

Exogenous application of plant bio-regulators improve yield and water use efficiency of maize under drought stress

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ABSTRACT

Moisture stress in rainfed areas has significant adverse impact on plant growth and yield. Exogenous application of plant bio-regulators (PBRs) plays an important role to mitigate drought stress in plants. A field experiment was conducted during rainy (*kharif*) seasons of 2018 and 2019 to explore the positive role of PBRs on the yield, economic feasibility and water use efficiency of maize grown under rainfed conditions. Ten treatments including PBRs, mulching and control were evaluated in a randomized block design. The result showed that foliar spray of salicylic acid (0.5 mM) with 1% urea solution improved the grain yield of maize by 24% as compared to control plot. Other PBRs such as glycine betaine, gibberellic acid and thiourea also increased the grain yield in the range of 10–21%. Application of 1% urea solution with PBRs synergise the effect of PBR to alleviate drought stress. The foliar applied PBRs maintained higher membrane stability index (73–78%), significantly increased relative water content (5–11%), chlorophyll content and decreased the proline content (11–21%) as compared to plants grown in control plot. Significant increase were noted in the rain and crop water use efficiency of maize with the foliar application of PBRs. Net returns were improved by 33% with application of salicylic acid (0.5 mM) + 1% urea as compared to control (₹ 49 × 10³ /ha). Thus, foliar spray of salicylic acid (0.5 mM) with 1% urea solution could be an economic feasible strategy to reduce the adverse effects of drought and may increase the maize yield and water use efficiency.

Key words: Drought stress; Plant bio-regulator; Rainfed maize; Salicylic acid; Water use efficiency

Millions of resource-strapped smallholders depend on maize (*Zea mays* L.) for their livelihoods, food security, and access to nutrition. It's also being used for biofuel and livestock feed (Shiferaw *et al.*, 2011). About 80% of the maize is cultivated in rainfed areas where plants often face drought stress (Aslam *et al.*, 2015). Drought stress is a major limiting factor for low productivity of maize. In rainfed situation, maize productivity is much below (2.2 t ha⁻¹) compared to global average of nearly 5.8 t ha⁻¹ (Erenstein *et al.*, 2022). Water scarcity, which is already a serious issue in many parts of the world and is predicted to get worse in the future, will make it difficult to increase production. The average per capita water availability in India has been decreasing, from 5,247 m³ in 1951 to 1,486 m³ in 2021 (CWC, 2019). Maize is widely grown from the

Southern to the Northern Hemisphere and from arid and semi-arid to humid and sub-humid areas. Water scarcity will increasingly constrain maize production.

Drought–stressed plants showed a steady decline in their relative water content (RWC) and stomata closure both limiting metabolic output, plant growth, and yield. The plants accumulate solutes in their cells to maintain the cell volume and resist against dehydration. This phenomenon is known as osmotic adjustment and has been observed different parts of plant like stem, leaves, roots, and fruits (Rahdari and Hoseini, 2012). To trigger this physiological response and improve plant tolerance, the exogenous application of plant bio–regulators such as salicylic acid (SA), glycine betaine (GB) and gibberellic acid (GA) and thiourea (GA) is a well-known mitigation strategy.

Exogenous application of plant bio–regulators (PBRs) has been tried under both controlled and actual field conditions to alleviate drought stress (Wakchaure *et al.*, 2018). PBRs facilitate growth, development, nutrient distribution, and source-sink transitions, which help plants to cope with the stress condition (Nathawat *et al.*, 2021). Although PBRs are spontaneously biosynthesized and stored in plant

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cells, their intrinsic accumulation is insufficient to protect plants from drought. In this situation, exogenous application can help to lessen the adverse effects.

Salicylic acid is a phenolic phytohormone signalling molecule that is endogenously synthesised. It acts as a regulator of the signalling networks in plants under abiotic and biotic stresses (Chavoushi *et al.*, 2019). Besides, the SA is a non-enzymatic anti-oxidant modifying plant tolerance to environmental stresses, particularly drought. Similar to this, glycine betaine (GB), a zwitterionic, N-methyl substituted glycine derivative builds up in a variety of plant species under various stresses and is discovered to be highly abundant in chloroplasts, where it is found to play a crucial role in controlling, protecting the thylakoid membrane and, ultimately, maintaining the level of photosynthesis (Alhoshan *et al.*, 2019). Shemi *et al.* (2021) observed 40.5 and 60.5% higher grain yield of maize upon foliar application of SA and GB, respectively, under the drought-stressed condition as compared to the values of control. Gibberellic acid, an another plant bio-regulator (PBR) altered the oxidative stress processes and antioxidant enzyme activity to reduce the harmful effects of drought stress. In addition to the antioxidant defense system, exogenous GA upregulated the glyoxalase system, which helped the plant survive under drought stress and improved biomass of wheat by 16-19% (Al Mahmud *et al.*, 2019). According to Bray (1988), function of ABA is to promote the accumulation of a group of purportedly protective proteins in tissues under water stress and desiccation. Thiourea is an another synthetic PBR having 36% nitrogen and 42% sulphur that gained importance in plant stress tolerance (Yadav *et al.*, 2020). Thiourea application increased seed yield of canola by 42.8% compared with control under high-temperature stress (Ahmad *et al.*, 2022). A comprehensive literature search revealed no studies in which potential synergistic effects of combining plant bioregulators with urea were tested. Therefore, the present study was conducted to evaluate the drought stress mitigation effect of plant bioregulator for increasing maize production with enhanced water use efficiency in drought prone areas.

MATERIALS AND METHODS

The study was conducted at the Central Research Farm of ICAR–Indian Grassland and Fodder Research Institute, Jhansi, India (25° 27' N latitude, 78° 33' E longitude and 270 m above mean sea level) during 2 years, i.e. 2018 and 2019. The region has a continental monsoon climate with an average (1939–2015) annual rainfall of 908 mm, majority of that falling from June to September. The study location was characterized by semi-arid climate with extreme temperature during summer (43 to 46°C) and winter (as low as 2°C). The rainfall received during the study period

is shown in Figure 1. The experimental soil was deep, moderately well drained with loamy in texture (35.3% sand, 46.2% silt and 18.5% clay). It had pH 7.4, electrical conductivity (EC) 0.16 dS/m, soil organic carbon 0.45%, KMnO_4 oxidizable N 208 kg/ha, 0.5 M NaHCO_3 extractable P 13 kg/ha and 1 N NH_4OAc extractable K 234 kg/ha in the top 15 cm soil.

The experiment was laid-out in a randomized complete block design with three replications. Ten treatments of the present study consisted of control; mulching; salicylic acid (0.5 mM); salicylic acid (0.5 mM) + 1% urea; glycine betaine (100 mM); glycine betaine (100 mM) + 1% urea; gibberellic acid (100 ppm); gibberellic acid (100 ppm) + 1% urea; thiourea 1000 ppm; potassium 1.0%. Exogenous foliar spray of SA, GB, GA, thiourea and potassium was sprayed at vegetative (V5) and reproductive (R1 silk) stages of maize. The foliar spray was accomplished using the manually operated knapsack sprayer fitted with a flat-fan nozzle using 500 L volume per hectare. No chemical was sprayed in the control plot. In mulched treatment, crop residue ($\sim 5 \text{ Mg ha}^{-1}$) was spread on the soil surface to reduce evaporation losses of moisture. Maize variety 'JM-216' was sown with seed rate of 25 kg ha^{-1} during first fortnight of July and harvested in the first fortnight of October in both the years. The plant spacing was $45 \times 15 \text{ cm}$. Maize crop received a common fertilizer dose of $90 \text{ kg N} + 60 \text{ kg P}_2\text{O}_5 + 30 \text{ kg K}_2\text{O}$ per hectare. Nitrogen was applied in 3 equal splits: basally in lines at sowing, and broadcast at V6 and VT stages. Phosphorus and potassium were band-placed basally at the time of sowing. Maize was raised as a rainfed crop. Weeds were managed through spraying of atrazine 50 WP at $0.75 \text{ kg a.i. ha}^{-1}$ followed by one hand weeding at 20-25 days after sowing. There were no major incidences of pests and diseases. Maize grain yield was adjusted to 14% moisture content.

Membrane stability index (MSI) of maize leaf was determined as per the method of Deshmukh *et al.* (1991). Leaf discs (100/mg) were carefully cleaned using tap and distilled water before being placed in two sets of test tubes each containing 10 mL of the distilled water. One set was kept in water bath at 40°C for 30 minutes, and the electric conductivity was noted as C_1 . Another set was kept in water bath to boil (100°C), and electrical conductivity was noted as C_2 . The following formula was used to compute the membrane stability index.

$$MSI (\%) = [1 - (C_1/C_2)] \times 100$$

Relative leaf water content (RLWC) was determined by the method of Barr and Weatherly (1962) as per following formula.

$$RLWC (\%) = \frac{\text{Fresh weigh} - \text{dry weight}}{\text{Turgid weight} - \text{dry weight}} \times 100$$

Proline concentration was determined as per method

suggested by Bates *et al.* (1973). However, the method developed by Arnon (1949) was used to determine the total chlorophyll content of leaves.

$$\text{Total chlorophyll (mg g}^{-1} \text{ FW)} = (20.2 \times A_{645}) + (20.2 \times A_{663}) \times \frac{V}{1000 \times W}$$

Where, A = absorbance; V = final volume of 80% acetone (in ml); W = Weight of plant tissue (in grams)

In the present study, water use efficiency was measured in two ways: rain water use efficiency (RWUE) and crop water use efficiency (CWUE). RWUE of crop was calculated by dividing the maize grain yield by the amount of rain received during crop growing season i.e. from planting to harvest. The CWUE was worked out from the seasonal crop consumptive use of water. The economic analysis was done by considering the variable production costs only. Benefit to cost ratio (BCR) was calculated by dividing net returns with total cost of cultivation. All the data were subjected to analysis of variance (ANOVA) using the general linear model procedures of the Statistical Analysis System (SAS 9.3). The F-test was used to determine significant effects of the treatments and least significant difference (LSD) was used to compare means at $\alpha = 0.05$.

RESULT AND DISCUSSION

Weather

The rainfall pattern of the experimental site was highly variable during both the years of study. Rainfall during crop growing period of 2018 was 893 mm which was almost double than rainfall received in 2019 (Fig 1). Moreover, the rainy days were 30 and 27 during 2018 and 2019 crop growing period, respectively. The number of rainy days were almost similar (30 in 2018 and 27 in 2019) in both the years despite of doubled rainfall in 2018 than 2019. The crop faced drought stress situation at post flowering stage in 2019, however, crop received some rainfall

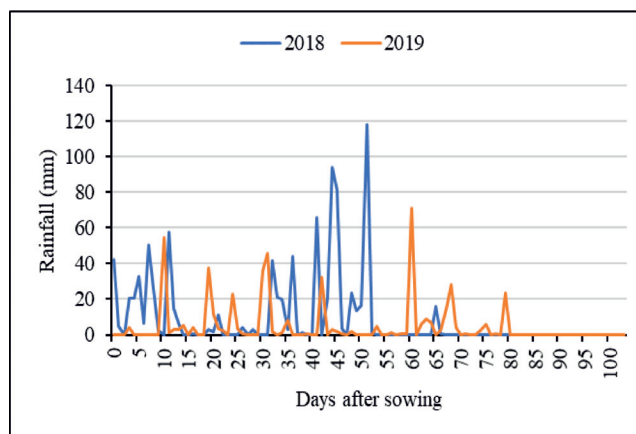


Fig. 1. Daily rainfall (mm) received during crop growing period of 2018 and 2019

during the same period in 2019. This reflects rainfall uncertainty (amount and distribution) in the central India and hence drought stress management practices are very vital for sustainable crop production and food security.

Crop yield

The treatments had significant effects on maize grain yield in both the years of experimentation (Table 1). In general, the grain yield in 2019 was slightly higher than 2018 despite less rainfall. Although rainfall was less in 2019 but distribution was good. In 2018, 350 mm rainfall was received in one week coinciding with the flowering stage causing water logging situation that hampered crop growth. Results showed that grain yield of maize during 2018 was significantly ($P < 0.05$) higher under mulching, SA, SA+1% urea, GB, GB+1% urea, GA+1% urea and thiourea applied plots compared with control. Almost similar trend was observed in 2019. Application of SA

Table 1. Effect of plant bio-regulators on yields and harvest index of maize

Treatment	Grain yield (t/ha)		Stover yield (t/ha)		Biological yield (t/ha)		Harvest index (%)	
	2018	2019	2018	2019	2018	2019	2018	2019
Control	3.44 ^c	3.72 ^d	9.1 ^c	9.4	12.6 ^c	13.1	27.4	28.4
Mulching	4.02 ^{ab}	4.48 ^{ab}	11.3 ^{ab}	11.4	15.3 ^{ab}	15.9	26.3	28.2
SA (0.5 mM)	4.15 ^{ab}	4.37 ^{abc}	10.9 ^{ab}	11.0	15.1 ^{ab}	15.3	27.5	28.5
SA (0.5 mM) + 1% urea	4.31 ^a	4.59 ^a	11.0 ^{ab}	11.1	15.3 ^{ab}	15.6	28.1	29.4
GB (100 mM)	4.14 ^{ab}	4.01 ^{bcd}	11.3 ^{ab}	10.4	15.5 ^{ab}	14.4	26.8	27.9
GB (100 mM) + 1% urea	4.17 ^{ab}	4.21 ^{abcd}	11.9 ^a	9.9	16.1 ^a	14.2	26.0	29.7
GA 100 ppm	3.77 ^{bc}	3.89 ^{cd}	10.7 ^b	9.8	14.4 ^b	13.7	26.1	28.4
GA 100 ppm + 1% urea	4.02 ^{ab}	3.98 ^{bcd}	11.7 ^{ab}	10.2	15.7 ^{ab}	14.2	25.6	28.1
Thiourea 1000 ppm	4.03 ^{ab}	4.36 ^{abc}	11.4 ^{ab}	10.5	15.5 ^{ab}	14.9	26.1	29.3
Potassium 1.0%	3.84 ^{bc}	4.08 ^{abcd}	10.8 ^{ab}	10.2	14.6 ^{ab}	14.2	26.2	28.7
P value	0.022	0.046	0.005	0.128 ^{ns}	0.004	0.074 ^{ns}	0.165 ^{ns}	0.618 ^{ns}

SA, salicylic acid; GB, glycine betaine; GA, gibberellic acid; ns, non-significant. Means followed by a superscripted similar lowercase letter within a column are not significantly different ($P < 0.05$) according to LSD test

(0.5 mM) +1% urea solutions spray increased grain yield of maize by 25.3 and 23.4% over control treatment (3.44 and 3.72 t/ha) in first and second year, respectively. Similarly, stover yield of maize significantly ($P < 0.05$) increased with the exogenous application of chemicals and mulching as compared to control in 2018. However, treatments had no significant effect on stover yield in 2019. Biological yield (grain + stover) followed the trend of stover yield. Harvest index was not influenced significantly ($P < 0.05$) by the treatments. Furthermore, application of SA, GB and GA along with 1% urea had a pronounced effect to mitigate the drought stress on crop yields.

The higher yield of maize may be the result of maintenance of photosynthetic activity owing to exogenous application of SA, GB, GA and thiourea. Foliar applied plant bioregulators such as SA, GB, GA are capable of quickly penetrating through leaves and transported to other organs, where it would boost stress tolerance (Hussain *et al.*, 2008). Accumulation of these organic solutes either actively or passively aids the plants in maintaining turgor pressure under water stress, protecting cellular compartments from damage brought on by dehydration, and retaining water within cells. Turgor maintenance is crucial for plant drought tolerance and may play a function in stomatal regulation and, in turn, photosynthesis (Ludlow *et al.*, 1985). Hussain *et al.* (2008) opined that, GB treated plants exhibit a slower decrease in leaf water potential during drought stress and develop wilting symptoms much later than untreated plants. SA treatment ameliorates the impact of abiotic stress through improving antioxidant system necessary to reduce oxidative damage and ion leakage from membranes (Yusuf *et al.*, 2008). Similarly, foliar spray of potassium is known to maintain K^+ balance and enzymes activities in the plant and thereby can alleviate the adverse effect of drought stress on important physiological functions in cereals (Pasala *et al.*, 2016). The higher yield of maize under mulching could be attributed to role of residue in conserving soil moisture coupled with enhanced nutrient supply through decomposition created conducive environment for plant growth and development (Choudhary *et al.* 2019; 2021).

Drought stress indicator

In the study, membrane stability index (MSI), relative leaf water content (RLWC), proline and chlorophyll content in the leaf were used as an indicator of drought stress in the plant. These parameters were measured at vegetative and reproductive stages but treatments had significant effect ($P < 0.05$) on reproductivity stage only (Fig 2). At vegetative stage, there was sufficient moisture in the soil, so treatments failed to exert any significant effect on these parameters except on total chlorophyll content.

Significantly higher MSI (78.3) was observed with foliar application of GB + 1% urea which was at par with SA (with and without urea), GA (with and without urea), GB

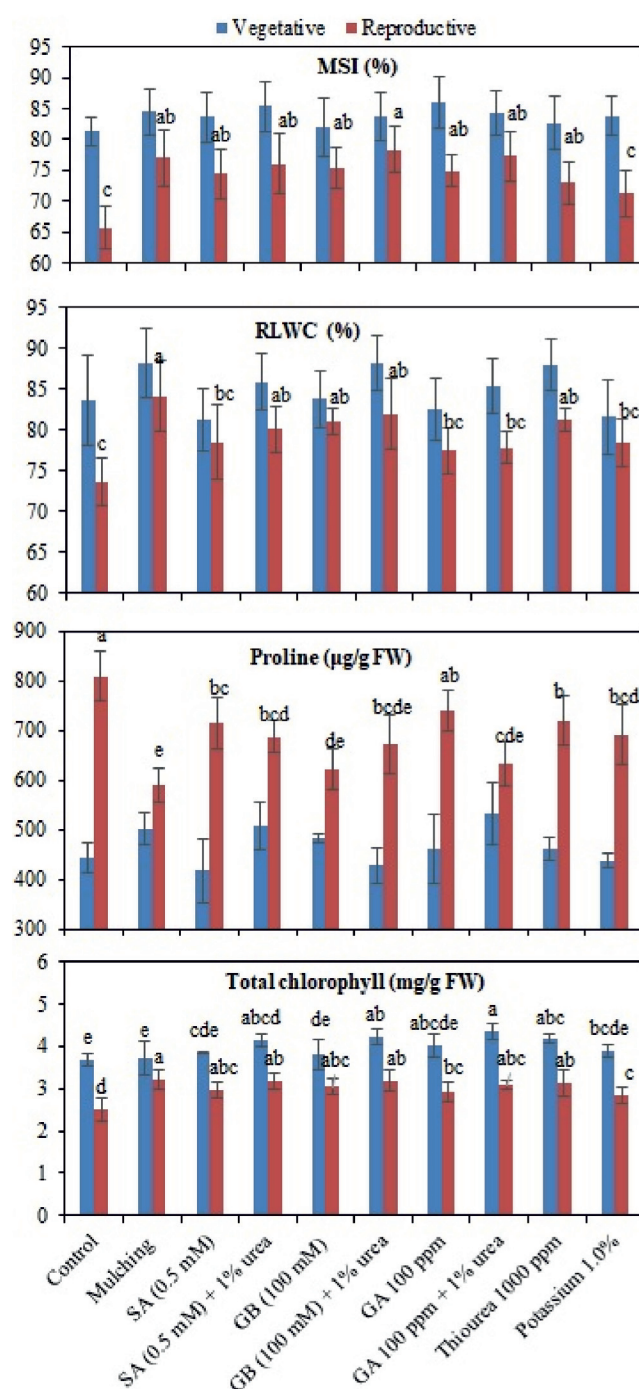


Fig. 2. Effect of different treatments on membrane stability index (MSI), relative leaf water content (RLWC), proline and total chlorophyll content at vegetative and reproductive stages of maize

SA, salicylic acid; GB, glycine betaine; GA, gibberellic acid. Bars represent mean values $\pm$ SD ($n = 3$). Bars sharing a similar lower-case letter within a stage are not significantly different ($P < 0.05$) according to LSD test

thiourea and mulching as compared to control (65.7) and potassium applied treatments plots (Fig 2). Moreover, the MSI in potassium applied plot was statistically at par with all other treatments except GB + 1% urea where MSI was higher. RLWC of mulched plants (84.2%) was higher than control (73.6%). Among exogenously applied chemicals, GB + 1% urea increased the RLWC by 11.4% as compared to control. This treatment was statistically at par with all other treatments. Exogenously applied chemicals decreased the free proline content in the leaves comparing with control (Fig 2). It was lowest under GB followed by GA + 1% urea. Plants grown under mulching recorded the least concentration of proline ($590 \mu\text{g g}^{-1}$ FW) in their leaves. Foliar application of different chemicals had significant effect on total chlorophyll content also at both vegetative as well as reproductive stages. Foliar application of 100 ppm GA + 1% urea followed by thiourea 1000 ppm, GB + 1% urea and SA + 1% urea significantly increased total chlorophyll contents over control (3.68 mg g^{-1} FW).

Drought significantly affects integrity of cell membranes manifested by lower values of MSI (Tas and Tas, 2007). Lower amount of ion leakage (high MSI) indicates the stability of leaf. Application of SA increases the accumulation of Ca^{+2} which can maintain membrane integrity. However, application of GB mitigates the plants from drought stress by decreasing the ROS production and lipid peroxidation that improves the membrane stability and chlorophyll (Kadioglu *et al.*, 2011). Potassium plays a key role in maintaining the cell turgor under drought stress (Bahrami-Rad and Hajiboland, 2017). The exogenous application of thiourea enhanced the RLWC which is consistent with another study conducted with cluster bean (Burman *et al.*, 2004). Thiourea being enriched with carbon and nitrogen, exogenous supplementation increased the chlorophyll pigments in drought-affected plants.

Water use efficiency

Different treatments had significant effect on water use efficiency during both the years of study (Fig 3). Irrespective of treatments, the values of CWUE were higher as compared to RWUE because in the former denominator was actual ET while total amount of rainfall in the later. In the second year, RWUE was about doubled than that of first year because of 1.9 times higher rainfall received in first year than second year. Foliar application of SA (0.5 mM) + 1% urea increased RWUE by 25.1 and 23.4% as compared to control plot (3.85 and 7.87 Kg/ha-mm) during 2018 and 2019, respectively. In first year, highest value of CWUE (10.8 Kg/ha-mm) was recorded in SA + 1% urea which was at par with all other treatments except control, and GA and potassium applied plots. However, GB, GA and potassium failed to exert significant effect on CWUE

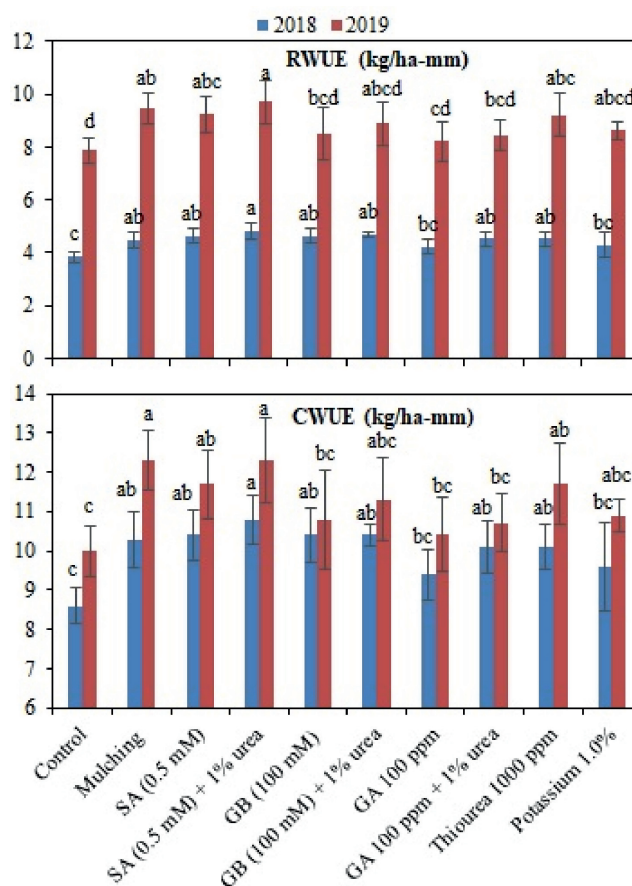


Fig. 3. Effect of different treatments on rain water use efficiency (RWUE) and crop water use efficiency (CWUE) of maize during 2018 and 2019

SA, salicylic acid; GB, glycine betaine; GA, gibberellic acid. Bars represent mean values $\pm$ SD ($n = 3$). Bars sharing a similar lower-case letter within a year are not significantly different ($P < 0.05$) according to LSD test.

in second year. Mulch applied plot was also recorded statistically similar RWUE and CWUE during both the years of experimentation. Higher WUE of maize under exogenous application of PBRs mainly occurred due to higher grain yield in these treatments. Fariduddin *et al.* (2003) also found higher water use efficiency in mustard with foliar application of SA. Wakchaure *et al.* (2018) stated that thiourea foliar application leads to 48.4% reduction in water consumption when plant exposed to water scarce condition and improved water productivity, quality and yield of onion.

Economics

The production cost and economic returns of different treatments are shown in Table 2. The production cost of maize varied from minimum with control ($\text{₹}26.9 \times 10^3 \text{ ha}^{-1}$) to maximum under GB + 1% urea ($\text{₹}30.8 \times 10^3 \text{ ha}^{-1}$). The maximum gross returns ($\text{₹}93.6 \times 10^3 \text{ ha}^{-1}$) and net

Table 2. Economic efficiency of different treatments in maize (average of two years)

Treatment	Total cost (₹ × 10 ³ /ha)	Gross returns (₹ × 10 ³ /ha)	Net returns (₹ × 10 ³ /ha)	BCR
Control	26.9	75.8 ^e	49.0 ^e	1.82 ^{ef}
Mulching	29.8	90.6 ^{ab}	60.7 ^{abc}	2.03 ^{bcd}
SA (0.5 mM)	28.4	90.2 ^{abc}	61.8 ^{ab}	2.18 ^{ab}
SA (0.5 mM) + 1% urea	28.4	93.6 ^a	65.2 ^a	2.29 ^a
GB (100 mM)	30.8	86.8 ^{bcd}	56.0 ^{bcd}	1.82 ^{def}
GB (100 mM) + 1% urea	30.8	88.8 ^{abc}	58.0 ^{bc}	1.88 ^{def}
GA 100 ppm	30.2	81.6 ^{de}	51.5 ^{de}	1.71 ^f
GA 100 ppm + 1% urea	30.2	85.6 ^{bcd}	55.4 ^{cd}	1.84 ^{def}
Thiourea 1000 ppm	28.6	89.1 ^{abc}	60.4 ^{abc}	2.12 ^{abc}
Potassium 1.0%	28.5	84.3 ^{cd}	55.8 ^{bcd}	1.95 ^{cde}
P value		0.0006	0.0010	0.0003

SA, salicylic acid; GB, glycine betaine; GA, gibberellic acid; ns, non-significant. Means followed by a superscripted similar lowercase letter within a column are not significantly different ($P < 0.05$) according to LSD test

returns (₹65.2 × 10³ ha⁻¹) were calculated in SA + 1% urea, being 23.4 and 33.1% higher over control, respectively (Table 2). All the treatments except GA (100 ppm) had significantly higher net returns as compared to control. Benefit to cost ratio was calculated by dividing the net returns value to the production cost in order to determine the economic efficiency. Similar to returns, highest benefit to cost ratio was recorded in SA + 1% urea (2.29) followed by SA (2.18) and lowest in CT-CR4 (1.16). In the present study, highest returns and BCR were achieved with the application of SA+1% urea. It is obvious that realization of higher net returns and BCR was the result of higher productivity. In agreement to our findings, increased economic profitability with SA, GB has also been reported by Alam *et al.*, (2022).

The results of the 2-years field experiment demonstrate that exogenous application of salicylic acid (0.5 mM) + 1% urea foliar spray could alleviate the negative effect of drought stress and improve the grain yield of maize in central India. The study suggests that the efficacy of plant bioregulators can be improved if applied with 1% urea solutions. Foliar spray of plant bioregulators was found effective in protecting leaf chlorophyll, and also maintained a higher level of membrane stability and relative water content in leaves. Water use efficiency was also improved with the application of plant bioregulators. Thus, foliar spray of salicylic acid (0.5 mM) with 1% urea solution could be a feasible strategy to reduce the adverse effects of drought and may increase the maize yield in rainfed regions.

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