_0 (*Table 2*). Application of nitrogen and cycocel as N_3C_3 had 58.5 % and 46.26 % more dry matter mobilization from shoots and stem in comparison with N_3C_0 (*Table 2*). It can be assumed that no N application resulting in reduced photo oxidation of chloroplast pigments, and reduced leaf senescence, which can be attributed to the remobilization and transfer of the stored assimilates in vegetative tissue to the grain. The stress tolerance efficiency of plants, such as low nitrogen, was dependent not only on the assimilation of dry matter and stem reserves, but also on the effective partitioning of these reserves to the grains. The highest of dry matter from shoots and stem reserves contribution to grain yield (48.05% and 27.10%, respectively) was observed in N_0C_0 (*Table 2*). The lowest of them (13.14 and 8.51%, respectively) was obtained in N_3C_0 (*Table 2*).

Application of nitrogen and cycocel as N_2C_3 had 32.66% and 14.63% in contribution of dry matter and stem reserves to grain yield, respectively, in comparison with N_2C_0 (*Table 2*). Mansuroglu *et al.* (2009) reported that cycocel compounds are able to increase the partitioning of assimilates to grain yield through the inhibition of gibberellin biosynthesis. These researchers showed that the export of the photoassimilates and the starch synthesis is reduced by the GA_3 application, so the beneficial action of low concentrations of plant growth retardants on plants could be explained by its gibberellins antagonistic action.

Seed yield is a function of interaction among various yield components that are affected differentially by the growing conditions and crop management practices (Cheema *et al.,* 2010). Nitrogen application and cycocel is an important input for wheat production. In the present study increasing levels of nitrogen fertilizer and cycocel improved grain yield, which seems to be the result of enhanced yield components (grain number per ear and 1000-grain weight) (*Table 1*). The highest of grain yield (11.65 $\mu g.g^{-1}$) were observed in N application as N_3 (1.068 g) and cycocel as C_3 (1.045 g) (*Table 1*). The lowest of mentioned osmolyte (0.767 and 0.77 g, respectively) were obtained in application of N as N_0 and cycocel as C_0 (*Table 1*). Piccinin *et al.* (2013) stated that the grain yield of wheat showed improvement when wheat plants were grown with a chemical N. Dolan *et al*. (2006) reported that higher nutrients availability and favourable soil conditions due to N could be possible reason for delayed grain filling period in N treated plots and it could be possible reason for lengthening of grain filling duration and increasing of grain yield. In grain crops, both current assimilation transferred directly to grains and remobilization of assimilates stored in vegetative plant parts contribute to grain yield (Arduini *et al.,* 2006) and may buffer the yield against unfavorable climatic conditions during grain filling (Tahir and Nakata, 2005). Furthermore, exogenous cycocel resulted in increased grain yield in wheat (Fathi and Jiriaie, 2014) and sunflower (Kumar and Haripriya, 2010).

CONCLUSIONS

The results showed that application of nitrogen and cycocel improved chlorophyll index, Fv/Fm, number of grains per ear, 1000-grain weight and grain yield. Whereas, dry matter mobilization from shoots and stem, contribution of remobilization from shoots to grain and stem reserve contribution in grain yield were decreased. It seems that 240 kg ha^{-1} urea and 1500 ppm cycocel can be recommended to improve wheat production.

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