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Salicylic acid and thiourea mitigate salinity and drought stress in wheat (*Triticum aestivum*)

TARAMANI YADAV[1, 2], GAJENDER YADAV[1], ASHWANI KUMAR[1], DINESH KUMAR[2] and R K YADAV[1]*

ICAR-Central Soil Salinity Research Institute, Karnal, Haryana 132 001, India

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ABSTRACT

Water and salinity stresses are the major environmental concerns limiting crop production, particularly in arid and semi-arid ecologies. Focused research is required to develop mitigating strategies to manage such stresses. Application of plant-bioregulators (PBRs) may be an effective option to manage water and salinity stresses. Therefore, a field experiment was conducted during 2016–17 and 2017–18 at semi-arid saline site of NW (north-western) India to assess the effectiveness of salicylic acid and thiourea in easing out the adverse effect of variable water deficit and irrigation water salinity regimes. Increasing water deficit and its salinity significantly ($P<0.001$) reduced all the studied growth parameters, viz. plant height, crop stand, no. of tillers, leaf area index and relative growth rate; and yield attributes like no. of effective tillers, spike length, grains/spike, grain weight/spike, 1000-grain weight, specific weight. Application of either salicylic acid or thiourea significantly improved growth parameters in comparison to control and thiourea proved more effective. Thiourea application improved grain yield by 3.96 and 17.36%, biological yield by 4.21 and 14.82%, effective no. of tillers by 3.77 and 11.91%, weight/spike by 2.83 and 15.17%, 1000-grain weight by 3.72 and 12.66% and specific weight by 2.04 and 8.84% compared to salicylic acid and control, respectively. This might be due to better nutrient uptake, water relations, enhanced CO_2 fixation and effective partitioning of assimilates. Thus, thiourea and salicylic acid application in wheat found better as compared to control under saline water stress.

Keywords: Salicylic acid, Salinity, Thiourea, Water deficit, Wheat

Wheat (*Triticum aestivum* L.) is the staple food of about 36% population of the world and one of the most important crops grown on salt affected areas (Kumar *et al*. 2022). Salinity is a devastating abiotic stress afflicting ~6.73 M ha area in India (Mandal *et al*. 2011). Recent predictions indicate that the extent of salt-affected soils may treble to 20 million hectares (m ha) by 2050, mainly due to expansion in irrigated area and injudicious use of water resources (CSSRI Vision 2050). Likewise, water deficit due to drought and soil salinization is probably more threatening environmental concern limiting crop production particularly in arid and semi-arid ecosystems (Yadav and Dagar 2016). Salinity and drought are critical stresses that negatively hasten morphological, physiological, biochemical and yield attributes to deteriorate crop production (Kumar *et al*. 2018).

Numerous plant bio-regulators (PBRs) and other plant hormones have been tried recently to enhance stress tolerance in crops (Srivastava *et al*. 2016). Among these PBRs, salicylic acid (SA) (phenolic nature) and thiourea (TU) play a crucial role in plant growth regulation under abiotic stresses (Yadav *et al*. 2020). Salicylic acid, an endogenously synthesized signaling molecule of phenolic nature, could enhance plant growth, photosynthesis and decrease ROS production under various abiotic stresses (Khan *et al*. 2015). Whereas thiourea, an analogue of urea containing nitrogen and sulfur, is a free radical scavenger that improves gas exchange, nutrient acquisition and assimilation by the root, sugar metabolism, proteins bio-synthesis and also modulates the pattern of gene expression regardless of the stress applied (Wahid *et al*. 2017). Considering the positive effects of salicylic acid and thiourea, a field experiment was conducted to determine their efficacy in improving morphological and yield characters of wheat under variable salinity and water deficit irrigation regimes to address the water scarcity and its quality problems in arid and semi-arid regions.

MATERIALS AND METHODS

An experiment was conducted at the research farm of ICAR-Central Soil Salinity Research Institute (ICAR-CSSRI), Nain, Panipat, Haryana, (29°19'10.0" N and 76°48'0.0" E) during 2016–17 to 2017–18 in split-split plot

[1]ICAR-Central Soil Salinity Research Institute, Karnal, Haryana; [2]ICAR-National Dairy Research Institute, Karnal, Haryana. *Corresponding author email: RK.Yadav@icar.gov.in

design with 3 replications of 3 sets of treatments. Treatments consisted of 3 irrigation regimes, viz. 60, 80 and 100% (I_1, I_2 and I_3, respectively) of crop ET_c (crop evapo-transpiration at identified crop growth stages) using FAO Penman-Monteith method (Doorenbos and Pruitt 1977) in main plot; 4 levels of saline irrigation water, viz. 2 (S_0), 4 (S_1), 8 (S_2) and 12 (S_3) dS/m by mixing of farm pond water (0.5–1.5 dS/m) and available saline groundwater from 2 tube-wells with 3–5 and 16–18 dS/m salinity in sub-plots. Two PBRs along with control (without PBRs) i.e. salicylic acid (G_1; 1 mM) and thiourea (G_2; 500 ppm) in sub-sub plots as seed priming (before sowing) and foliar application at booting (60–70 DAS) and milking stages (105–110 DAS) were also applied. Wheat variety KRL 210 was sown at 22.5 cm row spacing, on November 18 and 13, and crop was harvested on April 05 and 01 in 2017 and 2018, respectively. All standard agronomic practices were followed throughout the cultivation of the crop.

Crop stand per square meter was recorded at 30 days after sowing (DAS). Plant height of randomly selected 10 plants was measured at harvest. LAI was measured at different growth stages, viz. tillering, booting, flowering, milking and harvesting by portable Sun Scan Canopy analyser. Relative growth rate (mg/g/day) was measured at critical growth stages, viz. crown root initiation (21–25 DAS), tillering (40–45 DAS), booting (60–70 DAS), flowering (85–95), milking (105–110) and harvesting (139–140 DAS). Effective number of tillers/m^2 was recorded at physiological maturity and calculated the reduction percentage in effective tillers. Spike length (cm), number of grains/spike and grain weight/spike were taken at harvest from 10 randomly selected spikes. Whereas, 1000-grain weight, grain yield (t/ha), harvest index (%), specific weight (kg/hl) were recorded at harvest. Statistical analysis was done by analysis of variance (ANOVA) technique for split-split plot design using SAS 9.2 software (SAS Institute 2010) and comparison was made using Tukey's test at P≤0.05.

RESULTS AND DISCUSSION

Wheat growth, development, yield attributes and yield indicated significant differences amongst various deficit irrigation regimes and irrigation water salinity levels, and application of PBR's found effective in arresting the adverse effect of salinity and drought (Table 1–2; Fig 1).

Table 1 Effect of deficit irrigation, saline irrigation water and plant bioregulators on plant density, plant height and LAI of wheat

Treatment	Crop stand/ m^2 (30 DAS)	Plant height (cm)	Leaf area index				
			Tillering	Booting	Flowering	Milking	Flowering
Year							
2016–17	193.36	93.41^A	2.35^B	3.79^A	3.83^A	3.69^A	1.70
2017–18	193.68	91.22^B	2.45^A	3.59^B	3.66^B	3.62^B	1.63
SEd±	1.17	0.78	0.02	0.09	0.09	0.02	0.03
LSD (P=0.05)	NS	1.74	0.04	0.20	0.21	0.05	NS
Deficit irrigation regimes							
I_1 (60% ET)	193.095	89.19^C	2.34^B	3.65	3.58^C	3.57^C	1.56^C
I_2 (80% ET)	192.87	92.12^B	2.41^A	3.74	3.75^B	3.65^B	1.68^B
I_3 (100% ET)	194.58	95.64^A	2.45^A	3.68	3.91^A	3.74^A	1.76^A
SEd±	1.39	0.96	0.02	0.11	0.06	0.03	0.04
LSD (P=0.05)	NS	2.13	0.05	NS	0.13	0.06	0.08
Salinity levels of irrigation water							
S_0 (2 dS/m)	196.15^A	102.50^A	2.49^A	3.77^{AB}	3.98^A	3.80^A	1.78^A
S_1 (4 dS/m)	195.66^A	97.98^B	2.45^{AB}	3.77^{AB}	3.87^A	3.77^A	1.73^A
S_2 (8 dS/m)	194.17^A	89.28^C	2.40^B	3.64^{BC}	3.67^B	3.67^B	1.64^B
S_3 (12 dS/m)	188.1^B	79.51^D	2.26^C	3.55^C	3.46^C	3.39^C	1.52^C
SEd±	2.47	1.32	0.03	0.07	0.06	0.03	0.03
LSD (P=0.05)	4.90	2.62	0.06	0.13	0.13	0.06	0.06
Plant bioregulator							
G_0 (Control)	188.82^B	85.35^C	2.31^C	3.45^B	3.39^C	3.43^B	1.60^B
G_1 (SA)	196.24^A	94.53^B	2.42^B	3.76^A	3.82^B	3.75^A	1.69^A
G_2 (TU)	195.43^A	97.07^A	2.48^A	3.85^A	4.02^A	3.80^A	1.71^A
SEd±	2.16	1.14	0.03	0.06	0.06	0.03	0.03
LSD (P=0.05)	4.23	2.26	0.05	0.11	0.11	0.05	0.05

Data represented are means ±S.E.M of three independent replications. Two-way ANOVA followed by Tukey's test was used for analysis. Different letters denote significant differences at P<0.05.

Growth parameters: Crop stand/m^2 recorded at 30 DAS decreased 4.11% ($P<0.01$) with the application of 12 dS/m irrigation water (S_3) over control (S_0), but remained statistically similar under S_0 (196.15), S_1 (195.66) and S_2 (194.17) irrigation water salinity levels (Table 1). Seed priming with both PBRs i.e., SA and TU were equally effective and recorded higher (4.15 and 3.38%) ($P<0.01$) plant density, over control. Plant density was not affected by study years and varying deficit irrigation regimes. The wheat plant height at harvesting stage ($P<0.05$; 2.40%) was recorded significantly higher in year 2016–17 than 2017–18 (Table 1). I_2 (80% ET_c) and I_1 (60% ET_c) recorded 3.68 and 6.74%, respectively shorter plant than I_3 (100% ET_c). In general, increased salinity of irrigation water reduced the plant height and this reduction followed the decreasing trend as $S_0>S_1>S_2>S_3$. The reduction in plant height was observed in S_3, with the extent being 22.43, 18.85 and 10.94% compared to S_0, S_1 and S_2, respectively. The positive effect of both PBRs was observed on plant height enhancement by 10.76 and 12.07% with SA & TU, respectively over G_0 (control). Moreover, the application of TU recorded 2.69% higher plant height over SA.

LAI increased up to flowering stage and declined afterwards during both the years (Table 1). LAI was significantly higher in 2016–17 at all the growth stages, except tillering. Deficit irrigation declined LAI by 4.49, 8.44, 4.55 and 11.36% in I_1 (60% ET_c) than I_3 (100% ET_c) at tillering, flowering, milking and harvest stages, respectively. LAI significantly decreased with the increase in salt stress. However, the decrease was relatively more i.e. 9.24, 5.84, 13.07, 10.79 and 14.61% under S_3 (EC_{iw} ~12 dS/m) compared to S_0 (EC_{iw} ~2 dS/m) at tillering, booting, flowering, milking and harvesting, respectively. Application of PBRs increased LAI by 6.06, 10.29, 15.63, 10.06 and 6.25% at tillering, booting, flowering, milking and harvesting stages, respectively. The TU recorded significantly higher LAI over SA by 2.48 and 5.25% at tillering and flowering stages, respectively.The better crop performance during 2016–17 was due to receipt of 32.9% higher rainfall (93.30 mm) with favorable distribution than 70.2 mm in 2017–18. Particularly, the 85 mm rainfall received during booting stage in 2016–17 produced higher plant height (Table 1) and LAI at booting and milking stages in comparison to only 34 mm rainfall received at same stage in 2017–18. This resulted into lesser number (only 4) of saline water irrigations in 2016–17 as compared to 5 saline irrigations in 2017–18.

Also it helped to leach down more of the excess soluble salts, added with saline water irrigation, below root zone and nullified the effect of moisture deficit treatments. Enhanced grain and biomass yields and yield attributes of crops, due to congenial climatic conditions, have also been reported earlier (Wakchaure *et al*. 2016, Singh *et al.* 2018).

Year 2016–17 produced 5.7% higher effective tillers/m^2 in comparison to 2017–18. The percent reduction from total number of tillers to effective tillers was comparable during both the years (Fig 1). Deficit irrigation I_2 (80% ET_c) and I_1 (60% ET_c) produced 4.06 and 6.92% lesser effective tillers ($P<0.001$) than I_3 (100% ET_c). The percent reduction from total tillers to effective tillers increased from 9.2 to 10.9% and then further to 11.4% in I_3, I_2 and I_1 irrigation regimes, respectively. There was 0.53, 6.82 and 20.89% reduction in number of effective tillers at irrigation water salinity of 4 (S_1), 8 (S_2) and 12 dS/m (S_3) compared to 2 dS/m (S_0). Increase in irrigation water salinity increased the mortality of tillers as the per cent reduction from number of total tillers to effective tillers was progressively increased from 7.8 to 15.4 (Fig 1). Use of SA and TU increased numbers of effective tillers by 7.27 and 10.64% over control, suggesting the superiority of TU over SA. The per cent reduction from total tillers to effective tillers was also reduced by application of SA (9.9%) and TU (5.5%) as compared to control (12.9%). Depressed growth of wheat under deficit irrigation treatment I_1 (60% ET_c) could be due to alteration of cell turgor and growth via division, enlargement and differentiation, impaired germination and

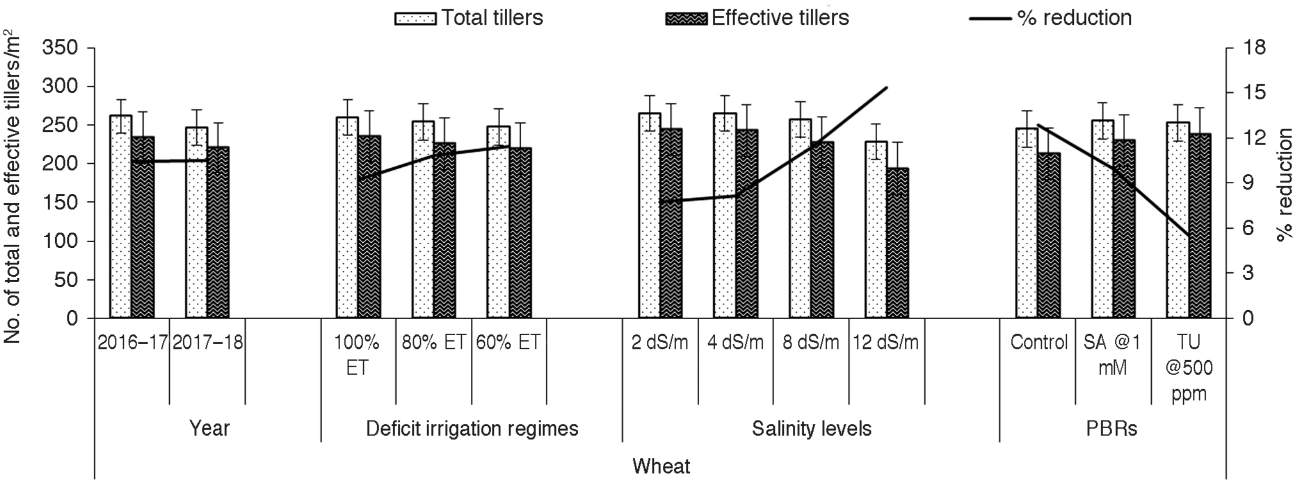

Fig 1 Effect of year, deficit irrigation, salinity levels and plant bioregulators on number of total and effective tillers. Vertical bars represent the standard deviation of the mean.

crop stand as reported earlier by Kaya *et al*. (2006). Fatima *et al.* (2018) also reported the cassation of plant growth and seed development in wheat due to to inactivation of adenosine diphosphate-glucose-pyrophosphorylase.

Yield and attributes: Deficit irrigation treatment I_2 (2.16%) and I_1 (3.19%) recorded lower (P<0.05) spike length than I_3 (8.79 cm). Significant decrement was also noted with increasing salinity levels from S_1 to S_2 (2.77%) and further to S_3 (15.38%). Application of G_2 produced greater spike length over G_1 and G_0 by 1.83 and 8.38%, respectively (Table 2). Grain/spike declined (P<0.0001) from I_3 to I_2 (4.29%) and further to I_1 (8.62%). Among irrigation water salinity treatments, significant decrease in grain/spike was recorded from 8 dS/m onwards. However, the maximum reduction (22.75%) was recorded under S_3 as compared to S_0. Application of both PBRs significantly improved grain/spike and TU (G_2) was better than SA. Similar effect of treatments was observed in weight/spike. Different deficit irrigation regimes, salinity levels and PBRs had significant effect on 1000-grains weight, but it remained unaffected by years of cultivation (Table 2). The lower 1000-grains weight was obtained under higher water deficit irrigation regimes (P<0.001). 1000-grains weight decreased significantly at irrigation water salinity of 8 dS/m S_2 (5.03, 4.65%) and further at 12 dS/m S_3 (19.14, 18.81%) as compared to S_0 and S_1, respectively. Use of PBRs showed significantly higher 1000-grains weight in comparison to control and TU proved better than SA. Bali *et al*. (2020) reported similar positive effects of SA and TU on yield attributes of wheat under high residual alkalinity of irrigation water. Drought stress disturbs physiological and biochemical processes i.e. damages photosynthetic apparatus and other enzymatic activities, which lead to reduction in crop yield (Dhansu *et al*. 2021). Moisture deficit reduces photosynthesis due to decrease in leaf expansion, impaired photosynthetic machinery, premature leaf senescence and associated reduction in all yields (Wahid *et al*. 2017). Water stress affected grain setting, shriveled grains and low specific weight of wheat grains.

Study year 2016–17 recorded 8.94% higher grain yield over 2017–18 (Table 2). Higher water stress in I_1 (60% ET_c) and I_2 (80% ET_c) resulted in lower yield (P<0.001), I_1 recorded 2.94 and 6.74% lesser grain yield compared to I_2 & I_3, respectively. The reduction in grain yield was observed,

Table 2 Effect of deficit irrigation, saline irrigation water and plant bioregulators on yield attributes of wheat

Treatment	Spike length (cm)	Grains/ Spike	Weight of spike (g)	1000-grain weight (g)	Grain yield (t/ha)	Harvest index	Hectoliter weight (kg/hl)
Year							
2016–17	8.67	43.46	6.77^A	42.67	4.63^A	42.20^A	79.43
2017–18	8.61	42.68	6.31^B	42.08	4.25^B	40.36^B	79.06
SEd±	0.08	0.42	0.09	0.33	0.04	0.35	1.00
LSD (P=0.05)	NS	NS	0.19	NS	0.09	0.79	NS
Deficit irrigation regimes							
I_1 (60% ET)	8.51^B	41.13^C	6.38^C	41.19^B	4.29^C	41.21	77.51^B
I_2 (80% ET)	8.60^B	43.08^B	6.54^B	42.17^B	4.42^B	41.19	79.04^B
I_3 (100% ET)	8.79^A	45.01^A	6.71^A	43.78^A	4.60^A	41.44	81.17^A
SEd±	0.10	0.51	0.10	0.40	0.05	0.43	1.22
LSD (P=0.05)	0.22	1.14	0.23	0.89	0.12	NS	2.72
Salinity levels of irrigation water							
S_0 (2 dS/m)	9.06^A	46.47^A	7.10^A	45.15^A	4.95^A	42.24^A	83.55^A
S_1 (4 dS/m)	9.04^A	46.02^A	7.02^A	44.97^A	4.92^A	41.03^B	83.04^A
S_2 (8 dS/m)	8.79^B	43.90^B	6.63^B	42.88^B	4.57^B	42.28^A	79.94^B
S_3 (12 dS/m)	7.65^C	35.90^C	5.42^C	36.51^C	3.32^C	37.51^C	70.44^C
SEd±	0.09	0.71	0.09	0.67	0.07	0.40	1.06
LSD (P=0.05)	0.17	1.40	0.17	1.33	0.14	0.79	2.09
Plant bioregulators							
G_0 (Control)	8.23^C	40.26^C	6.00^C	39.57^C	4.03^C	40.63^B	75.35^B
G_1 (SA)	8.76^B	43.80^B	6.72^B	42.98^B	4.55^B	41.63^A	80.37^A
G_2 (TU)	8.92^A	45.15^A	6.91^A	44.58^A	4.73^A	41.53^A	82.01^A
SEd±	0.07	0.61	0.07	0.58	0.06	0.34	0.91
LSD (P=0.05)	0.15	1.21	0.15	1.15	0.12	0.68	1.81

Data represented are means ±S.E.M of three independent replications. Two-way ANOVA followed by Tukey's test was used for analysis. Different letters denote significant differences at P<0.05.

at S_2 (4.57 t/ha) and subsequently at S_3 (3.32 t/ha), to the tune of 7.68 and 32.93%, respectively in comparison to S_0. Both PBRs improved (P<0.0001) wheat grain yield over control (G_0) and increment with SA and TU were 12.90 and 17.37%, respectively. All applied treatments except deficit irrigation regimes (P>0.05) had significant effect on harvest index (HI). Application of SA and TU increased HI by 2.46 and 2.22%, respectively over control. Deficit irrigation regimes, viz. I_1 and I_2 decreased the hectorliter weights 4.51 and 2.62% (P<0.05) than I_3 (100% ET). Reduction in hectoliter weight was also observed at irrigation water salinity level S_2 (4.28%) and further at S_3 (15.69%) over S_0. Both G_1 and G_2 increased (P<0.0001) the hectoliter weight compared to G_0 but found equally effective in their stress mitigating response.The adverse effect of salinity on growth of wheat might be due to several possible reasons such as growth inhibition, physiological water stress created by high salt concentrations and interference with nutrient absorption in the root zone (Yadav *et al*. 2020).

Higher Na^+ concentration in rhizospheric soil solution reduces the ability of plant to take up sufficient water creating water deficit within the plant and/or inability to regulate the net uptake of harmful ions, especially sodium and chlorides causing their toxicity and/or nutritional imbalance (El-Hendawy *et al*. 2017, Kumar *et al*. 2018). Decrease in wheat growth and yield components, have been reported earlier, as a function of increasing irrigation water salinity (Jiang *et al*. 2012, Yadav *et al*. 2020) and residual alkalinity (Bali *et al*. 2020). Salinity may also damage proteins, lipids and nucleic acids, and alter ER photosynthesis and respiration (Manai *et al*. 2014) and/or nutritional imbalance (Yadav *et al*. 2007), which affect plant growth and development. The increase in grain yield and yield attributes through application of PBRs could be due to enhanced CO_2 fixation, soluble protein content, nitrate reductase activity and effective partitioning of assimilates to the developing reproductive organs (Pooja and Sharma 2016). Seleiman and Kheir (2018) also showed positive response of TU on individual grain weight in wheat.

Salinity and drought stresses caused significant reduction in growth, development, yield and yield attributing traits in wheat. Increasing water stress from 100 ET_c to 80 ET_c and 60 ET_c reduced grain yield by 2.94 and 6.74%, respectively.Where as increasing salinity of irrigation water, viz. 4, 8 and 12 dS/m caused 0.61, 7.68 and 32.93% reduction, respectively. Both SA and TU helped to mitigate the adverse effect of water deficit and salinity stress in wheat growth attributes and TU found more effective than SA.

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