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Original Research Article

Effects of Exogenous Osmoprotectants on Physiological Characteristics of Wheat

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A B S T R A C T

A field experiment was conducted to evaluate the effect of different osmoprotectants on various physiological characteristics, growth and yield of wheat during *rabi* season of 2013-14 and 2014-15 under irrigated conditions of Ludhiana, India. The experiment was conducted in Randomised Complete Block Design, with eleven treatments consisting of three osmoprotectants applied at three different concentrations, viz., thiourea @ 20, 40 and 60 mM, potassium nitrate @ 1, 2 and 3% and sodium nitroprusside @ 400, 800 and 1200 µg/ml along with water spray and untreated control as additional treatments. During 2013-14, among all the osmoprotectants, foliar application of sodium nitroprusside @ 400 µg/ml, KNO_3 @ 2% and thiourea @ 20 mM recorded higher physiological parameters, i.e., relative chlorophyll content, stomatal content and photosynthetic rate, relative leaf water content and proline content of flag leaf than both water sprayed and untreated controls. However, exogenous osmoprotectants decreased canopy temperature of the crop. Grain yield also increased to the extent of 18.8% by foliar application of sodium nitroprusside @ 400 µg/ml , 17.9% by KNO_3 @ 2% and 14.9% by thiourea @ 20 mM during 2013-14. During excessive wet season of 2014-15, SNP @ 800 µg/ml, KNO_3 @ 3% and thiourea @ 40 mM performed better as compared to lower doses.

Keywords

Osmoprotectants, Physiology, Photosynthesis, Wheat, Yield

Introduction

High temperature stress is a major environmental stress that limits growth, metabolism and productivity of wheat. As the entire wheat growing region in India falls under tropical and subtropical climate, it is anticipated that climate will transform them into heat stressed and short season production environment (Beta and Gerats., 2013).The major reason is the sudden rise in temperature during grain filling period of wheat prior to maturity, a phenomenon called terminal heat stress. Mitra and Bhatia, (2008) have also reported decline in wheat productivity due to terminal heat stress in India. Photosynthesis is the most sensitive physiological process to high temperature which leads to disruption of photo system II, the most thermally liable component of electron transport chain. On the other hand, heat stress also results in reduction in chlorophyll content eventually leading to leaf senescence in which there is progressive loss of green leaf area during reproductive development of a crop (Nooden, 1988).

Hence, early leaf senescence results in photosynthesis rate that is too low to meet the plant economy. Indeed, relative water content is also substantially reduced during high temperature stress as evidenced by (Saairam *et al*., 2000).

The accumulation of osmolytes inside plants can overcome the negative consequences of abiotic stress on crop productivity and during the last 20 years it has received increasing attention. Osmolyte accumulation in plant cells decreases cell osmotic potential and maintains water absorption and cell turgor pressure, which might result in sustaining physiological processes, such as stomatal, photosynthesis and growth. Certain crop plants like wheat lacks significant amount of any osmolyte thereby exogenous induction of osmoprotectants in order to improve tolerance to heat stress is advocated. To tackle this problem, certain chemicals like thiourea, potassium nitrate and sodium nitroprusside can be used as foliar spray.

Thiourea is reported to be an efficient hydroxyl radical scavenger in plants and thus protects them against oxidative damage induced by high temperature stress (Anjum *et al*., 2011). Sodium nitroprusside (donor of NO), helps in enhancing the synthesis of antioxidant enzymes, thereby ameliorates high temperature stress (Bavita *et al*., 2012). Another chemical, potassium nitrate might play an important role in adaptation of cells to high temperature stress through its effect on water uptake, root growth, carbohydrate redistribution and starch synthesis in storage organs (Bardhan *et al*., 2007). However, little is known about the optimum concentrations of these chemicals to mitigate high temperature induced changes to wheat plants. It would be worthwhile to validate the effect of these osmoprotectants on physiological attributes of wheat to minimize the farmer's risk for reduced yield and low quality grain product under terminal heat stress.

Materials and Methods

Experimental site and weather

Wheat (*Triticum aestivum* L) variety HD-2967 was sown in field experiment during winter (rabi) season (November-April) of 2013-14 and 2014-15 at the research farm of Punjab Agricultural University, Ludhiana, India, situated at 30˚56 N latitude, 75˚52 E longitude and 247 m above mean sea level. The meteorological data for the crop season (8-11-2013/14 to 22-04-2014/15) on standard meteorological week (SMW) basis was obtained from meteorological observatory of the Punjab Agricultural University.

The weekly mean maximum air temperature during crop season ranged between 15.1 to 32.6°C during 2013-14 and 12.5 to 34.7°C during 2014-15, weekly mean minimum temperature ranged between 3.8 to 16.7°C during 2013-14 and 7.2 to 20.3°C during 2014-15. The weekly mean minimum temperature increased continuously from 7th (12-02-14 to 18-02-14) to 16th (16-04-14 to 22-04-14) SMW during 2013-14, respectively, which were about 1.0°C higher than respective normal weekly mean minimum temperatures. During 2014-15, the weekly mean minimum temperatures increased continuously from 7th (12-02-15 to 18-02-15) to 16th (16-04-15 to 22-04-15) SMW during 2013-14, respectively, which were about 2.5 to 3.5°C higher than respective normal weekly mean minimum temperatures.

The total amount of rainfall received during the crop seasons from November to April was 177 mm and 221 mm during 2013–14 and 2014–15, respectively. The amount of rainfall received during February and March (reproductive phase) was 36.7 and 35.0 mm during 2013-14 and 38.6 and 84.6 mm in 2014-15. Hence, it is easily understood that due to this increase in monthly minimum air temperatures, the experimental crop faced the problem of terminal heat stress severely during both the years, but more prominently during 2014-15.

Experimental design, treatments and crop management

The soil of experimental field was loamy sand in texture with pH of 7.8. The soil was low in both available N (189.4 kg ha^{-1}) and Walkley and Black organic carbon (0.16–0.30%) in 0–15 cm soil layer. The 0.5 mol/L $NaHCO_3$-extratable Olsen-P was medium (9.7 mg/kg) and the soil was sufficient (81–83 mg kg^{-1}) in 1 mol/L NH4OAC-extractable K. The nine treatments of osmoprotectants included thiourea @ 20, 40 and 60 mM, potassium nitrate @ 1.0, 2.0 and 3.0% and sodium nitroprusside @ 400, 800 and 1200 µg/ml. Two other treatments of water spray and untreated control was also included in the study. The experiment was laid out in a randomized complete block design with three replications. Two foliar sprays of the osmoprotectans were applied at weekly interval starting from anthesis of the crop. The first foliar spray was applied on 18th February in 2013-14 and on 21st February during crop season of 2014-15.

The crop was sown on 8th November in randomized block design using 100 kg seed ha^{-1} at 22.0 cm row spacing during both the years. The seed was treated with thiram @ 1.0g/kg seed against the seed borne diseases. Irrigations (75 mm depth each) were applied at four critical stages; crown root initiation, late tillering, boot and milk stages. Uniform application of 120 kg N as urea and 25 kg K/ha as muriate of potash was made on all the plots. Nitrogen was applied in two equal splits; at sowing and crown root initiation stage. The whole of P (as per treatment) and K fertilizers were applied at the time of sowing by broadcasting followed by soil incorporation.

Recorded traits

Physiological parameters

All physiological parameters were measured three days after both sprays and the data after last spray is discussed here. Relative leaf chlorophyll was recorded in the flag leaves by using a self calibrating SPAD chlorophyll meter (Minolta). The youngest fully expanded leaves of 5 plants were selected for SPAD measurement. Triplicate readings were taken on one side of the midrib of each single leaf blade, midway between the leaf base and tip of the leaf and then averaged.

Photosynthesis and gas exchanges activities of wheat were measured by using a portable photosynthesis system (Model LI-6400, LICOR, inc. Lincoln, Nebraska USA) during 1000-1300 hours under clear sunshine conditions The fully expanded leaves on two-third above part of plants were selected randomly in each experiment. The portable measuring system can simultaneously determine the net rate of photosynthesis (Pn), stomatal conductance (Gs) and transpiration rate (E).

Canopy temperature

Canopy temperature (°C) of the crop was determined with infrared thermometer (Model TECPEL 513, TAIWAN). The data were taken from the same side of each plot at 1m distance from the edge and approximately 50 cm above the canopy at an angle of 30° to the horizontal. Readings were made between 1300 and 1500 h on sunny days.

Relative water content

Fresh weight of the leaf samples was taken and then kept in distilled water for 4 hours to obtain turgid weight. The turgid weight was recorded after blotting the excess water on the surfaces of the samples. Dry weight was obtained after drying the samples in oven at 60 °C till constant weight obtained.The relative water content was then calculated as

RWC (%) = (fresh weight – dry weight) × 100 / (turgid weight – dry weight)

Proline content

Free proline was determined from the frozen fresh leaves according to the method of (Bates *et al*., 1973). A 0.5 g of fresh leaf tissue was homogenized with 10 mL of 3% aqueous sulphosalicylic acid and filtered homogenate through Whatman No. 2 filter paper. Two mL each of acid ninhydrin and glacial acetic acid were added to 2 mL of filtrate. The mixture was vortexed, heated at 100^{o}C in a water bath for 1 h and reaction terminated in an ice bath. The reaction mixture was extracted with 4 mL of toluene by vigorously vortexing for 15-20 sec. The chromophore containing free proline was aspirated and added to a test tube, warmed to room temperature and measured the absorbance at 520 nm. Same procedure was followed for blanks using 2 mL of 3% aqueous sulphosalicylic acid. A standard curve was constructed by running the proline standards (10 to 50 µg 2 mL^{-1}) along the unknown samples. The amount of free proline in the leaves was calculated using the formula:

µmoles free proline/g fresh weight =

$$\frac{(\mu g \text{ proline mL-1} \times \text{mL toluene})/115.5\ \mu g/\mu mole}{(g \text{ sample}/5)}$$

Statistical analyses

Data obtained from the experiment conducted under completely randomized block design were statistically analyzed by analysis of variance (ANOVA) as per standard procedure (Panse and Sukhatme, 1985). Wherever F values were found to be significant at a 5 per cent level of probability, least significant difference (LSD) values were computed for making comparisons among the treatment means.

Results and Discussion

Relative leaf chlorophyll

Leaf chlorophyll concentration is an important parameter that is measured as an indicator of photosynthetic potential. During 2013-14, the application of SNP 400 µg/ml, closely followed by 2% KNO_3 and thiourea 20 mM had resulted in significantly higher chlorophyll content, to the extent of 38.8, 32.2 and 27.3% as compared to untreated control, respectively (Figure1). However, a perusal of results of 2014-15, revealed the existence of significant differences between unsprayed and water sprayed control with SNP 800 µg/ml, 3% KNO_3 and thiourea 40 mM respectively and the increase over untreated control was to the extent of 35.8, 30.2 and 29.35%, respectively (Figure 1).

Gas exchange parameters

Stomatal conductance measures the rate of passage of CO_2 entering and the water vapour exiting. During 2013-14, the highest stomatal conductance was recorded with SNP at 400 µg/ml which was statistically at par with 2% KNO_3 and thiourea @ 20 mM (Figure 2). The increase over untreated conrol was to the extent of 66.6, 61.9 and 52.4 %, respectively. During 2014-15, however, due to above normal rainfall, foliar applications of SNP 800 µg/ml, 3% KNO_3 and thiourea 40 mM performed best.

Foliar spray of KNO_3, SNP and thiourea salts at suitable concentrations exerted conspicuous effects on photosynthesis of wheat (Figure 3). During 2013-14, both SNP 400 µg/ml and KNO_3 at 2% when sprayed recorded significantly higher photosynthetic rate than water sprayed and unsprayed controls. The increase over untreated control was to the extent of 44.3 and 43.9 %, respectively. On the other hand, both thiourea 60 mM and SNP 1200 µg/ml acted as growth retardants, decreasing net photosynthesis. During 2014-15, SNP 800 µg/ml, 3% KNO_3 and thiourea 40 mM performed significantly better than untreated control and the increase was to the tune of 55.1, 48.3 and 43.0 %, respectively.

Relative water content

Relative water content was also substantially increased with foliar application of osmoprotectants under investigation (Figure 4). These findings suggested that all these chemicals have a distinct property of improving leaf water status, particularly to turgor maintenance.

During 2013-14, highest relative leaf water content was recorded with SNP at 400 µg/ml, closely followed by KNO_3 at 2% and thiourea 20 mM, significantly better than unsprayed control and water sprayed treatments. The increase over untreated control was to the extent of 32.6, 31.4 and 24.5 %, respectively. However during 2014-15, SNP 800 µg/ml, 3% KNO3 and thiourea 40 mM performed better.

Canopy temperature

Although the difference was statistically non significant, foliar application of all the osmoprotectants reduced canopy temperature over the unsprayed control and water sprayed treatments (Figure 5). During 2013-14, lowest canopy temperature was recorded with SNP 400 µg/ml which was slightly less than 2% KNO_3 and thiourea 20 mM. A decrease of 12.8, 10.85 and 7.0 % has been noticed over untreated control, respectively In 2014-15, however, due to above normal rainfall, moderate concentrations of thiourea @40 mM and SNP @800 µg/ml, along with 3% KNO_3 performed better. In general, canopy temperature was lower during 2014-15 as compared to 2013-14 as there was more cooling of crop canopy by falling raindrops during the entire reproductive phase.

Proline content

Proline, an amino acid, generally accumulates in plants under stress condition and was found to be affected significantly due to application of different osmoprotectants in wheat. During 2013-14, the proline content was significantly higher with SNP 400 µg/ml and 2% KNO3 application as compared to untreated control (Figure 6).

The next best result was obtained with application of thiourea 20 mM. During 2014-15, however, SNP 800 µg/ml, closely followed by 3% KNO_3 recorded highest proline content. Furthermore, in this experiment, the treatment mean for proline content during 2013-14 was found to be higher than 2014-15.

Grain yield

Foliar spray of KNO_3, SNP and thiourea salts exerted conspicuous effects on grain yield of wheat (Table 1). During 2013-14, foliar spray of SNP at 400 µg/ml recorded significantly higher grain yield (5.49 t ha^{-1}) than water sprayed (4.73 t ha^{-1}) unsprayed control (4.62 t ha^{-1}) and the increase was 18.8 percent higher than untreated control. During same year, 2% KNO_3 recorded significantly and appreciably higher grain yield (5.45 t ha^{-1}) than water spray (4.73 t ha^{-1}) and unsprayed controls (4.62 t ha^{-1}) and the increase was 17.9 percent over untreated control. During 2013-14, thiourea 20 mM (lower dose) and during 2014-15, due to moderating the effect of chemical by excessive rainfall, thiourea 40 mM (moderate dose), was the best treatment for increasing grain yield. During 2014-15 foliar spray of SNP 800 µg/ml (5.34 t ha^{-1}) recorded the highest grain yield. However, SNP 1200 and 60 mM thiourea were found toxic to the plant and acted as growth retardant, decreasing the yield.

Results and Discussion

Higher relative chlorophyll content was observed for all the three chemicals at suitable concentration during both the years. Nitrogen has a direct role in synthesis of chlorophyll and uptake of other nutrients and therefore, all these osmoprotectants having N as their chemical constituent which resulted in better chlorophyll concentration and more SPAD values. Only about half of foliar applied chemicals penetrate the leaf in first 1-2 days due to waxy coating on leaf surface and rest is absorbed at a slower rate over a few days. But, intermittent rainfall occurred during reproductive period of crop lead to washing out of these chemicals counteracting their effect. Therefore, during 2014-15, higher concentrations of these chemicals were more effective.

In present investigation, results demonstrated that exogenous SNP improved the overall physiological processes of plant, which played a role in delaying leaf senescence. SNP might have functioned as a signalling molecule, which further activated the cellular antioxidant system. However, the effects of SNP depends on its concentration. Lower concentrations of SNP can triggerd beneficial reactions that counteracted oxidative and nitrosative damage to cell wall. Application of higher SNP concentration (1200 µg/ml) did not increased ROS damage, but even produced more cellular toxicity K+ is an essential element that plays vital roles in various aspects of plant cell growth and metabolism. So, it might have helped wheat plants in KNO_3 helps in recovering the impairment in photosynthetic CO_2 fixation occurs, activation of molecular O_2, and, thereby, prevention of oxidative degradation of chlorophyll and membranes Likewise, increased chlorophyll content due to thiourea application has been reported in several crops including clusterbean (Garg *et al*., 2006). Thiourea also maintained higher chlorophyll content as it exhibits cytokinin like activity. It is structurally similar to urea, except that the oxygen atom in urea is replaced by a sulphur atom in thiourea. The combined effect of N and S might have resulted in better chlorophyll content due to thiourea spray.

Stomatal conductance is directly related to the absolute concentration gradient of water vapor from the leaf to the atmosphere. It was higher under foliar spray of osmoprotectants because the good relative water content of leaves allowed the delay in leaf senescence and exerting a relevant aerodynamic resistance on water vapor fluxes. Similarly, the atmospheric humidity was highest through higher transpiration from spray of osmoprotectants. Another reason which is proposed is that when radiation is not limiting, nutrition and water are the major factors that influence stomatal conductance, unless the leaves are not senescing and nutrients like N, S and K were supplied to plants by these chemicals. Stomatal conductance is integral to leaf level calculations of transpiration. Hence, the rate of transpiration and stomatal conductance were higher under foliar spray of osmoprotectants in this study. Burman *et al* (2004) obtained similar results with thiourea application in clusterbean. Garcia matta and La Mattina (2001) also reported similar results after SNP application in wheat.

Net photosynthesis was significantly influenced by various osmoprotectants. Spray of KNO_3 supplied N and K which were effectively absorbed as anion and cation by plants, and might have delayed the synthesis of abscisic acid and promoted cytokinin activity, causing higher chlorophyll retention. This helped in securing higher photosynthetic activity in effective leaves. Increase in endogenous NO via application of SNP also improved the photosynthetic performance in plants, the improvement was associated with an increase in photosynthetic pigments. A higher internal CO_2 concentration to ambient CO_2 results in increased photosynthetic rate due to higher CO_2 assimilation at the tissue level. Higher stomatal conductance also led to increased rate of CO_2 diffusion into the leaf for photosynthesis and enhanced transpiration. Photosynthesis increased due to increase in all gas exchange parameters.

Kaya *et al*., (2015) repotred that foliar-applied thiourea was found to be effective in enhancing the leaf chlorophyll contents of maize plants that were positively associated with higher photosynthetic rate and hence higher biomass production.

Significant differences in relative water content of leaf were observed in different foliar spray treatments and untreated control. These osmoprotectants were able to maintain leaf water status. This result is similar to those reported by Ribeiro *et al*., (2009). Foliar application of KNO_3 plays a crucial role in plant water relations by regulating the osmotic potential and hydraulic conductivity of membranes and make changes in plant water permeability. As potassium is the main osmotic solute in plants, its accumulation in the cell has lead to osmotic water uptake and generated the cell turgor required for growth and for stomatal opening. Water channels and K^+ channel/transporters are functionally co-regulated as a part of plant osmoregulation to maintain appropriate cytosolic osmolality. Guo *et al* (2007) also showed a positive correlation between K absorption and water uptake and hence increased relative water content in Phaseolus vulgaris plants. Kaya *et al* (2015) also reported that foliar application of thiourea significantly enhanced leaf RWC.

There was non-significant reduction in canopy temperature of plants treated with osmoprotectants. The proposed reason is that canopy temperature is inversely related to rate of transpiration. As these chemical sprays increased transpiration, therefore canopy temperature was reduced in this study. Higher values of canopy temperature in control treatments might have occurred due to increased respiration and decreased transpiration resulting from stomatal closure. Relatively lower canopy temperature in plants treated with different osmoprotectants indicates a relatively better capacity for taking up soil moisture and for maintaining a relatively better plant water status by them.

Table.1 Effect of foliar spray of osmoprotectants on grain yield of wheat under terminal heat stress

Treatment	Grain yield (t ha^{-1})	
	2013-14	**2014-15**
No Spray	4.62	4.50
Water Spray	4.73	4.57
Thiourea 20 mM	5.31	5.01
Thiourea 40 mM	4.93	5.18
Thiourea 60 mM	4.51	4.61
KNO_3 1%	5.10	4.82
KNO_3 2%	5.45	5.21
KNO_3 3%	4.81	5.29
SNP 400 µg/ml	5.49	5.19
SNP 800 µg/ml	5.21	5.34
SNP 1200 µg/ml	4.48	4.45
LSD (p=0.05)	0.52	NS
SEm±	0.24	0.28

Fig.1 Effect of foliar spray of osmoprotectants on relative chlorophyll content of wheat

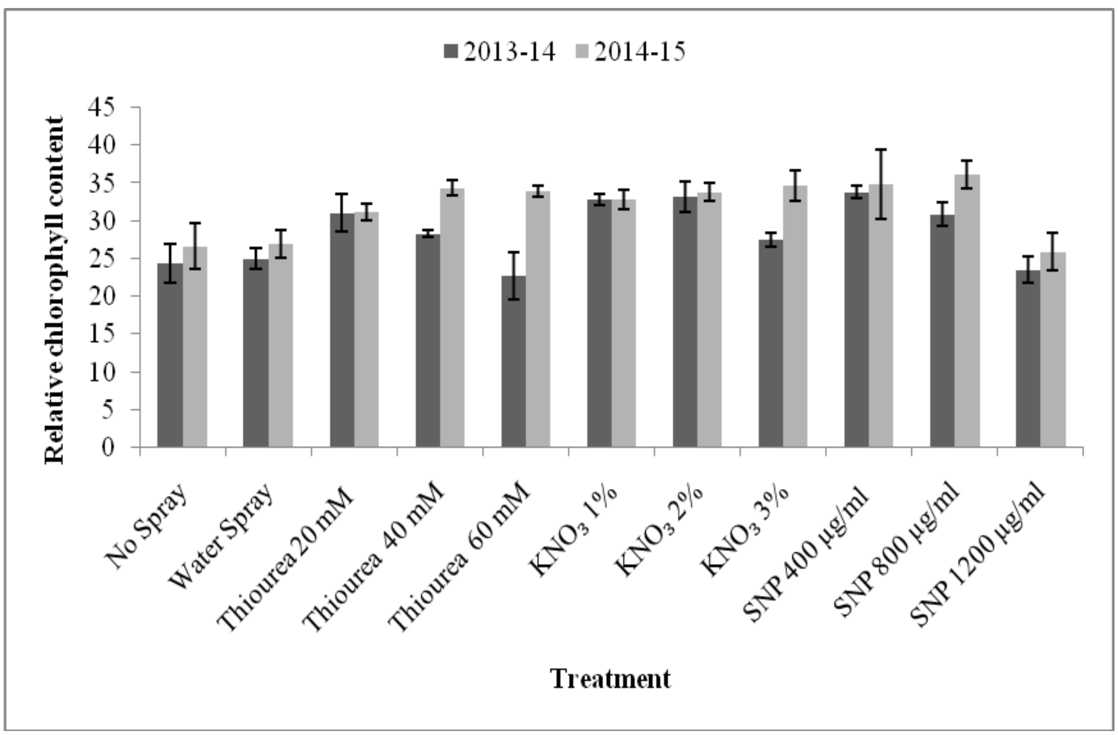


Fig.2. Effect of foliar spray of osmoprotectants on stomatal conductance of wheat

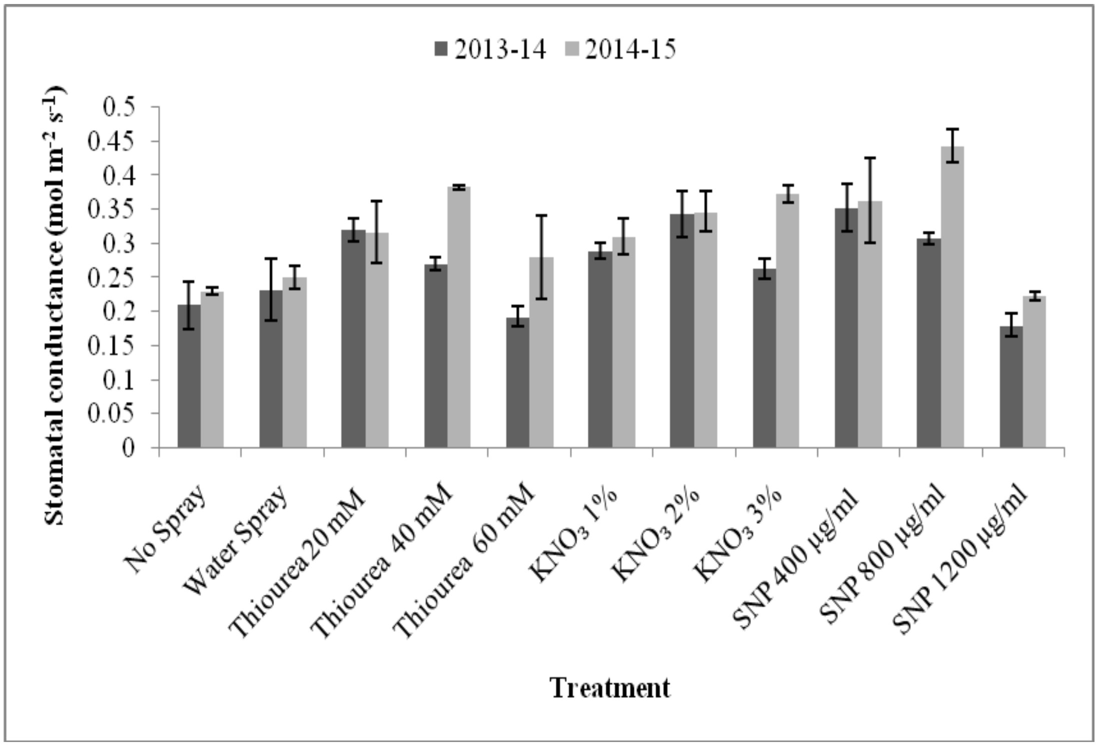


Fig.3. Effect of foliar spray of osmoprotectants on net photosynthesis of wheat

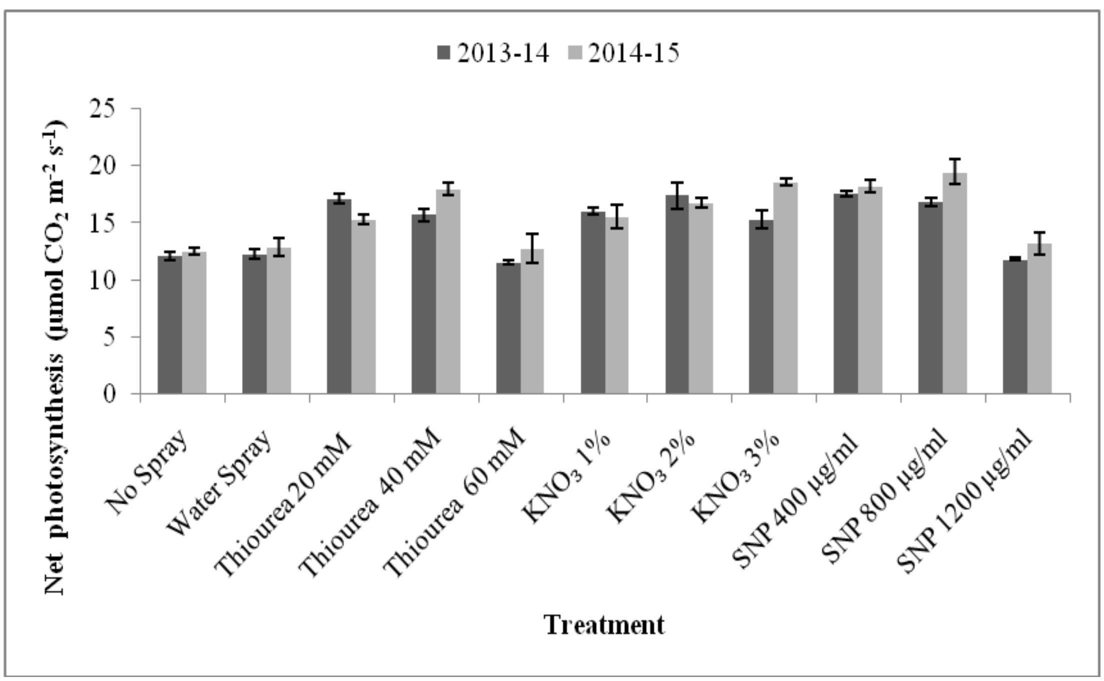


Fig.4. Effect of foliar spray of osmoprotectants on relative water content of wheat

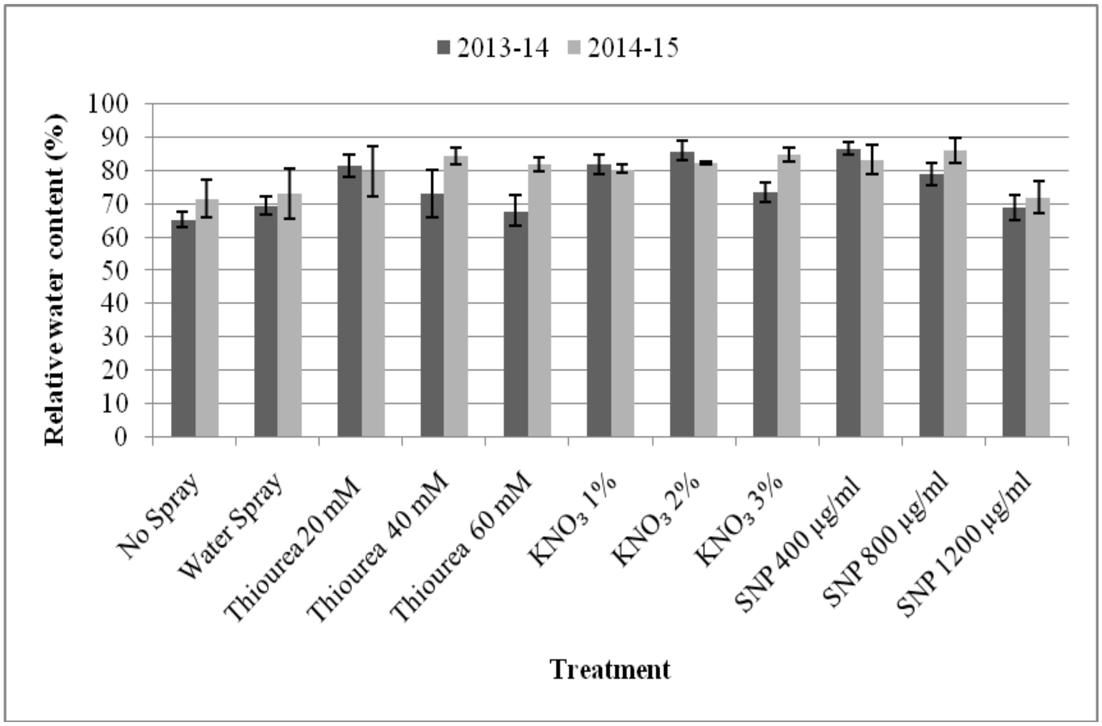


Fig.5. Effect of foliar spray of osmoprotectants on canopy temperature of wheat

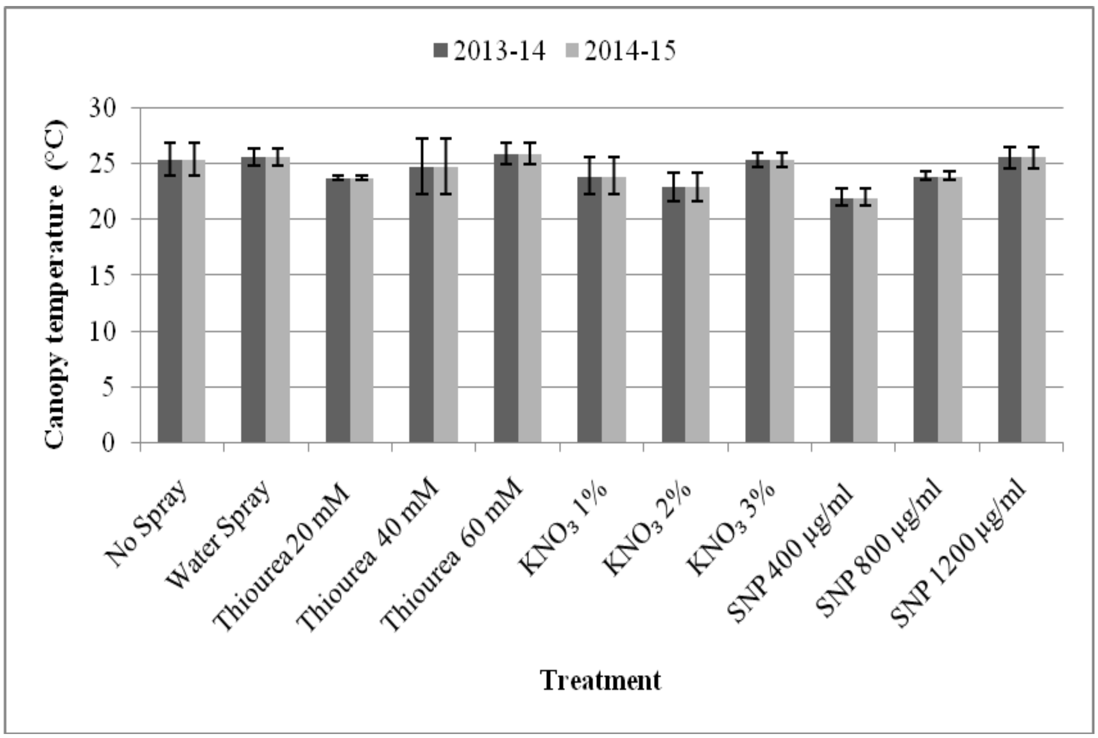
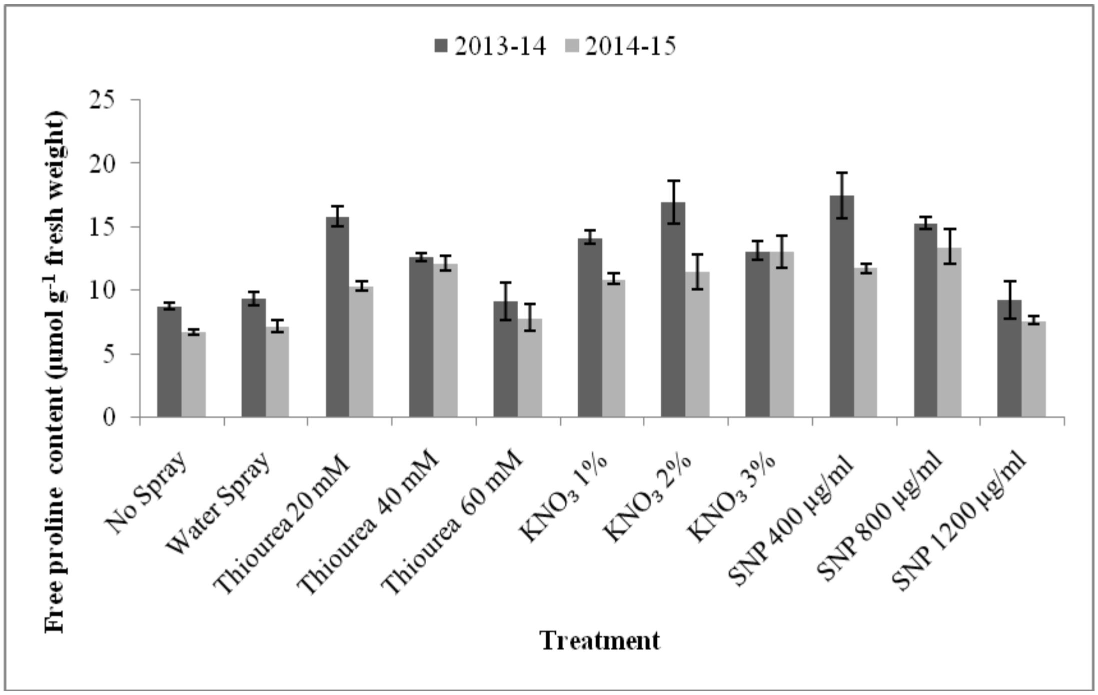


Fig.6. Effect of foliar spray of osmoprotectants on free proline content of wheat



Significant differences in leaf proline concentration was observed in plants treated with different osmoprotectants. As these chemicals led to enhancement of leaf water status resulting in accumulation of free proline. Foliar spray of KNO_3 might have induced proline synthesis and this accumulation of proline might have itself served as a compatible solute. It itself acted as a secondary osmoregulator inside plant. Similar results of increase in proline content due to foliar spray of KNO3 was also reported by Rao *et al* (2015) in mungbean. This result is also in confirmation with Anjum (2008), who reported an increase in free proline content with application of various bioregulators. Furthermore, in this experiment, the treatment mean for proline content during 2013-14 was found to be higher than 2014-15. This seasonal variation in proline content might occurred due to differences in soil moisture status during both the seasons. Less rainfall was recorded during 2013-14 as compared to 2014-15 as evident from the meteorological data recorded during the course of experiment. This might have subjected the crop to slight water stress resulting in higher accumulation of free proline in the leaves in 2013-14.

Grain yield increased with optimum concentrations of all three osmoprotectants which accelerated chlorophyll synthesis, enhanced photosynthesis rate and maintained higher relative water content. Increase in grain yield might be due to the collective role of N and K as N improves vegetative growth of wheat and K increases root growth and facilitates the translocation of nutrients and photo assimilates. Similar results has been reported by Yadav *et al*., (2005). The reason for increase in grain yield with thiourea spray might be that it can provide both sulfur and nitrogen to plants.

It may be concluded from this study that wheat crop needs to be fertilized with two foliar sprays of SNP 400 µg/ml and KNO_3

2% after anthesis to obtain maximum yield advantage. Foliar spray of thiourea @ 20 mM proved to be the next best feasible osmoprotectant. All the three chemicals, when sprayed at optimum concentrations positively influenced various physiological parameters i.e., chlorophyll content, gas exchange parameters, photosynthesis and proline content, besides reducing canopy temperature of wheat.

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