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Original Research Article

Alleviating the Adverse Effects of Water Stress in Wheat by Foliar Sprays of Bio-Regulators

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A B S T R A C T

Reduced supply of water is known to hamper important physiological and biochemical mechanisms leading to reduction in plant growth. Therefore, a field experiment was conducted for two years to evaluate the impact of water stress and bio- regulators on biochemical and physiological changes in wheat. Bio-regulators applied through exogenous sprays included: thiourea (1000 ppm), salicylic acid (200 ppm) and putrescine (10 ppm) at 40 DAS and 70 DAS. For water stress, irrigation was skipped at tillering and tillering + 50 % heading stage with no water stress under normal (20 November) and late sowing (20 December) conditions. Maximum reduction was observed under water stress at tillering + 50% heading stage than tillering alone under both normal and late sown conditions. Maximum / enhanced proline content (2.512 µg g^{-1} fresh weight), total chlorophyll content at 75 DAS (1.916 mg g^{-1} fresh weight), RWC at 75 DAS (81.1 %) and WUE (16.1 kg ha^{-1} mm^{-1}) was observed with the foliar application of thiourea (1000 ppm) with water stress under normal and late sown conditions.

Keywords

Putrescine, Thiourea (TU), Relative Water Content (RWC), WUE, Salicylic acid (SA), Total Chlorophyll Content, Water Stress, Yield, Bio-Regulators

Introduction

Wheat is one of the most important cereal crop of the world on account of its wide adaptability to different agro- climatic and soil conditions. Wheat contributes about 29 % of global food grain production. Since, higher temperature at critical plant development stages limits productivity at later sowings (Mahajan and Nayeem, 1990). Variation in the seedling date and temperature interact to influence growth, development and yield of wheat. Water, one of the major determinates of wheat growth governs realization of full potential of high yielding wheat cultivars. Soil moisture in the root zone at planting are insufficient to satisfy the crop water requirements for the whole season and the unfavorable distribution of rainfall and the year-to-year fluctuations generally lead to midseason droughts. These conditions lead to reduced tillering and number of grains per spike, while the grain weight may further suffer due to terminal heat stress caused by rising temperature at anthesis and onwards maturation of crop. Substantial yield losses have been observed in different crops due to reduced supply of water even for a short period of time (Pinheiro *et al.*, 2005). Reduced supply of water is known to hamper important physiological and biochemical mechanisms leading to reduction in plant growth.

Plant productivity is severely affected by abiotic stress *viz*., salinity, drought, high and low temperature. Out of the various abiotic stresses, high temperature is the second most important stress. Numerous plant bio-regulators and other plant hormones have been recently tried to impart stress tolerance to crop under water deficit (Srivastava *et al.,* 2016).

Salicylic acid plays diverse physiological roles in plants which includes thermogenesis, flower induction, nutrient uptake, ethylene biosynthesis, stomatal movement, photosynthesis and anti-oxidative enzymes (Hayat *et al.,* 2007). Thiourea, a sulphdryl compound has diverse biological activities (Jocelyn, 1972). Sahu and Singh (1995) reported that thiourea had a significant role in improving dry matter portioning towards sink in wheat and enhanced metabolic transport of sucrose to the grain via effect on phloem loading. Putrescine is a polyamines and tetramethylenediame (1,4- diaminobutane).

Increased putrescine level in stressed plants of adaptive significance because of their involvement in regulation of cellular ionic environment, maintenance of membrane integrity, prevention of chlorophyll loss, and stimulation of protein, nucleic acid and protective alkaloids (Sharma, 1999). Putrescine was found to enhance productivity in wheat under water stress conditions in pots (Gupta *et al.*, 2003). All these bio-regulators have been tested under varied water stress conditions.

Materials and Methods

Experimental Details

A field experiment was conducted for two years (2013-15) at the Research Farm of MPUAT, Udaipur of Rajasthan, India (24°35' N latitude, 73°42' E longitude, MSL 582.17m). The region falls under NARP agro-climatic zone IV a (Sub Humid Southern Plains and Arawali Hills) of Rajasthan. The region is typical sub-tropical with an annual rainfall of 600.8 mm, which is contributed by South West monsoon from june to September. The average monthly maximum and minimum temperature ranged between 21.4 to 36.6 °C and 4.8 to 21.7°C, respectively. The clay loam soil (sand, silt and clay, 38.1. 27.0, 35.1 % respectively) had pH 8.0, EC 1.05 dSm^{-1}; organic carbon 0.8 %, available N, P and K 295.3, 18.4 and 363.8 kg ha^{-1}, respectively.

Treatment combinations, in split plots design with four replications, consisted of; (i) six water stress, namely; normal sowing with no water stress, stress at tillering and tillering + 50 % heading and late sowing with no water stress, stress at tillering and tillering + 50 % heading and (ii) four levels of bio-regulators i.e thiourea (1000 ppm), SA (200 ppm) and putrescine (10 ppm). Different concentration of thiourea, SA and putrescine were prepared by dissolving 1000 mg, 200 mg and 10 mg in one litre of water to prepare 1000 ppm, 200 ppm and 1 ppm solution, respectively.

Two foliar sprays of each bio-regulator were made at 40 DAS and 70 DAS. The control plants were sprayed with water. For crop cultivation, the experimental field was initially ploughed. Wheat (Raj 4037) was drilled @ 125 kg ha^{-1} in rows 22.5 cm. the sowing date was 20th November and 20th December during 2013 and 2015.

A common irrigation was applied following sowing during each year to facilitate germination. Basal dose of half 60 kg N and 40 kg P_2O_5 per hectare was drilled at sowing while half 60 kg N was applied at first irrigation.

Biochemical and Physiological Parameters

Proline Estimation

Free proline in leaves (µg g^{-1} fr. wt.) was extracted at 75 DAS and determined by the method of Bates *et al.*, (1973). Its amount was calculated on fresh weight basis using the following formula:-

µmoles of proline g-1 fresh leaf tissue = [(µg proline / ml x ml toluene) / 115.5 µg / µ mole] / g sample / 5

Total Chlorophyll Content

Total chlorophyll content of leaves at 50 and 75 DAS were analysed by collecting fresh leaf samples from the crop. The total chlorophyll content was computed by following formula

Total chlorophyll (mg/g fresh weight of leaf) = (20.2(A 645) + 8.02 (A 663) / a x 1000 x W) x V

Where,

a = Length of light path in cell (1 cm)
V = Volume of extract and
W = Weight of leaf sample

Relative Water Content of Leaf

Fresh weight of the samples was taken at 50 and 70 DAS and then kept in distilled water for 4 hours (Barrs and Weatherly, 1992) to obtain turgid weight.

The relative water content (RWC) was then calculated by the formula:

$$\text{RWC (\%)} = \frac{\text{Fresh weight} - \text{oven dry weight of leaf}}{\text{Turgid weight} - \text{oven dry weight of leaf}} \times 100$$

Consumptive Use of Water

It was calculated based on the direct soil moisture determinations. The consumptive use of the crop was calculated as detailed by Dastane (1972).

$$Cu = \sum \mu$$

$$\mu = (E_0 \times 0.8) + \sum_{i=1}^{n} \frac{m1_i - m2_i}{100} (Ai \times Di) \times ER + GWC$$

Cu = Seasonal consumptive use of water in mm.

µ = Consumptive use during a given irrigation interval

E_0 = Evaporation (mm) from USWB class I open pan evaporimeter during interval from the day of irrigation to the day when sampling in wet soil was possible.

0.8 = A constant to be used with the USWB class I open pan evaporimeter.

n = Number of soil layers sampled in the root zone depth D.

$m^1{}_i$ = Soil moisture per cent in the i^{th} layer on the day when sampling in irrigated soil is possible.

$m^2{}_i$ = Soil moisture per cent in the i^{th} layer on the day just before the next irrigation.

Ai = Apparent specific gravity of i^{th} layer.

Di = Soil depth of the i^{th} layer in mm.

ER = Effective rainfall during the interval (mm).

GWC = Ground water contribution (the ground water contribution was considered to be zero as water table was below 20 m depth).

Water Use Efficiency

The ratio of crop yield (Y) to the amount of water depleted by the crop in the process of evapotranspiration (ET) was computed according to the following formula as suggested by Viets (1961).

$$\text{WUE (kg ha}^{-1}\text{ mm}^{-1}) = \frac{\text{Grain yield (kg ha}^{-1})}{\text{Seasonal consumptive use of water (mm)}}$$

Statistical Analysis

All the data were subjected to statistical analysis by adapting appropriate method of analysis for testing the significance of variation in experimental result. Wherever the F value was found significant at 5 % level of significance, the critical difference (CD) value was computed for making comparison among the treatment means. MS excel package was used to develop plots and correlation equations including analysis of weather and pooled data of crop yield attributes.

Results and Discussion

Grain Yield and Total Biomass/ Biological Yield

On pooled basis, grain yield was significantly reduced under water stress at tillering + 50 % heading (S_3) by 13.5 and 15.3 per cent over water stress at tillering (S_2) and no water stress (S_1), respectively under normal sowing. Under late sowing, water stress at tillering (S_5) and tillering + 50 % heading (S_6) resulted significantly lower grain yield by 7.8 and 15.4 per cent, respectively over no water stress (4547 kg ha^{-1}). Normal sown crop with no water stress (S_1) recorded significantly higher grain yield of 5691 kg ha^{-1} over late sown crop with no water stress (S_4) by 25.1 per cent.

Foliar spray of SA (200 ppm) and TU (1000 ppm) gave significantly higher grain yield by 4.3 and 9.2 percent, respectively over water spray (4603 kg ha^{-1}). Further, foliar spray of TU was significantly superior over foliar spray of SA by 4.7 per cent in this respect. Foliar spray of putrescine (10 ppm) did not influence grain yield.

Biochemical Studies

Maximum proline content was observed under late sown crop with water stress at tillering + 50 % heading stage (S_6) which was significantly higher over rest of the treatments. On pooled data basis, water stress at tillering + 50 % heading (S_3) registered significantly higher proline content by 19.8 and 55.3 per cent over water stress at tillering (S_2) and no water stress (S_1), respectively under normal sowing.

Under late sowing, water stress at tillering + 50 % heading (S_6) gave significantly higher proline content by 10.1 and 32.0 per cent over water stress at tillering (S_5) and no water stress (S_4), respectively. Water stress at tillering + 50 % heading (S_3) gave significantly lower chlorophyll content and relative water content at 75 DAS by 8.6, 12.3 per cent and 4.3, 7.5 per cent over water stress at tillering (S_2) and no water stress (S_1), respectively under normal sowing.

Foliar spray of SA (200 ppm) and TU (1000 ppm) recorded significantly higher proline content by 4.1 and 18.8 per cent, respectively over water spray (2.114 µg g^{-1} fr. wt.). Foliar spray of putrescine (10 ppm) did not influence proline content. Similarly, higher chlorophyll content and relative water content recorded with foliar spray of SA (200 ppm) and TU (1000 ppm) significantly at 75 DAS over water spray.

Consumptive Use of Water and Water Use Efficiency

Water stress at tillering (S_2) and tillering + 50 % heading (S_3) recorded significantly lower consumptive use of water by 52.3 and 100.5 mm, respectively over no water stress (S_1) under normal sowing (384.9 mm). Under late sowing, water stress at tillering (S_5) and tillering + 50 % heading (S_6) recorded significantly lower consumptive use of water by 61.3 and 91.9 mm, respectively over no water stress (S_4). Under normal sowing, water stress at tillering (S_2) and tillering + 50 % heading (S_3) recorded significantly higher water use efficiency by 12.7 and 14.0 per cent over no water stress (S_1). Under late sowing, water stress at tillering (S_5) and tillering + 50 % heading (S_6) recorded significantly higher water use efficiency by 12.7 and 15.7 per cent over no water stress (S_4).

Foliar sprays of bio-regulators did not influence consumptive use of water. Significantly higher water use efficiency was observed with the foliar spray of SA (200 ppm) and TU (1000 ppm by 1.2 and 6.6 per cent over water spray.

Grain Yield and Total Biomass/ Biological Yield

The grain yield and total biomass/biological yield of wheat responded significantly to foliar spray of thiourea and salicylic acid (Table). On the whole, the grain yield and biological yield were comparatively higher during the year 2014-15 compared to 13-14. The varied response in grain yield and biological yield was obviously due to cooler conditions during the wheat growth. Normal sown crop with no water stress (S_1) recorded significantly higher grain yield of 5691 kg ha-1 over late sown wheat with no water stress (S_4) by 25.1 per cent. Since the wheat was sown late (20 December) during both year, its grain development stage coincided with hot and drier environment (terminal heat) as further indicated temperatures during February and March and also by lower relative humidity.

The wheat experienced comparatively cooler and moist weather in vegetative (CRI and leaf stage) and warmer and dry weather in seed milking and grain development stages that was congenial for its better growth and productivity. Several studies have shown that higher temperature will negatively impact on grain filling at maturity. It was found that water stress at tillering + 50 % heading brought about a reduction of 15.3 per cent and that at tillering stage caused a reduction of 2.2 per cent in comparison to no water stress treatment under normal sowing (5691 kg ha^{-1}). Similar trends were observed under late sowing. However, the extent of reduction in grain yield was more due to water stress at tillering under late sowing in comparison to that under normal sowing. Thus, water stress at tillering + 50 % heading proved more injurious than water stress at tillering alone. Similar responses to water stress/ deficit have been reported by Maliwal *et al.*, (2000), Gupta *et al.*, (2003) and Meena (2015).

Foliar sprays of thiourea (1000 ppm) and salicylic acid (200 ppm) helps in improving grain yield of wheat. The bio-regulators those were used in this experiment had ability to fine tune the plant redox homeostasis which regulate root growth for improving plant water/ nutrient status, photosynthetic efficiency and source-sink homeostasis to enhanced crop yield (Ratnakumar *et al.,* 2016).

Table.1 Effect of water stress and foliar spray of bio-regulators on yield, biochemical parameters, CU and WUE of wheat (on pooled basis)

	Pooled (2013-15)						
	Grain yield	**Biological yield**	**Proline**	**Total Chl**	**RWC**	**CU**	**WUE**
Date of sowing and water stress							
Normal - no stress (S_1)	5691	14506	1.606	2.057	83.4	384.9	14.9
Normal - at tillering (S_2)	5566	14288	2.081	1.973	79.8	332.6	16.8
Normal -at tillering +50% heading (S_3)	4817	12825	2.495	1.803	77.1	284.4	17.0
Late - no stress (S_4)	4547	12017	2.060	1.954	79.0	339.5	13.4
Late - at tillering (S_5)	4193	11007	2.470	1.785	76.4	278.2	15.1
Late - at tillering +50 % heading (S_6)	3849	9914	2.721	1.613	71.3	247.6	15.5
SEm ±	63	123	0.023	0.008	0.5	4.4	0.2
CD (P=0.05)	182	356	0.067	0.023	1.5	12.8	0.4
Foliar spray of bio-regulators							
Water spray (F_0)	4603	11932	2.114	1.825	75.2	308.1	15.1
Salicylic acid 200 ppm (F_1)	4803	12588	2.201	1.876	79.0	313.2	15.4
Putrescine 10 ppm (F_2)	4675	12163	2.128	1.839	76.1	308.5	15.2
Thiourea 1000 ppm (F_3)	5028	13022	2.512	1.916	81.1	315.1	16.1
SEm ±	29	67	0.013	0.005	0.3	2.8	0.1
CD (P=0.05)	82	188	0.037	0.015	0.9	NS	0.2

The pooled data for two years indicate that the thiourea was the most effective in enhancing grain yield (9.2 per cent) followed by salicylic acid (4.3 per cent) over water spray. Thiourea has been widely used for enhancing plant growth, stress tolerance and crop yield (Pandey *et al.,* 2013). At the physiological level, these effects are due to co-ordinated regulation of plant source-to-sink relationship (Pandey *et al.,* 2013) and enhanced translocation of metabolites from source (leaves) to sink (pods). Salicylic acid in plant growth regulator that increases plant bio-productivity (Saavedra and Mex, 2007). Increase in grain yield with foliar spray of salicylic acid could be ascribed to the fact that crop yield is not an abstract entity but it is outcome of positive interaction between vegetative and reproductive growth of the crop.

Biochemical Studies

Proline is thought to play adaptive roles in mediating osmotic adjustment and protecting sub cellular structure in stressed plants (Ashraf and Foolad, 2007). Furthermore, Kavi Kishor *et al.,* (2005) suggested that proline which is accumulated under stress conditions might serve as a sink for excess reductants providing the NAD+ and NADP+ necessary for maintenance of respiratory and photothetic processes. Johri *et al.,* (2010) and Meena (2015) also reported higher proline content in wheat under water stress conditions. The result showed that total chlorophyll content and RWC (Table 1) reduced under water stress at tillering + 50 % heading stage over no water stress condition. Reduction occurred due to the variation to absorb water from the soil. Under the low moisture content in soil less water is available to plant to absorb which decrease the turgidity of the plant cell and RWC reduced. The RWC and intensity of the transpiration of leaves decreased. These results are in accordance with Sharifa and Muriefah (2013) and Meena (2015).

Application of SA and TU brought about significant improvement in biochemical studies *viz*., proline content, chlorophyll content and RWC over water spray. These improvements ultimately resulted in significantly higher grain yield under foliar spray of SA and TU as compared to water spray. However, during both the years, putrescine remained at par with water spray. Foliar application of thiourea results in accumulation of sugars which not only acts as a source of energy, but also increases fresh weight and provides the carbon skeleton to synthesize specific osmolytes such as proline and betaine that are used for adaptive and/or defensive responses against stresses, including salinity (Prado *et al.*, 2000). A number of studies depict that exogenous application of organic compounds have been found to be beneficial in ameliorating the adverse effects of stress on leaf photosynthetic pigments coupled with enhanced biomass production (Ali and Ashraf, 2011). Similarly, in the present study foliar-applied thiourea were found to be effective in enhancing the leaf chlorophyll contents of wheat plants that were positively associated with higher photosynthetic rate and hence higher biomass production. Increased chlorophyll content due to thiourea application has been reported in several crops including clusterbean by Solanki and Sahu (2007). The increase in chlorophyll content with leaf maturation (50 DAS) and their decline with the onset of senescence may be due to increased synthesis and degradation of chlorophyll, respectively. In plants such as barely, wheat, bean and tomato, during the oxidative stress, the amount of proline and sugar concentration were increased by the treatments of salicylic acid hormone. Increasing the amount of proline, sugars and also the osmosis gradient in the plants would lead to the resistance against loosing water, the contents of leaves and also accelerate the growth of plants in stress conditions (Tasgin *et al.,* 2006).

Consumptive Use of Water and Water Use Efficiency

The consumptive use (CU) of water decreased significantly with water stress at tillering and tillering + 50 % heading stage under normal and late sown crop (Table 1). Whereas, water use efficiency increased significantly with water stress at tillering and tillering + 50 % heading stage under normal and late sown crop (Table 1). The similar findings have been reported by Tomar and Singh (1992) and Dhaker (2014). Consumptive use of water was closely related to the amount of water applied through irrigation and also varied with number of irrigations. Normal sown crop with no water stress recorded higher consumptive use of water (380.3 mm) as compared to other treatments. Consumptive use of water was higher with no water stress due to more number of irrigations resulting in more moisture available to the crop and soil surface and increased evapotranspiration. The observations are in agreement with Maurya and Singh (2008). The highest water use efficiency (WUE) of 17.16 kg ha^{-1} mm was observed in normal sown crop with stress at tillering + 50 % heading and lowest (13.58 kg ha^{-1} mm) in late sown crop with no water stress (Table). The WUE depends on two factors *viz*., grain yield and consumptive use of water. The WUE was highest in treatment receiving water stress at tillering + 50 % heading stage mainly due to consumptive water use, which indicates the efficient water use at lower frequency of irrigation. Due to higher water availability in surface soil and consequently higher consumption in this treatment, water use efficiency was reduced. The result is supported by the findings of Ingle and Shelke (2007).

Foliar sprays of bio-regulators did not influence consumptive use of water. However, water use efficiency significantly increased with foliar spray of SA and TU over water spray by 1.2 and 6.6 per cent, respectively (Table). Water use efficiency is biomass production per unit of consumptive use of water. Thus, higher WUE with foliar spray of SA and TU might be due to increased growth parameters and ultimately higher biological and grain yield as compared to water spray but consumptive use of water remained unaffected by foliar spray.

From the present investigation, it is concluded that maximum reduction of grain yield was observed when the crop was exposed to the stress at tillering + 50 % heading. Bio-regulators like thiourea and salicylic acid were further identified to help in alleviation of water stress at 40 and 70 DAS under normal and late sown conditions. Thereby integrating the use of these bio-regulators with water stress at tillering + 50 % heading stage can substantially enhance the productivity, bio-chemical parameters vis-à-vis profitability from cultivation of wheat under water scarce conditions. Therefore, this practice can benefit the farmers.

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