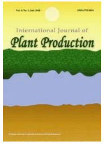

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Effect of exogenous application of ascorbic acid on physiological and biochemical characteristics of wheat under water stress

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Abstract

Wheat (*Triticum aestivum* L.) is widely cultivated in the Mediterranean zone where plants generally suffer from water stress during heading and reproductive stages. This research was carried out at the experimental farm of the faculty of agriculture, Kafrelsheikh University using two water treatments (water stress and well-watered), four N levels (0, 80, 160 and 240 kg ha^{-1}) and two ascorbic acid levels (0 and 200 mg l^{-1}). Water stress substantially reduced yield and related-traits compared with well-watered crop; however, AA application improved yield productivity and chlorophyll content was associated with the maintenance of leaf water status under water stress. Catalase (CAT) and peroxidase (POX) activity showed significant increases in plants treated with ascorbic acid (AA) under water stress. Among different nitrogen levels, the highest N level (240 kg N ha^{-1}) performed better during 2012/2013 and 2013/2014 seasons. Under water stress condition the higher N level (240 kg ha^{-1}), despite determining an increase in grain N content, did not imply an increase in NUE, due to a decrease in grain yield which positively related to N uptake. Exogenous application of ascorbic acid (AA) has been found very effective in mitigating the adverse effects of water stress, which might be attributed by activities of antioxidant enzymes (CAT and POX). In addition, inducing a stimulatory effect on all the yield-related traits. Maximum productivity and related traits were recorded under well watered condition treated by 240 kg N ha^{-1}. Nonetheless, exogenously applied ascorbic acid has better able to produce appropriate productivity under water stress.

Keywords: Wheat; Ascorbic acid; Water stress; Nitrogen use efficiency; Catalase; Peroxidase.

Introduction

Wheat (*Triticum aestivum* L.) is the national staple food for over one-fifth of human populace around the globe (FAO, 2014). The food and agriculture organization (FAO), during 2014-2015 growing season confirmed that 9.4 million tons of wheat was produced and it is estimated that up to 11 million tons will be produced in 2015-2016 growing season in Egypt. Meanwhile, approximately 8.1 million ha of the Egyptian soil is cultivated with wheat (FAO, 2015). It is noticeably that the Egyptian population increases, thus the demand for wheat will be increased annually. So it has to expanding the cultivated area with wheat; according to the economic Affairs sector, Ministry of Agriculture and Land Reclamation in Egypt (FAO, 2015). The wheat productivity could be increased through resistance to abiotic stresses (Siahpoosh and Dehghanianb, 2011).

Water stress has one of the greatest threats that emerged in many parts of the world especially in Egypt and is projected to double in future (Varga et al., 2015). Impaired water supplies are growing obstacles to wheat production worldwide (Gian et al., 2015). Water stress adversely affects growth, nutrient uptake, metabolism and the crop productivity (Gian et al., 2015). To overcome this problem, we have to find ways to increase the productivity of wheat to lessen the gap between required and consumption of population densities. The imposition of abiotic stress is associated with nutrient imbalance and production of reactive oxygen species (ROS), resulting in reduction in growth and yield (Batool et al., 2012).

Exogenous applications of antioxidants have been reported to markedly improve the inhibitory effects of water stress on plant growth and metabolism (Malik and Ashraf, 2012). Ascorbic acid (AA) as antioxidant plays a benefit impact on cell growth and division, differentiation and metabolism in plants (Athar et al., 2009). Xu et al. (2015) observed that foliar application of ascorbic acid ameliorates the adverse of water stress due to stomata closure, nutrient uptake, total chlorophyll content, protein synthesis, transpiration, photosynthesis and plant growth.

Nitrogen fertilization is a farmer's common practice to achieve acceptable yield, however its response relies on soil water availability than timing, amount and splitting of N applied (Cossani et al., 2012). Cossani et al. (2012) confirmed that the early vegetative growth processes such as tiller proliferation rely on the availability of water and N in wheat. Furthermore, water and N deficit around heading are known to induce flowering failure leading to reduction in grain number (Waraich et al., 2011). Nitrogen use efficiency may be affected by amount of applied N and water availability (Kibe et al., 2006), Likewise, many reports demonstrated a decline in NUE when N fertilizer rates are increased (Waraich et al., 2011), since N becomes less limiting at high rates. Hafez and Kobata (2012) indicated that nitrogen efficiency indices positively affected by N fertilizer rate. Nitrogen efficiency indices decrease with increasing N level under water stress (Zhang et al., 2015). Giuliani et al. (2011); Abou El-Hassan et al. (2014) stated that the application of high N levels may lead to less N uptake and low NUE due to high N losses.

Although a number of experiments have previously carried out to study the response of wheat to water stress, nonetheless, a little information is available concerning water stress around heading and reproductive stages. Moreover, the role of exogenous application of ascorbic acid on activities of antioxidant enzymes, growth, productivity and nitrogen use efficiency is imperative to study. Therefore, the main objective of this study was undertaken to examine the effect of ascorbic acid and N fertilizer application on productivity of wheat and NUE under water stress.

Materials and Methods

Plant materials

This research was undertaken at the experimental farm of the faculty of agriculture, Kafrelsheikh University, Egypt (31° 05' 54.3" N, 30° 57' 19.4" E) during two consecutive winter growing seasons in 2012–2013 and 2013–2014, respectively. Climatic data were collected from an agro-meteorological Sakha station located 2 km from the experimental site, as given in (Table 1). Soil samples were taken from 0 to 30 cm soil depth using a soil Auger to analyze N content by Kjeldahl method (Bremner, 1960). The soil was clayey and an average bulk density of 1.22 g cm^{-3} in upper 30 cm depth. This layer also contained 1.37% total organic matter, 0.15% total nitrogen (N), 35 mg kg^{-1} available phosphorus (P) and 255.3 mg kg^{-1} exchangeable potassium (K), EC (1.05 ds m^{-1}, 1:5), pH (8.1, 1:2.5) and 1.38 cm annual precipitation as average in both seasons.

Table 1. Monthly relative humidity (RH, %), wind speed (km day^{-1}), mean minimum and maximum air temperatures (T_{max} and T_{min}, respectively) during the two winter growing seasons.

Season / Month	2012				2013			
	Temperature (°C)		Wind speed (km day^{-1})	RH (%)	Temperature (°C)		Wind speed (km day^{-1})	RH (%)
	T_{max}	T_{min}			T_{max}	T_{min}		
Dec	17.7	11.6	155.5	37.8	16.9	11.4	156.0	30.7
Jan	22.6	11.0	158.7	41.4	23.5	9.4	142.0	41.4
Feb	23.3	11.9	167.9	45.5	22.0	11.0	152.1	45.1
Mar	22.5	14.7	129.3	49.2	24.0	16.2	123.5	47.9
April	29.1	17.0	89.1	52.5	27.4	17.2	88.2	55.4
May	33.8	18.2	111.7	61.1	31.9	17.8	96.3	64.1

max = maximum, min = minimum, RH = relative humidity.

Experimental design and agronomic practices

The experiment was laid out as a randomized complete block design (RCBD) of a split- split plot arrangement with three replicates. Two water treatments including (water stress and well-watering) were assigned in main plots and separated well to avoid infiltration when water treatments were applied. Four N fertilizer rates including (0, 85, 170 and 255 kg N ha^{-1}) were allocated to the subplots. Sub subplots were two levels of ascorbic acid (0 and 200 mg l^{-1}). The preceding crop was Maize (*Zea mays* L.) during the growing seasons. Calcium superphosphate (15.5% P_2O_5) was added at the rate of 125 kg ha^{-1} during seedbed preparation. N fertilizer was applied as ammonium sulphate (20.6% N) to each plot at three splits; 20% as basal dose at sowing stage, 40% at the beginning of tillering stage and the remaining 40% at end of stem elongation stage.

Water stress was achieved after three irrigations at sowing, tillering and stem elongation stages by withholding the water supply after ending of stem elongation stage till physiological maturity. Meanwhile, well-watered was achieved by the water supply for five irrigations (sowing, tillering, stem elongation, booting and reproductive stages. Ascorbic acid (AA) in the rate of 200 mg l^{-1} had foliar sprayed twice using hand atomizer and wetting agent at stem elongation and booting stages.

Locally recommended wheat (*Triticum aestivum* L., Cv.Masr-1) originated in Wheat department, Field Crops Research Institute, Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt, was planted on December 2nd during 2012/2013 and November 25th during 2013/2014 with row spacing of 12.5 cm and a seeding rate 140 kg ha^{-1}. Weeds were controlled by Topik 15% WP herbicide and mechanical control. The net experimental unit size (plot) was 10.5 m^2 (3 m width × 3.5 m long). Wheat grain yield (14% water content) obtained by harvesting the center (2 m × 2 m) of the experimental unit, but yield components were determined from two outer rows within each plot.

Morphological and Physiological measurements

Flag leaf area (cm^2) measurement and chlorophyll content (SPAD)

Ten plant samples from each plot were collected at heading stage, when the flag leaf fully was expanded to measure flag leaf area by Leaf Area Meter (Li-Cor 3100, Lambda Instruments Co., USA).

Hand-held chlorophyll meter (SPAD-502; Minolta Sensing Co., Ltd, Japan) was used to record SPAD readings from the topmost fully expanded leaves on each main stem at heading stage. SPAD values were measured at three different points along the flag leaf blade and then the readings were averaged to have a single value for a plant (Markwell et al., 1995).

Leaf relative water content

Relative water content was estimated using fully expanded leaves were weighted after harvesting to record fresh weight (FW). Turgid weight (TW) was determined after the leaves were rehydrated in distilled water into a closed container at 10°C in the dark for 24 h and weighted again. Dry weight (DW) was determined for the same leaves after oven-drying for 3 days at 80 °C. RWC was calculated using the following equation: RWC (%) = [(FW-DW) / (TW-DW)] × 100 (Jeon et al., 2006).

Biochemical Assays of Antioxidant Enzymes

The tested antioxidant enzyme activities were measured in plants, 0.5 g leaf material was homogenized at 0-4 °C in 3 ml of 50 mM TRIS buffer (pH 7.8), containing 1 mM EDTA-Na^2 and 7.5% polyvinylpyrrolidone. The homogenates were centrifuged (12,000 rpm, 20 min, 4 °C) and the total soluble enzyme activities were measured spectrophotometrically in the supernatant (Hafez, 2015). All measurements were carried out at 25 °C, using the model UV-160A spectrophotometer (Shimadzu, Japan). The enzyme assays were tested three times.

Activity of catalase (CAT) was determined spectrophotometrically according to (Aebi, 1984). Changes in the absorbance at 240 nm were recorded every 30 sec intervals for 3 min. Enzyme activity was expressed as the increase in absorbance min^{-1} g^{-1} fresh weight. Peroxidase (POX) activity was directly determined of the crude enzyme extract according to a typical procedure proposed by (Hammerschmidt et al., 1982). Changes in absorbance at 470 nm were recorded every 30 sec intervals for 3min. Enzyme activity was expressed as the increase in absorbance min^{-1} g^{-1} fresh weight.

Yield components

After the final harvest the grain, straw yield and yield components (number of grains $spike^{-1}$, number of spikes m^{-1} and 1000-grain weight) were determined for all plots.

The harvest dates at maturity were 10 May 2013 and 1 May 2014. Plant samples collected at harvest were separated into grain and straw and electric oven-dried at 70 °C for 72 h till constant dry weight, then were grounded in a mill to produce a fine powder which is needed for the N analysis using the standard procedure of micro-Kjeldahl digestion with Sulfuric acid.

Nitrogen uptake was calculated as it is described by (Craswell and Godwin, 1984) as follows:

$$Grains\ N\ uptake\ (kg\ ha^{-1}) = \frac{Grains\ N\ content\ (g\ kg^{-1}) \times Grains\ DM\ (kg\ ha^{-1})}{1000}$$

$$Straw\ N\ uptake\ (kg\ ha^{-1}) = \frac{Straw\ N\ content\ (g\ kg^{-1}) \times Straw\ DM\ (kg\ ha^{-1})}{1000}$$

Nitrogen use efficiency (NUE) was calculated as it is described by (Craswell and Godwin, 1984) as follows:

$$NUE\ (kg\ kg_N^{-1}) = \frac{GY_t - GY_c (kg\ ha^{-1})}{NF_t - NF_c (kg\ ha^{-1})}$$

where GY_t and GY_c express the grain yield at different N treatments and control, respectively. While NF_t and NF_c express the N applications for different N treatments and control, respectively.

Statistical analysis

Data obtained were subjected to an analysis of variance (ANOVA) procedures according to (Gomez and Gomez, 1984) using the MSTAT-C Statistical Software package. Different Means were compared using (Duncan, 1955), when the ANOVA showed significant differences ($P<0.05$).

Results and Discussion

Wheat grain yield is a result of shared factors of some yield-related traits, i.e. number of spikes per unit area, number of grains per spike and its weight. Water stress diminished the yield under all nitrogen applications due to marked reduction in traits relevant to yield, etc., in both seasons of study (Table 2). Some previous reports pointed that water stress diminished wheat yields by 30-58% (Waraich et al., 2011; Farooq et al., 2015).

Effect of treatments on flag leaf area and leaf chlorophyll

Flag leaf area and leaf chlorophyll were curtailed by water stress compared with well-watered treatment (Table 2). The minimum flag leaf area (34.3 and 32.4 cm^2 during 2012/2013 and 2013/2014, respectively) was recorded under water stress, while the maximum flag leaf area (39.6 and 36.8 cm^2 during 2012/2013 and 2013/2014, respectively) was recorded under well-watered treatment. Also, The minimum leaf chlorophyll content (42.6 and 41.6 during 2012/2013 and 2013/2014 respectively) was recorded under water stress which has been ascribed to loss of chloroplast membranes

(Flaxes et al., 2006), while the maximum leaf chlorophyll content (46.2 and 44.2 during 2012/2013 and 2013/2014, respectively) was recorded under well-watered treatment. This notable reduction due to loss of turgor pressure which lead to closeness of the pores and declined of photo-assimilates during low moisture content in soil (WU et al., 2008).

Table 2. Effect of water treatments, N levels and ascorbic acid levels on flag leaf area, chlorophyll content, number of grain spike^{-1}, number of spikes m^{-2} and 1000-grain weight, respectively) in 2012/2013 and 2013/2014 seasons.

Treatments	Flag leaf area (cm^2)		Chlorophyll content (SPAD)		No. of grains spike^{-1}		No. of spikes m^{-2}		1000-grain weight (g)	
Water treatments	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
WS	34.3^b	32.4^b	42.6^b	41.6^b	46.3^b	47.5^b	345.4^b	350.7^b	42.4^b	40.3^b
WW	39.6^a	36.8^a	46.2^a	44.2^a	50.3^a	50.1^a	349.8^a	355.9^a	45.5^a	44.6^a
N levels (kg ha^{-1})										
0	28.6^d	26.4^d	30.3^d	27.4^d	52.5^c	51.3^d	252.5^d	255.7^d	47.1^a	47.4^a
80	34.3^c	32.1^c	36.8^c	33.7^c	55.3^b	52.5^c	305.2^c	309.2^c	46.3^b	46.1^b
160	39.1^b	36.6^b	42.6^b	41.4^b	56.1^a	53.8^a	330.7^b	356.5^b	46.1^c	45.9^c
240	41.8^a	40.7^a	45.8^a	45.1^a	55.9^a	52.9^b	350.2^a	375.7^a	45.6^d	45.2^d
Ascorbic acid levels (mg l^{-1})										
0	35.3^b	33.6^b	42.2^b	43.5^b	50.2^b	50.1^b	345.8^b	340.3^b	42.1^b	41.5^b
200	41.8^a	40.4^a	47.4^a	46.8^a	54.7^a	53.4^a	349.2^a	347.2^a	45.6^a	44.8^a
W	*	*	*	*	*	*	*	*	*	*
N	*	*	*	*	*	*	*	*	*	*
AA	*	*	*	*	*	*	*	*	*	*
W × N	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
W × AA	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
N × AA	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
W ×N × AA	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Data within columns followed by different letters are significantly different at P<0.05; ns, no significant difference. WW: well-watered, WS: water stress.

Amounts of N applied enhanced flag leaf area and leaf chlorophyll content in both seasons which indicate the N impact in relieving stress effect such results were supported by (Hafez et al., 2014). Results in (Table 2) showed that the highest (240 kg N ha^{-1}) amount of N led to substantial increase in flag leaf area (41.8 and 40.7 cm^2 during 2012/2013 and 2013/2014, respectively) and leaf chlorophyll (45.8 and 45.1 during 2012/2013 and 2013/2014, respectively). These results are in conformity with the findings of (Makoto and Koike, 2006) who also reported that increasing amount of nitrogen applied leads to leaves expansion, increase photosynthetic rate and gas exchange parameters in wheat (Lopes and Araus, 2006). Our results cleared that water stress played an important role in photosynthetic capacity in addition, amounts of N apply could enhance it but could not change the declining tendency.

Likewise, in (Table 2) foliar spraying of ascorbic acid (200 mg l^{-1}) appreciably increased flag leaf area (41.8 and 40.4 cm^2 during 2012/2013 and 2013/2014, respectively) and leaf chlorophyll (47.4 and 46.8 during 2012/2013 and 2013/2014, respectively) compared to control treatment (without spray). Whereas, ascorbic acid induced growth improvement through increase flag leaf area (Dolatabadian and Jouneghani, 2009), which is the photosynthesizing tissue of the plant. Moreover, photosynthesis increased plant growth due to ascorbic application which regulates cell growth and division. These results are consistent with the findings published by (Dolatabadian and Jouneghani, 2009).

Effect of treatments on yield components

Pre and post heading stages are the most critical stages of wheat to water stress, leading to considerably reduction in number of grains per one spike (46.3 and 47.5), number of spikes m^{-2} (345.4 and 350.7) and 1000-grain weight (42.4 and 40.3 g), respectively as was found in both seasons (Table 2) compared with well-watered treatment in number of grains per one spike (50.3 and 50.1), number of spikes m^{-2} (349.8 and 355.9) and 1000-grain weight (45.5 and 44.6 g), respectively as was found in both seasons. This reduction attribute to declined photosynthesis, accelerated leaf senescence and sink limitations might be responsible for small grain number and weight under water stress (Farooq et al., 2015). Moreover, less grains number per spike was also due to decrease of number of spikelets per spike under water stress in both seasons of study (Madani et al., 2010). Increasing wheat yield in well-watered could be the result of favourable sink–source (Wei et al., 2010). Higher amounts of nitrogen applied during 2012/2013 and 2013/2014 seasons significantly increased number of spikes m^{-2} and number of grains $spike^{-1}$ while minimum 1000-grain weight was noted in higher amounts of nitrogen applied compared to control treatment. These observations are in line with reports in wheat subjected to different amounts of N applied by (Madani et al., 2010). Nevertheless, decrease of 1000-grain weight was linked to the increase of spikes per unit area induced by amounts of nitrogen applied and probably means that the level of carbohydrate supply may be the factor controlling grain weight which originating from photosynthesis (Marino et al., 2009). It was found markedly outperformed of the yield components, i.e. number of grains $spike^{-1}$ (8 and 9%), number of spikes m^{-2} (2 and 3%) and 1000-grain weight (7 and 8%) by adding 200 mg l^{-1} of ascorbic acid in both seasons, respectively compared with control treatment (without spray).The positive effect of ascorbic acid may be attribute to its crucial role in improvement flag leaf area and leaf chlorophyll content which in turn enhance vegetative and reproductive growth, carbohydrates accumulation and seed set (Smirnoff et al., 2000; Athar et al., 2009). The results of yield components showed that the higher grain yield was crucially due to the higher number of grains per one spike and number of spikes per m^2 and secondly, to the higher mean 1000-grain weight which are the vital yield components and directly associated with increased grain yield (Xu et al., 2015).

Effect of treatments on leaf relative water content

The leaf relative water content was measured at heading stage (Table 3). A decrease in the RWC in response to water stress has been noted compared to well-watered treatment in 2012/2013 and 2013/2014 seasons (Table 3). Higher RWC value 94.6% and 94.3% were recorded at well watered treatment in both seasons, respectively. Whereas, lower RWC value 82.4% and 81.8% were recorded at water stress treatment in both seasons, respectively. Low RWC could be due to a reduction of water supply to the leaves. Some authors showed that passive impacts of water stress on stomata closure and photosynthesis rate led to decline growth and yield (Ghotbi-Ravandi et al., 2014). Notably, The RWC increased significantly with increasing amounts of N applied in both seasons of study. The maximum RWC value 95.5 and 95.6% during the 2012/2013 and 2013/2014 respectively, were recorded at the highest amounts of applied N (240 kg N ha^{-1}) (Table 3). The minimum RWC values 85.2 and 85.8% were recorded at untreated N. Our results are in conformity with the findings of (Waraich et al., 2011) who stated that increasing amounts of N applied in wheat alters leaf relative water content. These changes in RWC could be attributable to alter in osmotic adjustment and photosynthetic rate. Foliar spraying of ascorbic acid significantly improved relative water content in water stressed wheat plants. The wheat plants sprayed with ascorbic acid had higher chlorophyll content, flag leaf area and relative water content and they produced higher biological yield than the non-sprayed plants. Higher RWC value 94.9% and 93.8% were recorded with 200 mg l^{-1} ascorbic acid treatment (Table 3). Whereas, lower RWC value 88.4% and 85.2% were recorded with unsprayed treatment. These results are consistent with (Ergin et al., 2014). These points out that ascorbic acid can ameliorate the negative effects of water stress by acting as a growth factor to accelerate shoot and root growth under water stress and reduce the loss of turgidity in flag leaves.

Table 3. Effect of water treatments, N levels and ascorbic acid levels on leaf relative water content (RWC), catalase activity (CAT) and peroxidase activity (POX), respectively) in 2012/2013 and 2013/2014 seasons.

Treatments	RWC (%)		CAT activity (mM g^{-1} H_2O_2 FW min^{-1})		POX activity (mM g^{-1} H_2O_2 FW min^{-1})	
Water treatments	2012	2013	2012	2013	2012	2013
WS	82.4^b	81.8^b	1.2^a	1.3^a	12.2^a	14.5^a
WW	94.6^a	94.3^a	0.8^b	0.9^b	7.4^b	8.9^b
N levels (kg ha^{-1})						
0	85.2^c	85.8^c	0.6	0.8	6.5	6.6
80	91.9^b	92.9^b	0.7	0.7	7.5	7.8
160	92.1^b	93.5^b	0.7	0.9	7.8	7.3
240	95.5^a	95.6^a	0.8	0.9	7.9	8.1
Ascorbic acid levels (mg l^{-1})						
0	88.4^b	85.2^b	0.7^b	0.9^b	6.8^b	7.5^b
200	94.9^a	93.8^a	2.6^a	2.7^a	17.6^a	18.4^a
W	*	*	*	*	*	*
N	*	*	ns	ns	ns	ns
AA	*	*	*	*	*	*
W × N	ns	ns	ns	ns	ns	ns
W × AA	*	*	*	*	*	*
N × AA	ns	ns	ns	ns	ns	ns
W × N × AA	ns	ns	ns	ns	ns	ns

Data within columns followed by different letters are significantly different at $P<0.05$; ns, no significant difference. WW: well-watered, WS: water stress.

In figure 1, there was an interaction effect between water treatments and foliar spraying on relative water content (RWC) of flag leaves in both seasons of study. The RWC was highly significant increased with water stress and well-water treatments exposed to foliar spraying by ascorbic acid in both seasons compared with unsprayed treatments. These results are consistent with (Ergin et al., 2014). These points out that ascorbic acid can ameliorate the negative effects of water stress by acting as a growth factor to accelerate shoot and root growth under water stress and reduce the loss of turgidity in flag leaves under water stress.

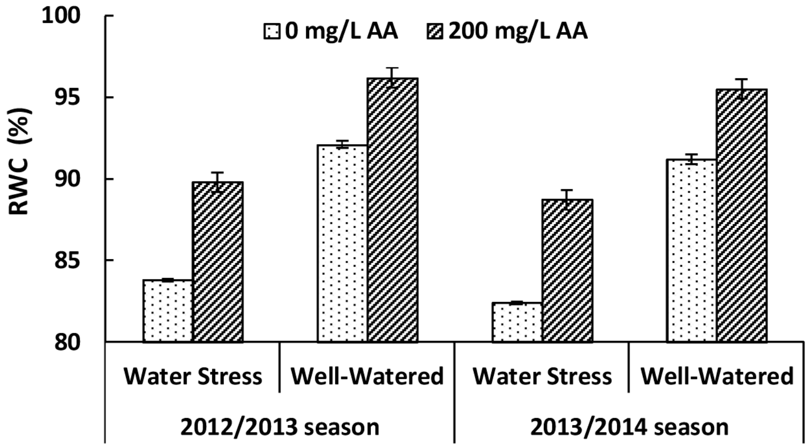

Figure 1. Effect of ascorbic acid application on leaf relative water content (RWC) under water stress and well-watered at heading stage in 2012/2013 and 2013/2014 seasons. The data are the mean ± SE of three replicates. Significant differences of the means according to Duncan's multiple range test ($P \leq 0.05$) are indicated with different letters.

Effect of treatments on activities of antioxidant enzymes

The activity of antioxidant enzymes increased under water stress (Table 3). Water stress increased CAT activity by 33% and 31% in both seasons, respectively, compared to the control. As well as, water stress increased POX activity by 40% and 38% in both seasons, respectively, compared to the control. Under the well-watered condition in (Figure 2), the application of 200 mg l^{-1} ascorbic acid (AA) led to highly increase in CAT activity by 80 and 85% in both seasons, respectively and POX activity by 62 and 70% in both seasons, respectively compared to the control. There was no significant difference among amounts of N applied on CAT and POX activity. Under the water stress condition also in (Figure 2), application of 200 mg l^{-1} ascorbic acid (AA) led to highly increase in CAT activity by 39 and 45% in both seasons, respectively and POX activity by 47 and 42% in both seasons, respectively compared to the control. High endogenous AA and high activities of antioxidant enzymes are a limiting factor to alleviate the harmful effects of ROS (oxidative stress) (Hafez et al., 2012). Furthermore, regulating processes of plant metabolism. It can be achieved by exogenous application of AA through foliar spraying (Batool et al., 2012; Malik and Ashraf, 2012). In the present study, activities of antioxidant enzymes (CAT and POX) in wheat plants were increased in response to water stress as well as after AA application. Whereas, AA application may act to protect plants from oxidative injury induced by water stress (Athar et al., 2009). The interaction between water treatments and foliar spraying were significant and the maximum CAT and POX activity under water stress treated with ascorbic acid (AA) in both seasons of study.

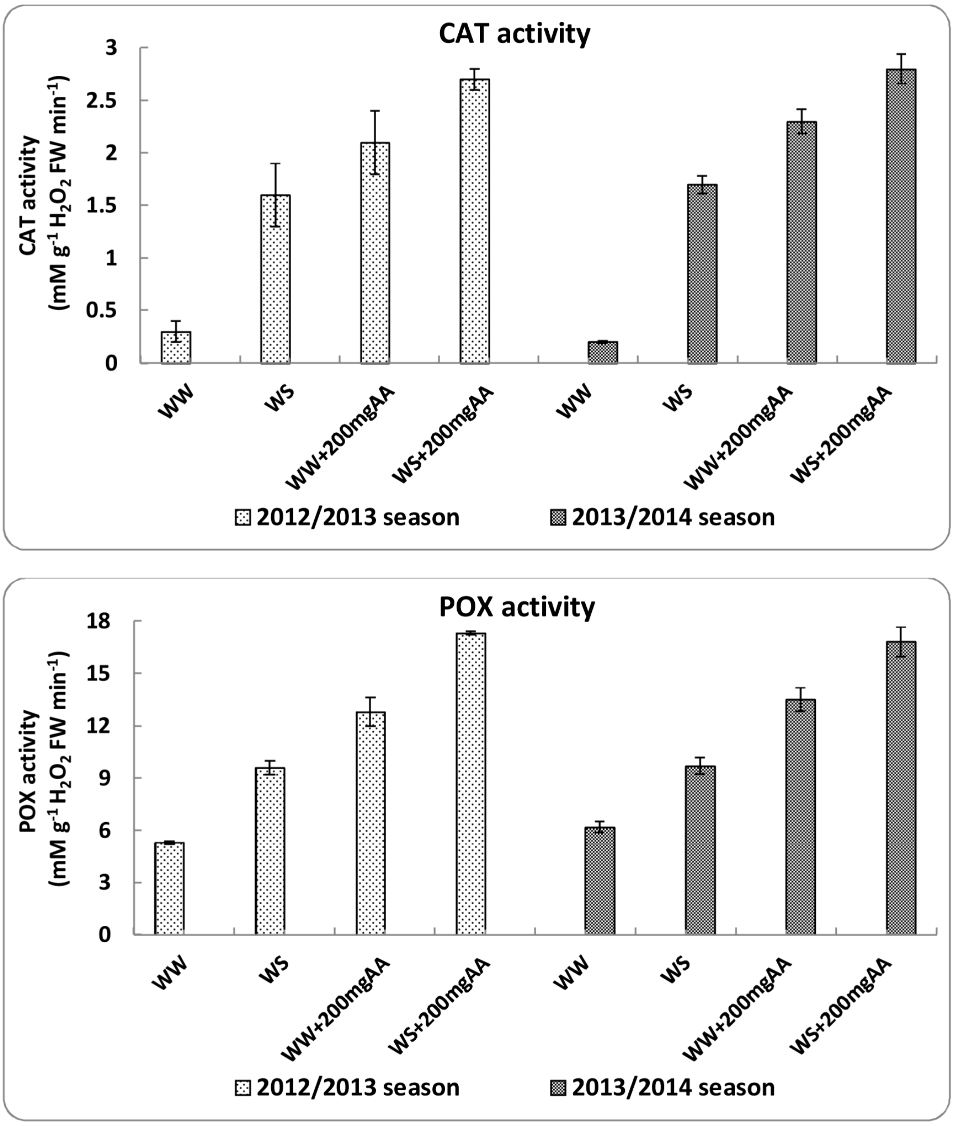

Figure 2. Effect of ascorbic acid application on activites of antioxidant enzymes catalase (CAT) and peroxidase (POX) under water stress and well-watered at heading stage in 2012/2013 and 2013/2014 seasons. The data are the mean ± SE of three replicates. WW: well-watered, WS: water stress, 200 mg AA: 200 mg l^{-1} of ascorbic acid.

Effect of treatments on Grain and straw yields

Grain and straw yields reduction were observed as a result of the induced water stress compared to well-watered treatment in both seasons. In our study (Table 4), grain yield varied between water stress (6950.5 and 6750.3 kg ha^{-1}, respectively in 2012/2013 and 2013/2014 seasons) and well watered (7320.5 and 7135.8 kg ha^{-1}, respectively in 2012/2013 and 2013/2014 seasons). While, straw yield varied between water stress (9750.5 and 9523.9 kg ha^{-1}, respectively in 2012/2013 and 2013/2014 seasons) and well watered (10478.8 and 10245.3 kg ha^{-1}, respectively in 2012/2013 and 2013/2014 seasons). It is noticeable that grain yield and straw yield gradually increased with the increase of amount of N applied in both seasons of study, accordingly (Athar et al., 2009).

Table 4. Effect of water treatments, N levels and ascorbic acid levels on grain yield, straw yield, grain N content and straw N content, respectively) in 2012/2013 and 2013/2014 seasons.

Treatments	Grain yield (kg ha^{-1})		Straw yield (kg ha^{-1})		Grain N (g kg^{-1})		Straw N (g kg^{-1})	
Water treatments	2012	2013	2012	2013	2012	2013	2012	2013
WS	6950.5^b	6750.3^b	9750.5^b	9523.9^b	13.2	13.1	4.8^b	4.7^b
WW	7320.5^a	7135.8^a	10478.8^a	10245.3^a	12.9	12.9	5.1^a	4.9^a
N levels (kg ha^{-1})								
0	5122.2^d	5051.7^d	8186.2^d	7378.4^d	10.2^d	10.5^d	3.1^d	3.1^c
80	6575.4^c	6329.5^c	9971.1^c	8211.3^c	12.1^c	12.4^c	4.2^c	4.1^b
160	7021.5^b	6786.1^b	10083.5^b	9448.7^b	13.1^b	12.9^b	4.7^b	4.3^b
240	7428.2^a	7132.5^a	10615.8^a	10304.4^a	13.4^a	13.2^a	5.5^a	5.1^a
Ascorbic acid levels (mg l^{-1})								
0	7255.8^b	7038.5^b	9965.3^b	9622.7^b	13.1^b	13.1^b	4.5^b	4.3^b
200	7720.4^a	7524.7^a	10278.6^a	9987.3^a	13.8^a	13.7^a	5.6^a	5.2^a
W	*	*	*	*	ns	ns	*	*
N	*	*	*	*	*	*	*	*
AA	*	*	*	*	*	*	*	*
W × N	ns	ns	ns	ns	ns	ns	ns	ns
W × AA	*	*	ns	ns	*	*	*	*
N × AA	ns	ns	ns	ns	ns	ns	ns	ns
W × N × AA	ns	ns	ns	ns	ns	ns	ns	ns

Data within columns followed by different letters are significantly different at $P<0.05$; ns, no significant difference. WW: well-watered, WS: water stress.

Nitrogen application (240 kg N ha^{-1}) tended to increase grain yield and straw yield by about 30% and 25%, respectively, as an average of fertilized versus unfertilized condition (Table 4).

External supply of ascorbic acid (AA) to wheat plants appreciably enhanced grain and straw yields in both seasons (Table 4). The application of ascorbic acid at 200 mg l^{-1} increased grain and straw yields by 8% and 4% when compared to control (without spray), respectively. The positive effect of AA on grain and straw yields may be attributed to its role in translocation of metabolites from leaves into reproductive organs. Moreover, synthesis of protein and nucleic acids, which improve grain and straw yields. These results agreed with the findings of (Dolatabadian and Jouneghani, 2009; Xu et al., 2015).

In figure 3, there was an interaction effect between water treatments and foliar spraying on grain yield of wheat plant in both seasons of study. The grain yield was significant highly increased with well watered treated by ascorbic acid in both seasons. There was no significant difference between water stress treated by ascorbic acid and well watered without foliar spraying by ascorbic acid in both seasons. The grain yield was the lowest significantly with water stress without ascorbic acid in both seasons.

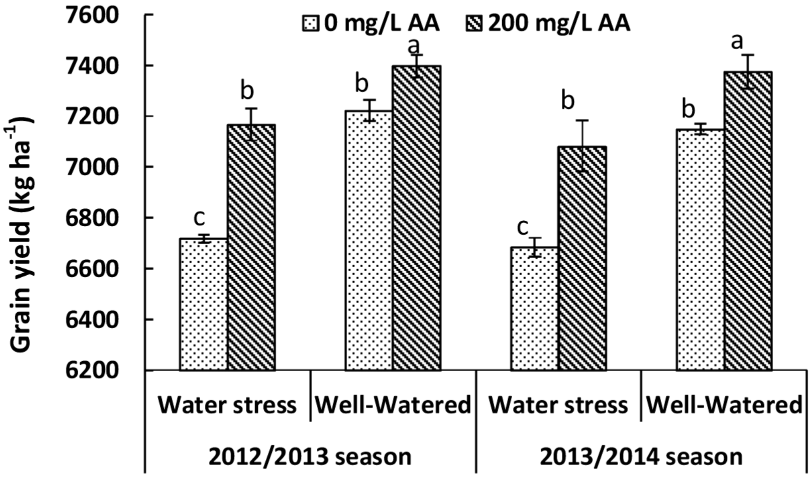

Figure 3. Effect of ascorbic acid application on grain yield (kg ha^{-1}) under water stress and well-watered in 2012/2013 and 2013/2014 seasons. The data are the mean ± SE of three replicates. Significant differences of the means according to Duncan's multiple range test (P≤0.05) are indicated with different letters.

In figure 4, there was an interaction effect between water treatments and foliar spraying on Total N content (grain N content + straw N content) of wheat plant in both seasons of study. There was not significant difference between both water stress and well watered treatments treated with foliar spraying by ascorbic acid in both seasons. Meanwhile significantly decreased with water stress without foliar spraying in both seasons.

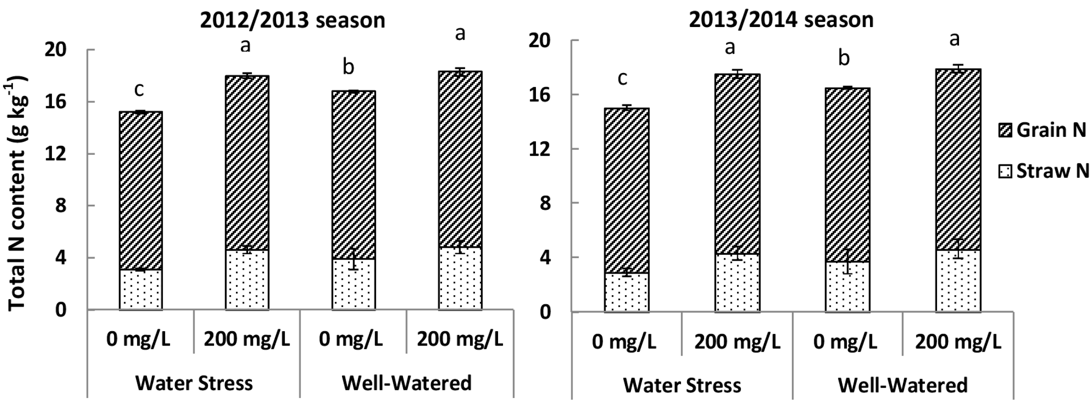

Figure 4. Effect of ascorbic acid application on grain and straw N content (g kg^{-1}) under water stress and well-watered in 2012/2013 and 2013/2014 seasons. The data are the mean ± SE of three replicates. Significant differences of the means according to Duncan's multiple range test (P≤0.05) are indicated with different letters.

Effect of treatments on plant N contents

Data regarding grain N content showed in both seasons of study (2012/2013 and 2013/2014) that water treatments had no significant effect on grain N content (Table 4). Indicating that grain N content was similar between water stress and well-watered treatments, with regard to water stress slightly increased grain N content (Table 4). Our results declared in (Table 3) that water stress significantly reduced straw N content (4.8 and 4.7 g kg^{-1}, respectively in 2012/2013 and 2013/2014 seasons) in relation to straw N content (5.1 and 4.9 g kg^{-1}, respectively in 2012/2013 and 2013/2014 seasons) in well watered treatment. In our study, it was found that under water stress higher rates of grain N accumulation and lower both carbohydrates accumulation and grain weight, leading to a negative correlation between grain N content and grain yield (Barati et al., 2015). Xu et al. (2006) confirmed that water stress enhanced N translocation from pre heading stage to grain, thus increasing remobilized N to N grain content. The accelerated senescence of the stressed plants lead to the increment in N absorbed in the water stress plants (Xu et al., 2006). Results of present study show the increase in the N rate up to 240 kg N ha^{-1} had a positive effect on grain N content. It can be seen from (Table 4) that the maximum grain N content (13.4 and 13.2 g kg^{-1}, respectively in 2012/2013 and 2013/2014) was obtained by adding 240 kg N ha^{-1}. Also, in (Table 4) illustrates the progressive changes in amounts of N applied on straw N content in both seasons of study. With the highest N level (240 kg N ha^{-1}); straw N content was higher (15 and 14%, respectively in 2012/2013 and 2013/2014) compared to lower N level (160 kg ha^{-1}). In addition, the highest N level (240 kg N ha^{-1}); straw N content was higher (44 and 43%, respectively in 2012/2013 and 2013/2014) compared to unfertilized treatment (Table 4). From our results, it can be understand that grain and straw N content in wheat relies on absorption of N from soil before heading and remobilization of stored vegetative N accumulated before heading (Pandey et al., 2001). Likewise, increasing N available and well-watered condition leading to higher contribution of N absorption after heading (Cossani et al., 2012). Foliar spraying by ascorbic acid at 200 mg l^{-1} gave higher grain N contents (8 and 7%, respectively in 2012/2013 and 2013/2014) compared to control (without spray).

As shown in (Table 4), foliar spraying by ascorbic acid at 200 mg l^{-1} gave higher straw N content (20 and 18%, in 2012/2013 and 2013/2014) in relation to control treatment(without spray) in both seasons. Ascorbic acid showed a positive effect on accumulated soluble proteins which play a vital role in osmotic adjustment and may be associated with absorption of nutrients (Batool et al., 2012; Malik and Ashraf, 2012).

Effect of treatments on plant N uptake

N uptake requires available water in the soil, as it is the factor which remobilize N to the soil-root interface. Therefore in our work (Table 5), water stress exhibited lower grain and straw N uptake than well-watered treatment in both seasons of study. This effect was more pronounced under higher amounts of N applied. Water stress (91.7 and 87.6 kg ha^{-1}, respectively in 2012/2013 and 2013/2014 seasons) significantly reduced grain N uptake in relation to well watered treatment (94.8 and 91.1 kg ha^{-1}, respectively in 2012/2013 and 2013/2014 seasons). As well as, water stress (47.3 and 44.1 kg ha^{-1}, respectively in 2012/2013 and 2013/2014 seasons) significantly reduced straw N uptake in relation to well watered treatment (52.1 and 49.5 kg ha^{-1}, respectively in 2012/2013 and 2013/2014 seasons). In agreement with this result, (Kibe et al., 2006; Xu et al., 2006) cleared that the N uptake by wheat is considerably decreased under water stress, even if N fertilizer is available in the soil. A similar response was found with less N fertilizer; but the variability between water treatments were less. Amounts of N applied significantly increased grain and straw N uptake in both seasons of study (Table 5). Amount of N (240 kg N ha^{-1}) resulted in higher grain (99.6 and 94.5 kg N ha^{-1}) and straw (58.5 and 52.8 kg N ha^{-1}) N uptake values in both seasons, respectively. The grain and straw N uptake values significantly increased at highest nitrogen level (240 kg N ha^{-1}) by 48 and 43% for grain N uptake and 57 and 55% for straw N uptake in both seasons compared to unfertilized plots, respectively. Also, grain and straw N uptake were negatively correlated with N harvest index under increasing N fertilizer level. N uptake by plants is expected to considerably take place pre heading. Thus, more 90% of grain N uptake is assimilated in wheat pre heading between root absorption and N remobilized (Albrizio et al., 2010). Cossani et al. (2012) believed that N uptake is associated with dry matter production of grains and straw.

Table 5. Effect of water treatments, N levels and ascorbic acid levels on grain N uptake, straw N uptake and nitrogen use efficiency (NUE) respectively in 2012/2013 and 2013/2014 seasons.

Treatments	Grain N uptake (kg ha^{-1})		Straw N uptake (kg ha^{-1})		NUE (kg kg^{-1} N)	
Water treatments	2012	2013	2012	2013	2012	2013
WS	91.7^{b}	87.6^{b}	47.3^{b}	44.1^{b}	21.7^{b}	20.2^{b}
WW	94.8^{a}	91.1^{a}	52.1^{a}	49.5^{a}	22.2^{a}	20.6^{a}
N levels (kg ha^{-1})						
0	51.3^{d}	53.4^{d}	25.7^{d}	23.3^{d}	-	-
80	79.8^{c}	78.9^{c}	42.2^{c}	34.1^{c}	23.1^{a}	20.9^{a}
160	92.2^{b}	87.9^{b}	47.6^{b}	41.1^{b}	16.8^{b}	15.8^{b}
240	99.6^{a}	94.5^{a}	58.5^{a}	52.8^{a}	14.6^{c}	13.6^{c}
Ascorbic acid levels (mg l^{-1})						
0	95.1^{b}	91.5^{b}	49.2^{b}	46.8^{b}	21.2^{b}	20.1^{b}
200	104.1^{a}	103.3^{a}	54.2^{a}	51.5^{a}	23.6^{a}	22.7^{a}
W	*	*	*	*	*	*
N	*	*	*	*	*	*
AA	*	*	*	*	*	*
W × N	ns	ns	ns	ns	ns	ns
W × AA	ns	ns	ns	ns	*	*
N × AA	ns	ns	ns	ns	ns	ns
W × N × AA	ns	ns	ns	ns	ns	ns

Data within columns followed by different letters are significantly different at $P<0.05$; ns, no significant difference. WW: well-watered, WS: water stress.

It is obvious from (Table 5) that foliar spraying wheat plants with ascorbic acid (200 mg l^{-1}) significantly increased grain and straw N uptake in both seasons compared to control treatment (without spray). Spraying of AA increased grain N uptake by (10 and 12%, respectively in both seasons) compared to control treatment and increased straw N uptake by (9 and 10%, respectively in both seasons) compared to control treatment. The exogenous application of AA might increase the absorption and translocation within the leaf in addition to enhancement of the biosynthesis of photosynthetic pigments and improvement the nutritional status of wheat (Batool et al., 2012; Malik and Ashraf, 2012).

Effect of treatments on nitrogen use efficiency

The results showed that NUE of wheat affected by water treatments, amount of N applied and foliar spraying (Table 5). Water stress had slightly lower NUE (21.7 and 20.2 kg grains kg^{-1} N, respectively in 2012/2013 and 2013/2014 seasons) than well-watered (22.2 and 20.6 kg grains kg^{-1} N, respectively in 2012/2013 and 2013/2014 seasons). Water stress genes contribute to greater NUE because they improve biomass production over an extended range of soil moisture availability and weather conditions (Cossani et al., 2012). In addition, the traits for water stress improved deep root penetration (to access water and nutrients) and stomata closure to decrease the transpiration, all of which are relevant to NUE as well (Cossani et al., 2012). NUE decreased as amount of N applied increased. While NUE at N_{80} was (23.1 and 20.9 kg of grain kg^{-1}, respectively in both seasons of study), it dropped to (16.8 and 15.8 kg of grain kg^{-1}, respectively in both seasons of study) for N_{160} and (14.6 and 13.6, respectively in both seasons of study) for N_{240}. Poor NUE is a result of the soil-plant system leaking mineral-N when it exists in excess of what plants can use (Li et al., 2004). High amounts of N applied at early vegetative phase could result in more loss due to crop requirements is not high and the crop was unable to assimilate all the N absorbed. In addition, leaching, volatilization, surface runoff and immobilization from days after applying of N to N absorption (Hafez and Kobata, 2012). Consequently, amounts of N applied with plant N requirements may result in lower NUE. Giambalvo et al. (2004) showed that there was a little bit increment in available N absorption at higher N rates, but there was a decrement in soil N retention efficiency which led to a net reduction in NUE. Spraying of ascorbic acid increased NUE by (12 and 13%, respectively in 2012/2013 and 2013/2014 seasons) compared to control (without spray) treatment. Foliar spraying by ascorbic acid contributes to greater NUE because AA improve grain yield compared to untreated plots Foliar spraying AA has ameliorative effect on grain yield and relevant traits might be attribute to stimulatory influence of AA on vegetative growth resulting in higher photosynthesis rate and distribute of assimilates to grains (Chen and Gallie, 2008).

In figure 5, there was an interaction effect between water treatments and foliar spraying on nitrogen use efficiency (NUE) of wheat plant in both seasons of study. The NUE was highly significant increased with well watered treated by ascorbic acid in both seasons. While significantly decreased with water stress without foliar spraying. In addition, it was obvious that water stress supplemented with foliar spraying by ascorbic acid significantly outperformed than well-watered treatment without foliar spraying in both seasons.

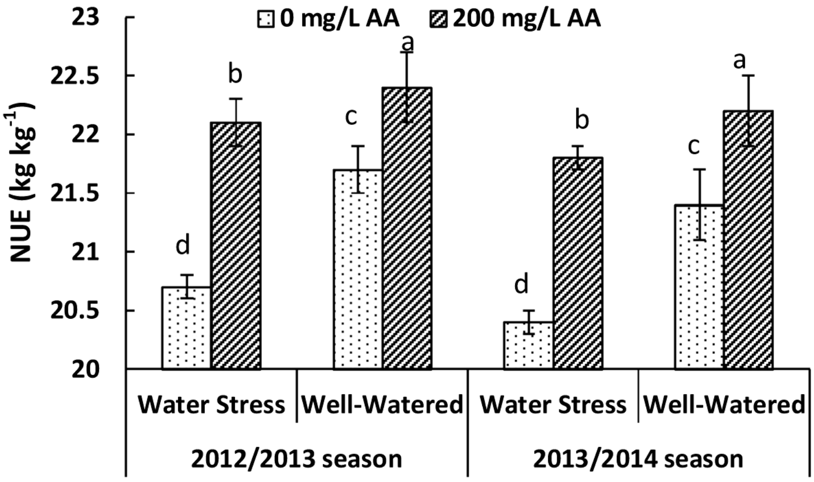

Figure 5. Effect of ascorbic acid application on NUE (kg ha^{-1}) under water stress and well-watered in 2012/2013 and 2013/2014 seasons. The data are the mean ± SE of three replicates. Significant differences of the means according to Duncan's multiple range test (P≤0.05) are indicated with different letters.

Conclusion

To sum up, farmers adopt any techniques due to notable improvement in wheat productivity. From the observations of physiological and biochemical analyses. The present study indicate that adverse effects of water stress on yield-related traits, leaf water status, chlorophyll content, nitrogen uptake and nitrogen use efficiency were substantially improved by foliar spraying of ascorbic acid. NUE decreased as amount of N applied increased. Under water stress increasing amounts of N applied led to an increase in grain N content and slightly lower NUE compared with well-watered condition. The results of this research confirm this approach, exogenous application by ascorbic acid led to a significant increment in all the studied parameters under water stress by up-regulating the antioxidative defense system in wheat plants. This research requires more studies on wheat plants under wider scale to more determination the availability of adding ascorbic acid for increasing the yield-related traits of wheat in Mediterranean areas.

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