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Ameliorating drought stress in sugarcane (*Saccharum* spp.) using biostimulants

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In India, abiotic stress, particularly drought, is known to affect sugarcane cultivation. Among various management strategies, application of biostimulants, especially those from seaweeds, offer promising results in containing yield loss due to drought stress. Here, we tested the efficacy of Sea6 liquid biostimulant formulation LBS6 in field condition along with conventional management practices for comparison purpose using two popular commercial varieties of sugarcane (Co 86032 and Co 0212). Drought stress imposed during formative phase of the crop and foliar application of sea6 liquid formulation was applied at 60, 90 and 120 days after planting (DAP). The effect of liquid formulation on mitigating drought stress was assessed by studying the growth, physiological, yield and yield traits at important pheno-phases of the crop. Results showed that, in Co 86032 shoot population of control was 100.5×10^3 ha^{-1} and drought was 85.1×10^3 ha^{-1} with mean reduction of 15.5% over control. Drought stress induced 11.4, 12.4, 9.8 and 5.0% reduction in plant height, leaf area index (LAI), chlorophyll content (SPAD value) and photochemical efficiency, respectively. In Co 0212, drought induced 10.15, 9.4, 10.5, 9.5 and 5.1% reduction in shoot population, plant height, LAI, SPAD value and photochemical efficiency, respectively. Under drought condition, in Co 86032, foliar application of KCl (2.5%) and seaweed extract LBS6(2 mL L^{-1}) was observed comparatively higher cane yield of 92.9 t ha^{-1} and 89.5 t ha^{-1}, with 18 and 16.5% yield improvement over untreated drought plot, respectively. In Co 0212, foliar application of KCl (2.5%) and seaweed extract LBS6 (2 mL L^{-1}) recorded 99 and 93.5 t ha^{-1} with 18.5% and 15.2% yield improvement over untreated drought plot, respectively. Among the two varieties, Co 0212 performed better under drought situation with KCl (2.5%) spray demonstrating better physiological efficiency under stress, closely followed by of sea6 formulation LBS6 (2 mL L^{-1}). Further, the drought management practices did not affect the juice quality parameters in both the tested varieties.

Keywords: Abiotic stress, *Gracilaria*, *Kappaphycus*, Seaweeds bioformulation

Sugarcane is grown in over 5.1 M ha in India, with an average productivity of 75 t ha^{-1}, being the second largest producer in the world[1] Out of total area under sugarcane cultivation, 35% is endowed with ample irrigation facilities, while the remaining 65% is only partially irrigated, with decline in average productivity to 41 t ha^{-1}. Nearly 70% of sugarcane crop in India experiences moisture stress, thus affecting the potential productivity of sugarcane varieties. Lack of adequate irrigation is mainly because of limited availability of water in lift irrigated areas, canal closure during summer in many of canal irrigated tracts, and drought which occur in a cyclic manner[2]. Initial establishment and tillering are the two crucial stages of water requirement, which in turn determines the yield potential of sugarcane. Limited water availability during the months of April to July is one of the reasons for low productivity, especially formative growth stage (60-150 days) has been identified as the critical water demand period and stress during this phase had a direct influence on cane yield and juice quality[3]. An estimated 250 tonnes of water is required for production of one tonne of sugarcane and the total water requirement of sugarcane crop varies from 1850 to 2500 mm. Yield reduction up to 60% has been recorded in a typical drought year. Water stress especially during summer months coincides with the formative phase, which affects yield through reduction in tiller productivity, number of millable canes, individual cane weight, and juice quality of sugarcane[4]. Drought accompanied by high temperature is more detrimental to sugarcane growth and productivity as reported by Gomathi *et al.*[5]. The situation is expected to be more challenging as the sugarcane cultivation has already been extended to semi-arid tracts of frequent droughts. Therefore, several attempts have been made to overcome or reduce the adverse impact of drought on sugarcane. Even though there are several management practices to mitigate the ill effect of

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drought stress in tropical India apart from usage of tolerant varieties, ameliorative strategies specific to different levels of drought is lacking.

In sugarcane, external application of abscisic acid (1×10^{-5} M) exerted a regulatory role on stomatal diffusive resistance and helped in maintaining relatively high water potential[6]. Foliar application of 2.5% urea has been demonstrated to be useful as a short term mitigating measure under moisture stress condition in sugarcane[7,8]. Similarly, potassium (K) which is known to maintain turgidity of the cells also helps in proline accumulation which is desirable under moisture stress. Taking clue from this, foliar spray of 2.50% K was attempted and application of K increased growth and cane yield of sugarcane under stress K ions by regulating the opening and closing of stomata which governs the transpirational loss of water was reported by Chand *et al*.[9] and Manikam *et al*.[10]. Combination of soaking sets in saturated lime water, trash mulching and foliar spray of 2.50% urea + 2.50% KCl were effective in increasing drought tolerance potential in sugarcane as reported by Sundara[8] and Gomathi *et al*.[11].

Seaweed is complex manure containing all the major and minor plant nutrients, several auxins and gibberllins, antibiotics, a wide range of vitamins and amino acids (alginic acid) and carbohydrates. It was reported that the seaweed compost added to soil increased the uptake of major nutrients; liquid seaweed fertilizer is a unique combination of nitrogen, phosphorus, K, trace elements, alginates and simple sugars in dissolved form[12]. In sugarcane, efficacy of two seaweed saps *viz*., *Kappaphycus* and *Gracilaria* on growth, yield and quality aspects was tested through sett treatment as well as foliar application. Results showed that cane length and number of internodes significantly influenced cane yield by 40% with *Kappaphycus* application at 5% application[13]. Among the Sea6 bio formulations tested in plant and ratoon crops, LBS6 at the rate of 1.0 mL L^{-1} recorded significantly higher plant growth parameters, yield attributes, thereby increasing cane yield by 22.2 and 19%, respectively[14]. In the present study, we tested the efficacy of LBS6 on mitigation of drought stress in popular sugarcane varieties.

Materials and Methods

Research trial for testing the drought ameliorative effect of sea weed formulation

Two commercial varieties (Co 86032 and Co 0212) with six treatments (T_1: control; T_2: drought stress without management practices; T_3: drought stress water spray at 60, 90 and 120 days after planting (DAP); T_4 & T_5: drought stress + foliar application of Sea6 liquid formulation LBS6 @1 and 2mL L^{-1}, respectively at 60, 90 and 120 DAP; and T_6: drought stress + foliar application of 2.5% KCl at 60, 90 and 120 DAP) were planted in strip plot design at ICAR-SBI, Coimbatore research farm as well as at farmers' field for confirmation. Drought stress imposed was during formative phase (60 to 120 DAP) of the crop by withholding irrigation for a period of 60 days. The soil moisture from random samples in the drought treatment was estimated at 90 and 120 DAP through gravimetric method, the percentage of soil moisture depletion was worked out as 40 and 52.8%, respectively as compared to control. Shoot population, plant height, leaf area index (LAI) and biomass accumulation was recorded at formative (FP), grand growth (GGP) and maturity phases (MP) by following routine procedure. Chlorophyll content (SPAD value), chlorophyll *a* fluorescence or photochemical efficiency (F_v/F_m) and chlorophyll stability index (CSI) was determined in the physiologically active leaf at formative phase. Cane yield attributes and juice quality was assessed at maturity phase of the crop. Yield and yield traits were assessed at harvest (360 DAP).

Confirmatory trial at farmers' field for testing sea6 formulation efficacy

A trial was conducted at farmers' field (Madathukulam taluk, Tiruppur district) with the variety Co 0212 and six treatments as mentioned previously. Efficacy of foliar application of Sea6 bioformulation LBS6 was assessed by recording shoot population, plant height, LAI and biomass accumulation at FP, GGP and MP of the crop. Cane yield attributes and juice quality was assessed at MP.

Statistical analysis

Data was subjected to analysis of variance; varieties being the main effect, while drought stress and drought management strategies were the sub effects. Means were compared based on the least significant differences at probability level $\alpha = 0.05$. Graphs were plotted in Microsoft Excel 2016.

Results and Discussion

Drought management practices on biometric parameters

Sugarcane, in general possess fairly high crop growth rate compared to other cultivated crops. After the initial canopy formation, stalk elongation contributes for crop growth rate, which is in turn

determined by the variety as well as environment. As cane length is an important parameter of final sink size in sugarcane, any reduction in plant height would result in reduced commercial yield. A strong positive relationship between stalk elongation and water content was noticed in earlier studies in sugarcane by Zhao *et al.*[15] and Gomathi *et al.*[5]. Reduction in shoot population, plant height and leaf area were quite obvious due to drought stress particularly in sensitive genotypes as reported in sugarcane[16-18]. Further they reported that the continuous water stress decreased the leaf expansion, thus suggesting the mechanism of leaf size determination under drought.

In present study, the crop growth parameters *viz*., shoot population, plant height, LAI, and total dry matter production (TDMP) recorded at different phenophases revealed that the irrespective of the varieties, these parameters were significantly influenced by drought stress. Data on shoot population showed that irrespective of the treatments, Co 0212 recorded higher values of 118.3×10^3, 103.5×10^3 and 94.8×10^3 ha^{-1} at FP, GGP and MP, respectively as compared to Co 86032 (106.1×10^3, 91.1×10^3 and 87.7×10^3 ha^{-1}) [Fig. 1A$_{(i)}$]. In Co 86032, drought induced significant reduction in shoot population of 25.2, 34.2 and 23.9%, at FP, GGP and MP, respectively, as compared to control, whereas the reduction in Co 0212 was 35.0, 33.3 and 22.7%. Foliar spray with 2.5% KCl (T_6) followed by LBS6 (T_5) caused comparatively less reduction in shoot population than that observed under drought (T_2), showing their efficacy in mitigating the effects of stress. Irrespective of varieties and treatments, plant height followed a linear trend from FP towards MP, while in general Co 0212 plants were taller than Co 86032 [Fig. 1A$_{(ii)}$]. Varieties, treatments and their interactive effect were statistically significant for plant height. Among the treatments 2.5% KCl spray (T_6) was found effective in maintaining higher plant height under drought situation in both the tested varieties, while this drought ameliorative effect was followed by foliar application of 2 mL L^{-1} of LBS6 (T_5).

Irrespective of varieties and treatments, LAI recorded highest values at GGP in Co 86032 (3.45)

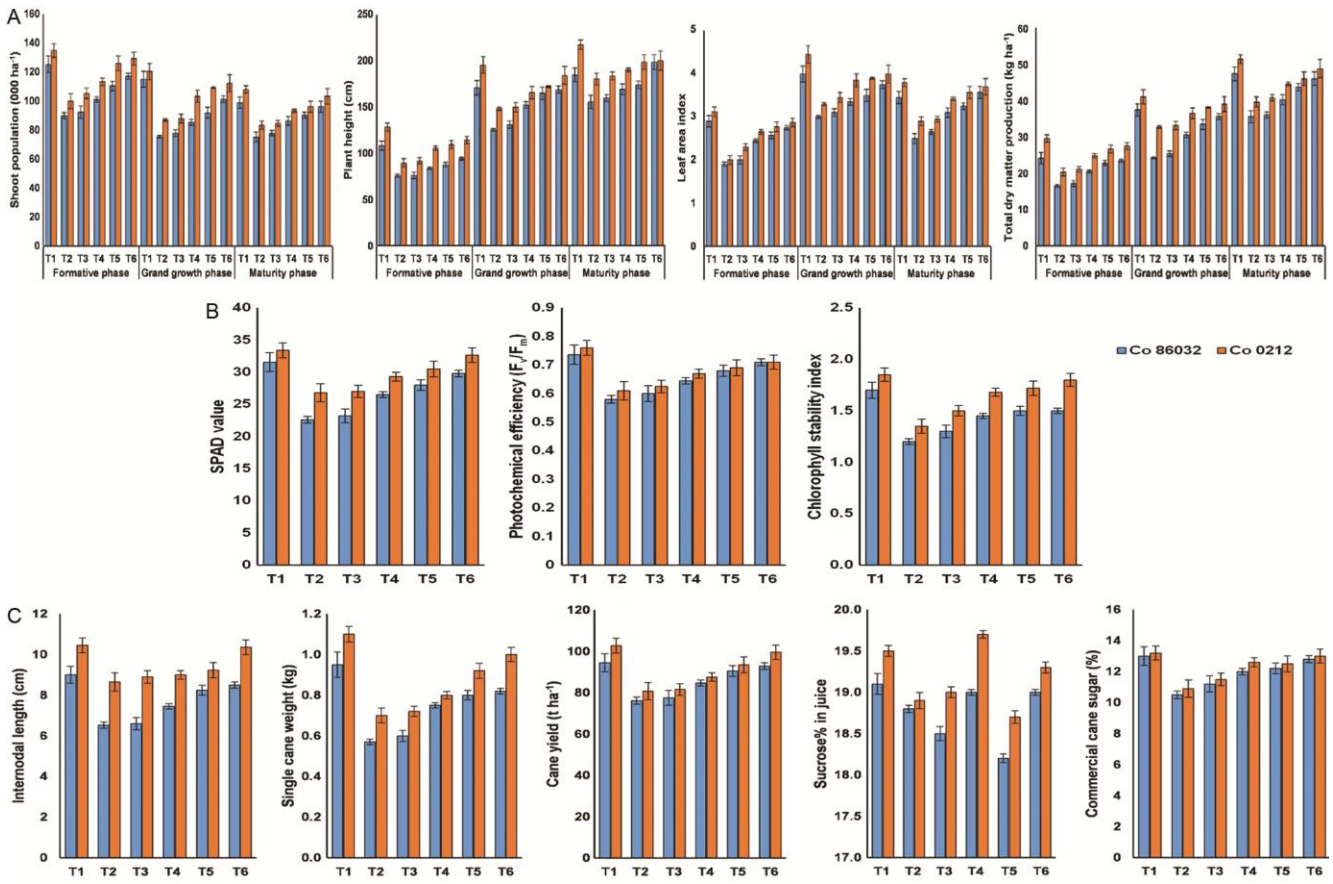

Fig. 1 — Impact of drought management practices on (A) growth traits; (B) physiological traits; and (C) yield and quality of sugarcane varieties

and Co 0212 (3.85) which declined during MP (Co 86032: 3.04 and Co 0212: 3.39) [Fig. 1A$_{(iii)}$]. Irrespective of the varieties, foliar application of 2.5% KCl spray (T_6) was found effective in maintaining higher LAI under drought condition, followed by foliar application of 2 mL L^{-1} of LBS6 (T_5). Treatments, varieties and their interactive effect were statistically significant for LAI. Irrespective of varieties and treatments, TDMP showed an increasing trend from FP (2.07 kg m^{-2} in Co 86032; 2.41 kg m^{-2} in Co 0212) to MP (3.67 kg m^{-2} in Co 86032; 4.45 kg m^{-2} in Co 0212) [Fig. 1A$_{(iv)}$]. Drought induced significant reduction in TDMP at FP (31.90% in Co 86032; 30.00% in Co 0212), GGP (34.20% in Co 86032; 20.01% in Co 0212) and MP (26.80% in Co 86032; 21.65% in Co 0212), indicating that drought recovery was faster in Co 0212 compared to Co 86032. Such genotypic differences in the ability to maintain leaf area and LAI might be associated with drought tolerance through the maintenance of high leaf water potential. In both the varieties, foliar application of 2.5% KCl spray (T_6) was found effective in maintaining higher TDMP under drought condition and this was followed by foliar application of 2 mL L^{-1} of LBS6 (T_5). Application of KCl spray on improvement on growth parameters *viz*. LAI and biomass accumulation in sugarcane has been reported earlier[8-10]. Similarly, there are reports available on improvement in plant height, LAI and TDMP value due to sea sap application in sugarcane under normal condition[14].

Drought management practices on physiological traits

In sugarcane, decline in SPAD value and F_v/F_m ratio are the sensitive and readily reliable non-destructive traits and it was routinely used to screen genotypes for drought tolerance potential in large germplasm pool as reported by Gomathi *et al.* (2013)[18] and Gomathi *et al* (2020)[5]. In present study, drought induced 28.30, 19.76 and 29.40% reduction in SPAD value, photochemical efficiency (F_v/F_m) and CSI, respectively in Co 86032, while in case of Co 0212 the reduction was 19.7, 19.13 and 27% over control indicating the drought tolerance potential of the latter (Fig. 1B). Foliar application of 2.5% KCl was effective in mitigating drought effect on SPAD value (5.5 and 2.2% reduction over control in Co 86032 and Co 0212), F_v/F_m ratio (3.53 and 6.5% reduction over control in Co 86032 and Co 0212, respectively) and CSI (11.1 and 2.7% reduction over control in Co 86032 and Co 0212). Foliar application of Sea6 bioformulation LBS6 (2 mL L^{-1}) showed 11.3, 7.6 and 11.1% reduction in SPAD value, F_v/F_m ratio and CSI in Co 86032, respectively, while in Co 0212 the reduction was 8.7, 9.2 and 7.2% over control, respectively. Results of physiological data indicated that among the drought management treatments, 2.5% KCl spray closely followed by Sea6 bioformulation LBS6 (2 mL L^{-1}) was effective in maintaining better physiological efficiency under drought situation. Similar improvement in SPAD value due to sea sap foliar application was reported in sugarcane under normal condition[14]. As the Sea6 bioformulation is rich in K (5%), the turgor potential of the leaf sap is maintained under stress. As K ions plays major role in regulating the opening and closing of stomata which governs the transpiration loss of water and thus application of potassium has been found to increase the sugarcane growth during water stress and finally cane yield[10,14]. These finding confirms the present results.

Drought management practices on cane yield attributes and juice quality

Plant growth especially crop yield in sugarcane is highly sensitive to water stress and showing drastic reduction in yield attributes due to drought during the formative phase[19]. Data on yield and yield attributes indicated that drought stress resulted in 33.30, 40.00 and 19.41% reduction over control in internodal length, single cane weight (SCW) and cane yield, respectively in Co 86032, while in Co 0212; the reduction was 17.2, 36.3 and 19.0% over control (Fig. 1C). Traits such as synchronized tillering, early canopy closure and rapid stem elongation contribute to higher cane yield during drought[5,20]. This variation in these parameters causes genotypic differences in cane yield which were in agreement with the present finding. However, the reduction was comparatively less in case of 2.5% KCl sprayed plots Co 86032 (5.5, 13.7 and 1.6%) and Co 0212 (2.4, 9.0 and 3.1%). Foliar spray of seaweed bioformulation LBS6 (2 mL L^{-1}) was also found effective in ameliorating drought stress to maintain better yield attributes in both the tested varieties. Under drought condition, in Co 86032, foliar application of KCl (2.5%) and seaweed extract LBS6 (2 mL L^{-1}) resulted in comparatively higher cane yield of 92.9 and 89.5 t ha^{-1}, respectively with 18.0 and 16.5% yield improvement over untreated drought plot (Fig. 1C). In Co 0212, foliar application of KCl (2.5%) and LBS6 (2 mL L^{-1}) recorded 99.0 and 93.5 t ha^{-1}, respectively with 18.50 and 15.20% yield improvement over untreated drought plot. Despite the drastic reduction under drought stress, cane yield and its attributes

significantly improved through foliar application of KCl and seaweed bioformulation LBS6. Application of potassium has been found to increase the sugarcane growth during water stress and finally increased the cane yield[8-10]. The present experiment results also confirm the earlier findings.

Reduction in juice quality due to drought has been reported in sugarcane by Gururajarao & Singh[21]. However, such a drastic difference or reduction in juice quality due to drought stress was not observed in the present study. In general, drought led to the inversion of sucrose into hexose forms (glucose and fructose) thereby resulting in poor juice quality. Juice quality assessment at 12th month indicated that drought treatment did not cause significant difference in sucrose% in juice, whereas commercial cane sugar % (CCS%) varied considerably in both the tested varieties [Fig. 1C$_{(iv, v)}$]. It indicated that as compared to control, there was not much difference in the juice quality, which was not affected by foliar application of Sea6 bioformulation. Similar findings were also reported in sugarcane grown under normal condition by Gomathi *et al.*[14].

Drought management practices on economics of Co 86032 and Co 0212

Analysis of the cost of cultivation, gross and net return from one hectare of sugarcane subjected to drought and other mitigation treatments revealed that in both tested varieties, control (T_1) had higher gross and net return as compared to the other treatments (Table 1). Under drought condition, foliar application of 2.5% KCl recorded higher net returns of Rs. 82,141 ha^{-1} (Co 86032) and Rs. 1,01,103 ha^{-1} (Co 0212) with BCR of 1.44 and 1.55, respectively. Seaweed bioformulation LBS6 (2 mL L^{-1}) recorded comparatively higher gross returns of Rs. 74,277 ha^{-1} (Co 86032) and Rs. 82,826 ha^{-1} (Co 0212) with BCR of 1.40 and 1.45, respectively. The treatment T6 (2.5% KCl spray) recorded additional benefit of Rs. 38,533 ha^{-1} in Co 86032 and Rs. 44,386 ha^{-1} in Co 0212, as compared to untreated drought plot (T_2), followed by T_5 (Seaweed bioformulation LBS6 2 mL L^{-1}) (Rs. 30,669 ha^{-1} in Co 86032 and Rs. 26,109 ha^{-1} in Co 0212). From this study, it may be concluded that the foliar application of 2.5% KCl and seaweed extract LBS6 (2 mL L^{-1}) under drought condition could increase cane yield with higher economic returns and benefit: cost ratio as reported in earlier studies in sugarcane under normal condition[14].

Seaweed bioformulation spray mitigates ill effects of drought stress in terms of sugarcane growth, yield and economic aspects

Drought resulted in 26.9, 19.3, 25.0, 27.5 and 26.8% reduction over control in shoot population, plant height, SPAD value, LAI and TDMP of Co 0212 at FP, respectively. However, the reduction was comparatively less in the treatment T6 (7.7, 7.1, 8.5, 8.0 and 5.7%) closely followed by T_5 (9.2, 10.2, 9.9, 13.7 and 13.9%). Among the drought management treatments, 2.5% KCl spray was found to be effective in maintaining better physiological efficiency under drought situation, followed by Sea6 bioformulation LBS6 (2 mL L^{-1}). Similar trend was observed at grand growth phase (GGP) and maturity phase (MP) as well. Data on yield and its attributes indicated that drought stress induced 24.9, 20.8, 18.9, 28.1 42.6 and 31.7% reduction over control in the number of millable canes (NMC), cane length, number of internodes, internodal length, single cane weight (SCW) and cane yield, respectively. However, foliar application of 2.5% KCl and Sea6 bioformulation LBS6 (2 mL L^{-1}) were found to be effective in mitigating drought stress, resulting in significantly higher yield of 105.2 and 103.1 t ha^{-1} with minimum reduction over control of 19.2 and 20.1%, respectively. The treatment T_6 and T_5 showed 16.2 and 14.0% yield improvement over untreated drought plot (T_2). Under drought condition, foliar application of 2.5% KCl spray

Table 1 — Effect of drought and different drought management strategies on the cost of cultivation, gross and net returns, benefit cost ratio and additional benefit obtained in terms of currency INR (₹)

Treatment	Cost of cultivation (₹ ha^{-1})		Gross return (₹ ha^{-1})		Net return (₹ ha^{-1})		Benefit cost ratio		Additional benefit (₹ ha^{-1})	
	Co 86032	Co 0212	Co 86032	Co 0212	Co 86032	Co 0212	Co 86032	Co 0212	Co 86032	Co 0212
T_1	1,78,425	1,78,425	2,69,301	2,92,954	90,876	1,14529	1.50	1.64	NA	NA
T_2	1,73,400	1,73,400	2,17,008	2,30,117	43,608	56,717	1.25	1.32	NA	NA
T_3	1,74,400	1,74,400	2,20,855	2,32,425	46,455	58,025	1.26	1.33	2,847	1,308
T_4	1,81,525	1,81,525	2,41,316	2,49,638	59,791	68,113	1.32	1.37	16,183	11,396
T_5	1,83,625	1,83,625	2,57,902	2,66,451	74,277	82,826	1.40	1.45	30,669	26,109
T_6	1,82,600	1,82,600	2,64,741	2,83,703	82,141	1,01,103	1.44	1.55	38,533	44,386

[*Details of the treatment are mentioned in Materials and Methods; 'NA' denotes 'not applicable']

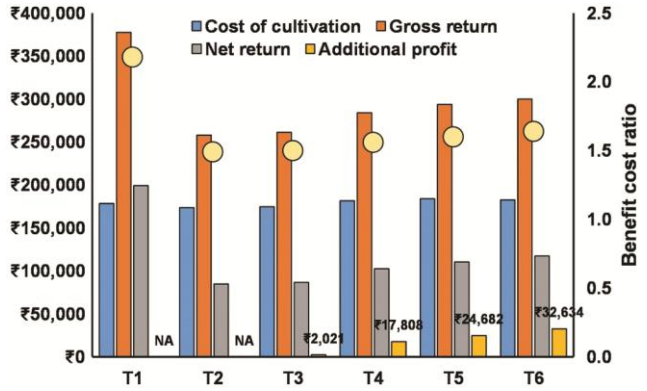

Fig. 2 — Drought management practices on economics of the variety Co 0212 at farmer's field experiment.

recorded higher net returns of Rs. 1,17,136 ha^{-1} compared to untreated drought plot (T_2), with BCR of 1.64 and additional benefit of Rs. 32,634 ha^{-1}, which was closely followed by seaweed bioformulation LBS6 (2 mL L^{-1}) with gross returns of Rs. 1,10,682 ha^{-1}, BCR of 1.60 and additional benefit of Rs. 25,682 ha^{-1} (Fig. 2). Results obtained from research trials were thus validated in the farmer's field that the routine supplemental application of 2.5% KCl and Sea6 bioformulation LBS6 (2 mL L^{-1}) at specific phenophases was effective in mitigating drought stress in sugarcane variety Co 0212.

Conclusion

Results from the research trial and its validation at farmers' field indicated that supplemental application of 2.5% KCl and seaweed bioformulation LBS6 (2 mL L^{-1}) significantly and positively influenced growth, physiology and cane yield under drought condition. In most of the traits observed, seaweed bioformulation LBS6 (2 mL L^{-1}) was either on par or second only to 2.5% KCl spray in ameliorating the ill effects of drought on sugarcane. Since LBS6 has 60 g kg^{-1} of K in bioavailable form, in present study, we tested its efficacy on mitigation of drought stress in popular sugarcane varieties. The influence of such bioformulations on the growth and physiological traits at important growth phases have significant effect on the final cane yield under drought stress, thereby may be recommended as one of the best drought management strategies in sugarcane.

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Conflicts of interest

Authors declare no competing interests.

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