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Differential growth, yield and biochemical responses of maize to the exogenous application of *Kappaphycus alvarezii* seaweed extract, at grain-filling stage under normal and drought conditions

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ABSTRACT

Maize is sensitive to water stress at flowering and grain-filling stages and thus strategies for the mitigation of stress and improving the yield sustainably would be beneficial. Two factorial pot experiments were carried out to assess the efficacy of a biostimulant product obtained from *Kappaphycus alvarezii* in conjunction with recommended rate of fertilizers (RRF), in mitigating moderate and severe stress in maize. The plants were subjected to normal as well as water deficit conditions following which *Kappaphycus alvarezii* seaweed extract (KSWE) was applied foliarly only once at the grain-filling stage along with a suitable control (water spray + RRF). Growth and yield parameters along with antioxidant enzymes, metabolites and reactive oxygen species content in leaves were studied. The results revealed that water deficit conditions (moderate and severe) significantly ($p < 0.001$) reduced the yield in both the trials, by 22 and 74% as compared to plants receiving the normal irrigation schedule, while KSWE increased the yield by 14% and 23% ($p < 0.01$), respectively. KSWE applied once at the grain-filling stage increased seed yield only under normal condition and not under stress conditions in both the seasons. The increase in seed yield observed under the KSWE treatments was predominantly through the increase in all the yield attributes, especially, cob length, the number of grains per cob and length of grain fill, however, the 100-seed weight remained unaltered. Increased activities and concentrations of enzymatic and non-enzymatic antioxidants due to KSWE application with concomitant reduction in reactive oxygen species, such as $\cdot O_2^-$ and H_2O_2 levels, both under normal and stressed conditions, resulted in a lesser degree of oxidative stress in KSWE treated plants. Thus, the results revealed that a single application of KSWE, even at a late crop stage can be beneficial under normal irrigated condition.

1. Introduction

Maize is an important food crop and in the major cultivation belts of India, United States, China and other parts of the world, the crop is predominantly cultivated under rain-fed conditions [1–3]. It is well known that water deficit reduces the yield of maize [4] as the crop is vulnerable to stress at flowering and grain-filling stage [5,6]. The scenario of higher temperatures and increasing trend of evapotranspiration following global climate change [7–10] is perceived as one of the potential threats to the productivity of maize as it would result in greater incidence of drought in most of the regions where the crop is grown, especially in the semi-arid regions, where there is severe depletion of ground water as well as other irrigation resources. Further, the variation in the spatio-temporal distribution of rainfall in the rain-fed areas often results in dry spells, especially during the monsoon season which prevails in Indian subcontinent. This phenomenon would manifest into transient drought like situations severely impacting maize productivity in rain-fed areas. Thus sustainable strategies need to be adopted for mitigating such abiotic stresses in addition to using tolerant varieties.

Du Jardin (2015) [11] defined plant biostimulant as “any substance or microorganism applied to plants with the aim to enhance nutritional efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrient content”. Seaweeds are an important source of biostimulants and the extracts or products obtained from them have been

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used to demonstrate their efficacy in enhancing growth and yield sustainably in various crop plants in particular maize [12–15]. In addition, seaweed extracts (SWE) obtained from *Kappaphycus alvarezii*, *Jania rubens, Sargassum muticum*, *Ascophyllum nodosum*, *Ecklonia maxima, Ulva rigida* have been reported to alleviate various abiotic stresses such as cold, drought and salinity [16–21]. Recently, we have identified critical growth stages in maize which elicit optimal response to foliar applications of a *Kappaphycus alvarezii* seaweed extract (KSWE) [22] wherein two applications of the KSWE at V5 and V15 stage were shown to have beneficial effect on productivity. Further, in another report we provided empirical evidence implicating the glycine betaine present in KSWE as one of the important constituent imparting tolerance to maize plants grown under water deficit conditions [23]. In the above experiments as well as in other reports of the available literature, *Ascophyllum*, *Ecklonia* SWE as well as other commercial extracts were applied mostly during the vegetative stage of the plant growth cycle in maize. Further, to the best of our knowledge, none of the reports available in the literature have correlated the beneficial effects of SWE application with yield under drought, except, Trivedi et al. (2018) [23]. In addition to quaternary ammonium compounds like glycine betaine, KSWE also contains plant growth regulators, carrageenans, phenolic compounds, micro nutrients which either alone or in combination might play a major role in improving crop productivity in addition to functioning in stress alleviation. As mentioned earlier, maize plants are susceptible to drought conditions occurring at the grain-filling stage. Therefore, we wanted to investigate whether KSWE (commercially sold as 'Aquasap') has the potential to alleviate stress resulting from water deficit conditions when foliar applied at the grain-filling stage in relation to control plants (water + RRF) under normal irrigation conditions. In addition, we also wanted to analyse the behaviour of antioxidants as well as the enzymes involved in quenching reactive oxygen species at the end of the stress period so as to interpret the response of the enzymes owing to KSWE application and relate these parameters with yield. Hence, experiments were carried out in consecutive seasons with the objective to study the mitigating effects of a single foliar application of KSWE under drought conditions simulated by two types of altered irrigation regimes at grain-filling.

2. Materials and methods

2.1. Study area and experimental design

Two pot experiments were carried out in the net house facility of the institute situated in Bhavnagar (21°44′57.6″N latitude, 72°08′39.3″E longitude), Gujarat. The experiments were carried out during the *rabi* season (November–March) of 2013–14 and 2016–17. The treatments consisted of evaluating KSWE at 10% concentration on maize plants at their grain-filling stage (occurring at 70 and 79 days after sowing (DAS) in the two trials) along a water sprayed control in combination with two different irrigation regimes (normal and water deficit condition). The plants were subjected to a single foliar spray with 10% concentration of KSWE (v/v) at grain-filling stage while plants without KSWE were sprayed with an equivalent amount of water, as our previous experiments [14,15] with different concentrations of KSWE (0, 2.5, 5, 7.5, 10 and 15%) revealed that KSWE when applied at 10% concentration elucidated optimal response in maize under different agro-ecological conditions. Foliar application of KSWE as a mist was carried out with a hand held sprayer. Two to three drops of adjuvant (Dhanuvit) was added to KSWE and water (control) while spraying to facilitate adhesion and prevent drifting. The plants were subject to water deficit treatment immediately after the foliar application of the KSWE, prior to which all the plants received irrigation as per the regular schedule at the rate of two litres of water applied manually every third day as a drench.

In the first season experiment (E1), under normal irrigation regime (I+), the plants continued receiving water till harvest as per the schedule mentioned above, while under stressed condition (I−), the plants were watered at a lesser frequency, i.e., every fifth day from the date of application of KSWE and the process was continued till harvest. A longer with-holding period of 13 days was given in the second season experiment (E2) to test the validity of the results obtained in E1 under a longer period of severe stress. Normal irrigation was resumed to the plants after 13 days stress period in E2 till harvest.

Table 1
Initial soil characteristics of the potting mix soil used in the experiments. Data shown as the mean ± SD, n = 3. WHC – water holding capacity.

Parameters	Values (mean ± sd)
EC (dS/m)	0.224 ± 0.006
pH	7.88 ± 0.07
WHC (%)	57.2 ± 3.7
Available N (mg/kg)	48.6 ± 2.3
Available P (mg/kg)	6.59 ± 0.39
Available K (mg/kg)	384 ± 8
Ca (mg/kg)	1686 ± 234
Mg (mg/kg)	789 ± 61
Na (mg/kg)	35.4 ± 3.0
Organic C (%)	1.71 ± 0.3

Each treatment was replicated five and eight times in E1 and E2, respectively and was laid out in a completely randomized design. A pot with a single plant constituted a replication. Each pot was filled with 32 kg of potting mix consisting of 1:2:1 ratio of black soil, red soil and farmyard manure. In addition, chemical fertilizers were applied at the recommended rate of 120:60:40 kg ha^{-1} of N: P_2O_5: K_2O through urea, single super phosphate and sulphate of potash, respectively. The physico-chemical composition of the soil is shown in Table 1. The maize variety used in the study was Sugar-75 (Syngenta). Four seeds were sown in each pot, which after successful germination was thinned to a single plant per pot. The meteorological data during both the growing seasons is given in supplementary table 1 (Table S1).

2.2. Preparation of KSWE

KSWE was prepared as per the procedure described in Singh et al. (2016) [15]. *Kappaphycus alvarezii* seaweed that was cultivated along the coast of Mandapam in Tamil Nadu, India was used for extract preparation. The seaweeds were initially washed with seawater to remove adhering foreign particles and the wet biomass was directly milled in a grinder (without addition of water) and the pulp was centrifuged in order to obtain the extract. Preservatives (0.02% propylparaben; 0.2% methyl paraben and 0.1% potassium benzoate) were added to the extract to prevent deterioration. The same amount of preservatives was also added to water in control treatment. The extract thus obtained was considered as 100% concentration of KSWE. KSWE approximately had 4% of soluble solids (w/v). From the above extract 10% solution was prepared by appropriate dilution with water. Each plant was sprayed with a hand held sprayer with 40 ml of 10% KSWE or water as per treatment. Thus KSWE was foliar applied on maize plants at a concentration 10% (v/v). The composition of KSWE was described earlier in Layek et al. (2015) [14] and the same batch of the extract was also used in the present experiments.

2.3. Sample collection

Biochemical analyses were carried out on leaf samples that were collected at 0, 5 and 15 DAT (days after treatment) in E1, while at 13 DAT in E2. The samples were collected immediately before spraying and prior to watering at 0 day (0 d) while the samples were collected just before irrigation at 5 d and 15 d respectively in E1 and in a similar way at 13 d in E2. The leaves were snap-freezed immediately in liquid nitrogen and stored at minus 80 °C until further analysis.