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Drought alleviatory potential of *Kappaphycus* seaweed extract and the role of the quaternary ammonium compounds as its constituents towards imparting drought tolerance in *Zea mays* L.

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Abstract

Kappaphycus seaweed extract (KSWE) has been reported to enhance the yield of several crops under normal conditions. The detection of the quaternary ammonium compounds (QACs) glycine betaine and choline chloride (GBCh) in KSWE impelled us to explore its efficacy on growth and yield under soil moisture deficit conditions. Another objective was to elucidate the extent to which the QACs have a role in mitigating drought stress, if at all. Thus, a factorial experiment was carried out on maize plants, wherein five foliar treatments (KSWE at 10 and 15%; GBCh at equivalent concentration to that found in 10 and 15% KSWE, respectively, designated as 10 and 15% Eq-GBCh; and water spray control) were subjected to three soil moisture stress levels (well-watered, WW; moderately stressed, MS; and severely stressed, SS). Observations were recorded for growth and yield parameters, pigments, photosynthetic attributes, antioxidant enzymes, and quality of grains. The results revealed the ability of KSWE to alleviate soil moisture stress, 10% KSWE being effective in increasing the seed yield under WW and MS conditions while 15% being optimal under SS condition. The percent increases in seed yield over their respective controls under WW, MS, and SS conditions at the optimal KSWE concentrations were 13.5, 21.7, and 36.4%, respectively, indicating higher grain yield response to KSWE treatments at higher stress levels. The yield advantage under stress could be attributed to minimal damage of photosystem in KSWE-treated plants as evidenced by higher pigment content, photosynthetic rate, reduced photoinhibition, and lipid peroxidation by enhanced protection against reactive oxygen species. The protein content in grains was enhanced by KSWE application under all stress groups compared to their respective controls. Although the predominant role of GBCh in KSWE towards drought mitigation and yield response was apparent, the results also connoted towards the role of other constituents in KSWE acting in unison along with GBCh, which should be investigated further.

Keywords Rhodophyta · Biostimulant · Maize · Moisture stress · Antioxidant defense system · Glycine betaine · Choline chloride

Introduction

Soil moisture deficit leading to drought is one of the major constraints in agriculture towards achieving higher crop productivity (Kumar et al. 2017). Unpredicted drought conditions are increasing day by day due to a gradual rise in global warming and climate change, which makes crop production more susceptible to severe water stress (Rasul et al. 2011; Nawaz et al. 2015). In contrast, food demand is increasing continuously. Among all cereals, maize is the most preferred food in southern and eastern Africa, some parts of Asia, Central America, and Mexico (Ranum et al. 2014; Singh et al. 2016). By 2050, its demand is expected to double, but its production has been predicted to decrease by 10% due to several factors (CGIAR research programme on maize 2015). Drought and lower soil fertilities are the two major reasons

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behind lesser crop yields (Edmeades and Deutsch 1994). Maize is least tolerant to water stress (Muchow 1989). The instance of drought 1 week before silking and 2 weeks after silking in maize has been reported to decrease the grain yield by 53% (Aslam et al. 2013).

Soil moisture deficit affects a number of morphological and physiological processes in plants. It decreases transpiration, photosynthesis, and reproductive processes and leads to the accumulation of compatible solutes, adjustment in root growth, reduction in the expansion of aerial organs, and transcriptional regulation of several genes (Mansori et al. 2016). Under drought condition, photosystem II (PSII) of the photosynthetic machinery is more affected compared to photosystem I (PSI) which in turn generates more high-energy free electrons in leaves. A diminution in CO_2 fixation induces more production of reactive oxygen species (ROS), which damage cell organelles like chlorophyll and cell membranes as well as macromolecules like nucleic acids, proteins, and lipids eventually leading to a reduction in growth and crop yield. Sustainable technologies are therefore needed to increase or maintain the maize production even under unfavorable conditions like water deficit.

Quaternary ammonium compounds like glycine betaine and choline chloride are compatible solutes and have been found to have drought alleviation potential (Chen and Murata 2002). Glycine betaine has been reported to be localized in plastids, chloroplasts, and cytosol upon foliar application to plants (Park et al. 2006). It is synthesized in plants by either oxidation of choline or by *N*-methylation of the glycine (Chen and Murata 2002). It offers protection to PSII against abiotic stress in transgenic plants at concentrations of the order of μmol g^{-1} fresh weight (Chen and Murata 2008). Besides its effect on the acceleration of repair of the photodamaged PSII under abiotic stress, it is also responsible for inducing expression of various genes including those involved in synthesis of transcription factors, components of membrane-trafficking, and ROS-scavenging enzymes like catalase and other genes whose products are involved in stress tolerance (Park et al. 2006; Chen and Murata 2008).

Seaweed is an organic source whose agricultural use as a plant biostimulant has been reported by many researchers (Beckett and van Staden 1989; Atzmon and van Staden 1994; Jayaraj et al. 2008; Spann and Little 2011; Calvo et al. 2014; Colla and Rouphael 2015; Singh et al. 2016; Rouphael et al. 2017a). Their role as stress alleviator under moisture stress has been reported by several researchers, viz., Zhang and Ervin (2004) in bentgrass, Spann and Little (2011) in sweet orange, Xu and Leskovar (2015) in spinach, Elansary et al. (2016) in medicinal plant species, Mansori et al. (2016) in sage, and Martynenko et al. (2016) in soybean. The seaweed extracts are a concoction of several constituents which include various macro- and micronutrients, plant growth regulators, and quaternary ammonium compounds. These constituents have been purported to be primarily responsible for the observed beneficial crop responses, but despite many studies, the precise role of individual constituents and the mechanism of their action are still unclear (Khan et al. 2009; Craigie 2011; Calvo et al. 2014; Mondal et al. 2015). Understanding the mechanism will help to develop more efficient and effective ways for its application to get an optimum response.

While most studies are based on extracts of cold water seaweed species like *Ascophyllum nodosum*, the tropical growing *Kappaphycus alvarezii* has come into prominence in the recent years on account of being able to derive multiple products including the use of its extract as crop biostimulant (Mondal et al. 2013). *Kappaphycus* seaweed extract (KSWE) has been reported to enhance the productivity of several crops like rice (Pramanick et al. 2014), blackgram (Pramanick et al. 2016), greengram (Raverkar et al. 2016), soybean (Rathore et al. 2009), and maize (Layek et al. 2015). KSWE has a low associated carbon footprint and can reduce global warming potential per unit of crop production upon its use (Ghosh et al. 2015; Singh et al. 2016). In our earlier study, quaternary ammonium compounds (QACs) like glycine betaine and choline chloride in the KSWE were quantified, and their role in enhancing the yield of maize was established (Mondal et al. 2015). Since these compounds are known osmoticants providing abiotic stress tolerance to the plants, it was hypothesized that the KSWE might also have drought-alleviating potential.

Thus, an experiment was designed to assess the effect of KSWE on maize productivity under water deficit condition and also ascertain the extent to which these QACs might be responsible for the alleviatory effect and the possible concentrations that may be effective under different soil moisture stress regimes.

Material and methods

Preparation of KSWE

KSWE was prepared through the patented procedure of Eswaran et al. (2005). Seaweed cultivated along the sea coast of Tamil Nadu State, India, was used as a source of the extract. After initial washing of the seaweeds to remove the adhering foreign particles, it was ground and centrifuged to obtained pristine sap (extract) in the form of filtrate. It was further preserved by adding a mixture of 0.02% propylparaben, 0.2% methylparaben, and 0.1% potassium benzoate (Singh et al. 2016; Trivedi et al. 2017). This extract was considered as 100% concentrated seaweed sap from which further dilutions were prepared as per the treatments. Analyses of constituents like minerals, inorganic ions, quaternary ammonium compounds, and plant growth regulators were carried out according to Mondal et al. (2015). The detailed composition of

seaweed extract is published in Layek et al. (2015) and Singh et al. (2016). The same batch of extract was also used in the present experiment (Table 1).

Study area and experimental design

The pot experiment using maize (sweet corn, variety Sugar 75, Syngenta) was conducted at the net house facility (21° 44′ 57.6″ N, 72° 08′ 39.3″ E) of CSMCRI, Bhavnagar district, Gujarat, India, during *Kharif* season (July to October 2014). Treatments were distributed in three groups as per the degree of moisture stress i.e., WW, MS, and SS. This was initiated by irrigating the pots immediately after germination with two liters of water was applied to each pot in every 48 h (WW), 72 h (MS), and 96 h (SS). Each group contains five common treatments: T1, water spray—control; T2, 10% KSWE; T3, 15% KSWE; T4, 10% glycine betaine (GB) + choline mixture (GBCh); and T5, 15% GBCh, which was applied as finely atomized foliar spray at 25 days after sowing (DAS; first), 59 DAS (second), and 78 DAS (third). Each treatment was replicated three times and laid out in completely randomized design (CRD). All the pots were filled with 32 kg of soil to which chemical fertilizers at the recommended rate of 120:60:40 kg ha^{-1} of $N/P_2O_5/K_2O$ were applied uniformly to all the treatments through urea, single super phosphate, and sulfate of potash, respectively. The initial soil of the experiment was sandy loam in texture, having pH 7.88 and electrical conductivity 0.22 dS m^{-1}. Available N, P, and K were 109, 15, and 166 kg ha^{-1}, respectively. Organic carbon was 0.41%. Four seeds were sown in each pot, which after successful germination was thinned to single plant per pot.

Table 1 Composition of KSWE (Singh et al. 2016)

Sr. no.	Constituent	KSWE (mg L^{-1})
1	Indole 3-acetic acid (IAA)	26.52
2	Zeatin	19.65
3	GA_3	23.65
4	Choline	57.30
5	Glycine betaine	79.33
6	Sodium	198.0
7	Potassium	33,654
8	Calcium	321.0
9	Magnesium	1112.0
10	Zinc	4.7
11	Manganese	2.1
12	Iron	86.1
13	Chromium	32.0
14	Copper	0.65
15	Nickel	3.45
16	Phosphorous	17.45

Growth and yield attributes

Growth, yield, and other yield attributes were measured in between the experiment and also at harvest. Plant height was measured at different time intervals (32, 60, and 86 DAS) including one prior to treatment application (23 DAS). Plant height was measured as the distance from the soil surface to the last leaf collar (at 32 and 60 DAS) or with tassel (at 86 DAS). Fresh, sundried, as well as oven-dried weights of different plant parts (grain, leaf, stem, and root) were recorded after harvest. Oven-dried weight expressed in g $plant^{-1}$ is represented in tables as dry matter accumulation (DMA). Root volume was also recorded by water displacement method. Dried and dead leaf numbers at harvest were also counted and shown in Table 2. Length and width of each leaf were recorded at 32 DAS. Measurements on the cob were done just after harvest of fresh cobs. The grains were separated from the cobs after sun-drying and expressed as g $plant^{-1}$ (Table 4).

Photosynthetic parameters

Photosynthetic rate and F_v/F_m ratio were measured using an infrared gas analyzer system (IRGA; Model Li-6400XT, Li-Cor, USA) at the photosynthetic photon flux density (PPFD) of 1000 μmol photons m^{-2} s^{-1}. External CO_2 was maintained at the constant value. Initial values of the minimum (F_0) and maximum (F_m) fluorescence yield were recorded in the dark state of leaves (before dawn). Chlorophyll index (CI) was measured using a chlorophyll content meter (Model CCM-200, Opti-Sciences Inc., USA). Total chlorophyll, chlorophyll *a*, chlorophyll *b*, and carotenoids were determined according to Arnon (1949) using 480, 510, 645, 652, and 663 nm wavelength.

Plant sampling

Uniform sampling from three replicates of each treatment was carried out after the third spray frozen immediately in liquid nitrogen and stored at − 80 °C prior to analysis.

Enzyme extraction

All operations were carried out at 0–4 °C. For glutathione reductase (GR) and catalase (CAT) assays, leaf tissue (0.1 g fresh weight) was frozen in liquid nitrogen and homogenized in a mortar using an extraction buffer (pH 7.5) containing Tris (62.5 mM), EDTA (0.1 mM), Triton X-100 (0.2%), polymethylsulphonyl fluoride (PMSF) (1 mM) and dithiothreitol (DTT) (2 mM). The homogenates were thoroughly vortexed and centrifuged at 18,000×*g* for 30 min at 4 °C. The supernatant was collected and stored at − 80 °C till further analysis. For ascorbate peroxidase (APX) assay, the extraction was carried out in 62.5 mM potassium phosphate extraction

Table 2 Growth parameters of maize crop as affected by different foliar treatments in all three water regimes

Water regimes	Treatments	Plant height (cm)				Dry matter accumulation (g plant^{-1})				Root volume (cc plant^{-1})	Leaf RWC (%) (27 DAS)	No. of dry leaves per plant (86 DAS)	Leaf length (cm) (32 DAS)	Leaf width (cm) (32 DAS)
		23 DAS (before treatment)	32 DAS	60 DAS	86 DAS	Root	Leaf	Stem	Total					
Well-watered (WW)	Control	28.8^{a}	42.0^{bcd}	239.0^{bc}	242.0^{abc}	16.5^{ab}	30.1^{a}	30.6^{b}	77.2^{ab}	96.7^{ab}	76.3^{abc}	7.0^{bcd}	37.4^{cd}	3.2^{abc}
	10% KSWE	27.5^{a}	46.6^{a}	251.7^{a}	257.7^{a}	18.0^{a}	29.2^{a}	33.8^{a}	81.0^{ab}	103.3^{a}	81.9^{ab}	6.7^{bcd}	45.3^{abc}	3.0^{abc}
	15% KSWE	27.2^{a}	44.2^{ab}	230.7^{cd}	237.0^{bcd}	14.0^{abcd}	27.3^{ab}	31.0^{b}	72.3^{abc}	86.7^{abc}	84.2^{ab}	5.7^{cd}	44.1^{abc}	2.8^{bc}
	10% Eq-GBCh	26.7^{a}	44.7^{ab}	246.7^{ab}	248.0^{ab}	15.8^{ab}	26.4^{ab}	31.4^{b}	73.6^{abc}	90.0^{ab}	85.9^{a}	4.7^{d}	46.5^{ab}	2.9^{abc}
	15% Eq-GBCh	27.3^{a}	44.6^{ab}	249.8^{ab}	256.0^{a}	15.7^{ab}	31.0^{a}	35.0^{a}	81.6^{a}	103.3^{a}	85.1^{a}	8.0^{bcd}	46.8^{ab}	3.0^{abc}
Moderately stressed (MS)	Control	19.7^{bcd}	38.1^{ef}	229.5^{cd}	233.3^{bcd}	9.7^{de}	24.5^{ab}	26.2^{cd}	60.4^{d}	70.0^{c}	63.7^{de}	8.0^{bcd}	37.4^{cd}	2.7^{c}
	10% KSWE	21.0^{b}	41.2^{cd}	242.3^{abc}	251.0^{ab}	14.0^{abcd}	28.3^{a}	28.6^{bc}	70.9^{bc}	93.3^{ab}	80.2^{ab}	7.3^{bcd}	51.9^{a}	3.1^{abc}
	15% KSWE	20.0^{bcd}	40.1^{cde}	222.0^{d}	227.0^{cd}	11.0^{cde}	23.5^{ab}	29.2^{bc}	63.8^{cd}	83.3^{abc}	80.7^{ab}	7.3^{bcd}	45.1^{abc}	2.7^{c}
	10% Eq-GBCh	20.3^{bc}	42.7^{bc}	238.7^{bc}	240.7^{abc}	13.2^{bcd}	29.4^{a}	29.0^{bc}	71.5^{abc}	93.3^{ab}	74.6^{bc}	8.0^{bcd}	50.7^{a}	3.3^{abc}
	15% Eq-GBCh	20.0^{bcd}	39.5^{de}	232.0^{cd}	235.0^{bcd}	15.3^{abc}	28.6^{a}	29.3^{bc}	73.2^{abc}	96.7^{ab}	69.5^{cd}	9.0^{bc}	43.2^{abc}	3.2^{abc}
Severely stressed (SS)	Control	15.5^{bcde}	32.8^{g}	199.5^{fg}	202.0^{e}	7.2^{ef}	19.3^{bc}	22.5^{ef}	48.9^{e}	53.3^{d}	59.4^{e}	13.0^{a}	34.8^{d}	3.0^{abc}
	10% KSWE	13.8^{e}	32.1^{g}	193.0^{gh}	202.3^{e}	4.8^{f}	14.9^{c}	18.4^{g}	38.1^{f}	33.3^{e}	70.0^{cd}	9.3^{bc}	39.6^{bcd}	3.6^{ab}
	15% KSWE	14.5^{de}	36.6^{f}	212.0^{e}	225.3^{cd}	10.8^{cde}	25.0^{ab}	24.8^{de}	60.7^{d}	80.0^{bc}	77.0^{abc}	8.3^{bcd}	47.1^{ab}	3.0^{abc}
	10% Eq-GBCh	13.5^{e}	33.1^{g}	186.0^{h}	194.3^{e}	6.8^{ef}	16.1^{c}	21.3^{f}	44.3^{ef}	56.7^{d}	69.5^{cd}	9.3^{bc}	48.7^{ab}	3.6^{a}
	15% Eq-GBCh	15.2^{cde}	33.6^{g}	207.3^{ef}	220.7^{d}	12.7^{bcd}	27.1^{ab}	24.9^{de}	64.6^{cd}	80.0^{bc}	59.9^{e}	10.0^{b}	43.2^{abc}	2.9^{abc}
Analysis of variance table														
Water regimes (factor A)		***	***	***	***	***	***	***	***	***	***	***	ns	*
Foliar treatments (factor B)		ns	**	**	**	**	*	***	***	***	***	**	***	**
Water regimes × foliar treatments (A × B)		ns	**	***	***	***	**	***	***	***	*	ns	*	*

Values represented are mean of three independent replicates. Values followed by different superscript letters in the columns are significantly different using Student-Newman-Keuls test

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

buffer (pH 7.0) containing 2 mM ascorbate in addition to containing all the above ingredients as described earlier except Tris. Ascorbate was added to prevent inactivation of APX during its isolation. Total protein was quantified by method of Bradford (1976) using bovine serum albumin (BSA) as a standard.

Antioxidant enzyme assays

Glutathione reductase assay Glutathione reductase (GR) (EC 1.6.4.2) activity was measured according to Edwards et al. (1990). The GSSG (oxidized glutathione)-dependent oxidation of NADPH was monitored by the decrease in absorbance at 340 nm at 25 °C. Briefly, the assay mixture with a final volume of 1 mL contained 100 mM *N*-2-hydroxyethyl-piperazine-*N*-2-ethanesulfonic acid [HEPES] (pH 7.8), 1 mM EDTA, 3 mM $MgCl_2$, and 0.5 mM GSSG. The reaction was initiated by the addition of NADPH (8 mM) and extract (10–25 μl). Corrections were made for non-enzymatic reduction of GSSG by NADPH. The activity was calculated using an extinction coefficient of 6.22 M^{-1} cm^{-1} for NADPH. One unit of GR is defined as 1 μmol NADPH oxidized per minute per mL at 25 °C and expressed as unit per mg protein.

Catalase assay Catalase (EC 1.11.1.6) activity was measured following the procedure described by Aebi (1984). Catalase (CAT) activity was assayed spectrophotometrically at 25 °C in a 1-mL reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 10–25 μL of extract, and 10 mM of H_2O_2 at final concentration. The reaction was started by the addition of hydrogen peroxide and the activity measured by monitoring the decrease in absorbance at 240 nm over 150 s against an extract-free blank. Enzyme activity was calculated using the molar coefficient 0.043 mM^{-1} cm^{-1} with one unit of catalase defined as 1 μmol of H_2O_2 decomposed/consumed per minute per mL at 25 °C and expressed as unit activity per mg protein.

Ascorbate peroxidase assay Total APX (EC 1.11.1.1) activity was measured according to Nakano and Asada (1981). The assay depends on the decrease in absorbance at 290 nm as ascorbate was oxidized. The reaction mixture at final concentration contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM EDTA, 1.2 mM H_2O_2, and 10–25 μL of enzyme extract in a final assay volume of 1 mL. The concentration of oxidized ascorbate was calculated by using the extinction coefficient of 2.8 mM^{-1} cm^{-1}. One unit of APX was defined as 1 mM mL^{-1} ascorbate oxidized per minutes and expressed as unit per mg protein.

Determination of lipid peroxidation

Lipid peroxidation was determined by thiobarbituric acid (TBA) method as described by Hodges et al. (1999). Total malondialdehyde content was measured in three replicates of each treatment. Leaf tissues (0.1 g fresh weight) were homogenized in a mortar and pestle with 4 mL of 80:20 (*v*/*v*) ethanol/water and 5% PVPP followed by centrifugation at 3000×*g* for 10 min. A 1-mL aliquot of the supernatant was added to a test tube with 1 mL of either (Aebi, 1984) −TBA solution comprised of 20.0% (*w*/*v*) trichloroacetic acid and 0.01% butylated hydroxytoluene or (Arnon, 1949) +TBA solution containing the above plus 0.65% TBA. Samples were then mixed vigorously, heated in a water bath maintained at 95 °C for 25 min, cooled, and centrifuged at 3000×*g* for 10 min. Absorbance was read at 440, 532, and 600 nm using a spectrophotometer. Malondialdehyde equivalents were calculated in the following manner:

$$[(\text{Abs } 532_{+\text{TBA}})-(\text{Abs } 600_{+\text{TBA}})-(\text{Abs } 532_{-\text{TBA}}-\text{Abs } 600_{-\text{TBA}})] = A$$

$$[(\text{Abs } 440_{+\text{TBA}}-\text{Abs } 600_{+\text{TBA}})\ 0.0571] = B$$

$$\text{MDA } \textit{equivalents } \left(\text{nM mL}^{-1}\right) = (A–B/157000) \times 10^6$$

Hydrogen peroxide estimation

Hydrogen peroxide (H_2O_2) content was estimated according to Velikova et al. (2000). Fresh leaf tissue (0.1 g) was crushed in liquid nitrogen and homogenized in 0.1% *w*/*v* TCA solution followed by centrifugation at 10,000 rpm for 10 min. Of supernatant, 0.5 mL was added to a separate test tube containing 0.5 mL of 10 mM phosphate buffer (pH 7.0) and 1 M potassium iodide (KI). The absorbance was read at 390 nm. H_2O_2 concentration [mM H_2O_2 g^{-1} fresh weight (FW)] in the sample was determined from a calibration curve prepared using known concentrations of H_2O_2.

Relative water content (%)

Leaf relative water content (RWC) was measured according to Barrs and Weatherley (1962). Three similar-sized leaf portions were collected from the youngest leaf each time from every plant of every treatment for determination of RWC. Leaf samples were wiped with tissue paper to remove foreign particles. Fresh weight (FW) was recorded immediately and samples were placed in 10-mL deionized water for 8 h at room temperature. Leaves were then gently blotted dry with tissue paper and the turgid weight (TW) was recorded. Leaf samples were then transferred to the oven for complete drying at 70 °C for 48 h and later dry weight (DW) was recorded. RWC was than calculated as per following formula:

$$\text{RWC } (\%) = [(\text{FW}-\text{DW})/(\text{TW}-\text{DW})] \times 100$$

Nutrient quality parameters

Macronutrients N, P, and K were determined by digesting the plant samples with sulfuric acid-selenium-salicylic acid mixture as described by Novozamsky et al. (1983) followed by estimation through SKALAR The San++ continuous flow analyzer where N was estimated by colorimetric Berthelot reaction (Krom 1980; Searle 1984) and P was estimated by formation of a blue-colored phosphomolybdenum complex by reduction with ascorbic acid. K was estimated by fame photometric method described in *Plant Analysis Procedures* (Temminghoff and Houba 2004).

Secondary macronutrients Ca and Mg and micronutrients (Fe, Zn, Mn) were determined by digesting the plant samples in the di-acid mixture (nitric acid and perchloric acid) as described by Miller (1998) followed by estimation through inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 2000, PerkinElmer). Carbohydrate was estimated by anthrone method (Yemm and Willis 1954), and protein was determined by multiplying the factor 6.25 with nitrogen content (Jones 1941).

Statistical analysis

The experiment was laid out in CRD with three levels of water regimes (WW, MS, and SS) and five foliar treatments (water, KSWE (10 and 15%), and Eq-GBCh (10 and 15%)) which were forming 15 combinative treatments (5 × 3) as mentioned above. Analysis of variance was carried out using MSTAT C software (Michigan State University, USA) employing two-factor CRD, and the significance of the two main effects and their interaction were determined at 5% level of confidence. For the interactions which were found significant, post hoc comparison of means was carried out using Student-Neuman-Keuls range test and the mean separation significance was tested at the level of $p < 0.05$.

Results

Growth and photosynthetic parameters

Growth and photosynthetic parameters of maize crop are presented in Tables 2 and 3. The mean plant height measured just before applying the foliar treatments (23 DAS) revealed significant variations among WW (27 to 29 cm), MS (20 to 21 cm), and SS conditions (14 to 16 cm), while there were no significant differences among the foliar spray treatments within the same soil moisture regimes. Periodic observations till harvest revealed that the diminution in the plant height could be rescued up to moderate soil moisture stress by application of KSWE as well as 10% Eq-GBCh when compared to WW condition. This was, however, not possible at severe stress levels, wherein the plant height decreased markedly compared to WW condition (Table 2). The treatments receiving GBCh in equivalent concentrations as their KSWE counterparts were statistically at par at either of the concentrations for final plant height attained under stressed conditions.

The volume and DMA in roots varied significantly due to the main effects of stress as well as foliar spray treatments. Their interaction was also found significant ($p < 0.001$). Striking differences in root volume were obtained by application of 10% KSWE under MS and 15% KSWE under SS conditions which were 33.3 and 50.1% higher over their respective controls. Both 10 and 15% Eq-GBCh did not increase root volume over control in the WW condition. Under SS condition, only the highest concentration of Eq-GBCh was superior over its control with respect to root volume. In case of DMA in roots, 15% Eq-GBCh treatments were superior compared to their controls under MS and SS conditions, both of which were at par with KSWE having an equivalent concentration of these compounds in the respective stress groups.

Under WW condition, 10% KSWE and 15% Eq-GBCh were found better than its control for DMA in the stem; however, they were at par with each other. Under MS and SS, none of the foliar spray treatments were better over their respective control for this parameter. None of the KSWE or Eq-GBCh levels resulted in higher leaf DMA under WW, MS, or SS, compared to their respective controls. No improvement in total DMA (excluding cob) was observed by foliar treatments compared to its control under WW condition. However, beneficial differences were observed by 10 and 15% KSWE under MS and SS conditions, respectively. Ten percent KSWE was at par with 10% Eq-GBCh under MS, while 15% KSWE was at par with 15% Eq-GBCh under MS and SS conditions, respectively. Even though there was no change in leaf biomass, significantly higher leaf RWC was observed by application of the foliar spray treatments over their respective controls under stressed regimes, except in the case of 15% Eq-GBCh under MS and SS conditions (Table 2).

The total number of leaves formed did not vary due to either different stress conditions or the different foliar spray treatments and ranged from 13 to 15 leaves per plant. However, the number of dried and dead leaves per plant at harvest varied significantly due to the two main effects, viz., stress conditions as well as foliar treatments. Analysis of the main effect of foliar spray treatments revealed that the number of dried leaves per plant was least (7.1) in 15% KSWE-treated plants which was closely followed by 10% Eq-GBCh (7.3) and 10% KSWE (7.7), all of which were at par to each other but significantly lower than the control that recorded 9.3 for this parameter. The significantly lower number of dried leaves found due to KSWE treatments compared to control apparently led to the prolonged longevity of the photosynthetic machinery which might have resulted in higher photosynthate formation. Although the length of leaf recorded at 32 DAS

Table 3 Photosynthetic parameters of maize crop as affected by different foliar treatments in all three water regimes

Water regimes	Treatments	Chlorophyll index (CI)			Chlorophyll *a* (mg g^{-1} FW)	Chlorophyll *b* (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)	Photosynthetic rate (72 DAS)	F_v/F_m ratio (72 DAS)
		32 DAS	60 DAS	86 DAS						
Well-watered (WW)	Control	35.5^{de}	29.6^{ab}	36.1^{cd}	0.771^{cde}	0.269^{cd}	1.117^{abcd}	50.3^{def}	12.0^{def}	0.633^{ef}
	10% KSWE	46.6^{a}	34.1^{a}	47.9^{a}	0.884^{a}	0.378^{ab}	1.434^{a}	60.8^{a}	17.9^{a}	0.789^{ab}
	15% KSWE	42.3^{bc}	34.0^{a}	44.4^{ab}	0.831^{abc}	0.319^{bc}	1.260^{abc}	55.9^{abcd}	13.4^{bcde}	0.729^{bc}
	10% Eq-GBCh	42.5^{bc}	34.1^{a}	44.6^{ab}	0.880^{a}	0.291^{cd}	1.339^{abc}	50.3^{def}	15.6^{b}	0.747^{b}
	15% Eq-GBCh	45.4^{ab}	34.6^{a}	44.3^{ab}	0.894^{a}	0.260^{cd}	1.374^{ab}	51.5^{cdef}	14.5^{bcd}	0.707^{bcde}
Moderately stressed (MS)	Control	33.4^{def}	20.9^{de}	26.1^{ef}	0.672^{f}	0.223^{de}	1.026^{cd}	46.4^{efgh}	10.6^{f}	0.620^{f}
	10% KSWE	43.5^{bc}	28.7^{bc}	36.6^{cd}	0.777^{cde}	0.409^{a}	1.341^{abc}	57.9^{abc}	15.4^{bc}	0.657^{cdef}
	15% KSWE	36.4^{d}	25.4^{bcd}	38.5^{bc}	0.802^{bcd}	0.337^{abc}	1.243^{abc}	59.1^{ab}	13.8^{bcde}	0.745^{b}
	10% Eq-GBCh	40.5^{c}	24.1^{cd}	31.7^{cde}	0.792^{bcd}	0.256^{cd}	1.237^{abc}	43.5^{fgh}	15.8^{b}	0.779^{ab}
	15% Eq-GBCh	42.47^{bc}	26.4^{bc}	30.5^{de}	0.859^{ab}	0.398^{ab}	1.339^{abc}	47.1^{efg}	15.9^{b}	0.646^{def}
Severely stressed (SS)	Control	27.8^{h}	13.1^{g}	9.4^{g}	0.573^{g}	0.189^{e}	0.916^{d}	39.7^{gh}	8.7^{g}	0.660^{cdef}
	10% KSWE	30.6^{fgh}	18.8^{ef}	11.4^{g}	0.743^{def}	0.277^{cd}	0.624^{e}	45.1^{efgh}	11.5^{ef}	0.713^{bcd}
	15% KSWE	33.6^{def}	21.4^{de}	25.1^{ef}	0.731^{def}	0.343^{abc}	1.333^{abc}	52.4^{bcde}	14.7^{bc}	0.765^{ab}
	10% Eq-GBCh	30.0^{gh}	16.3^{fg}	22.5^{f}	0.708^{ef}	0.320^{bc}	1.101^{bcd}	38.9^{h}	11.5^{ef}	0.788^{ab}
	15% Eq-GBCh	32.6^{efg}	15.7^{fg}	25.0^{ef}	0.745^{def}	0.343^{abc}	1.101^{bcd}	43.7^{fgh}	12.8^{cdef}	0.832^{a}
Analysis of variance table										
Water regimes (factor A)		***	***	***	***	*	***	***	***	***
Foliar treatments (factor B)		***	***	***	***	***	***	***	***	***
Water regimes × foliar treatments (A × B)		***	ns	***	ns	***	***	*	***	***

Values represented are mean of three independent replicates. Values followed by different superscript letters in the columns are significantly different using Student-Neuman-Keuls test

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

did not vary due to the main effect of stress conditions, it varied due to foliar treatments and their interaction (Table 2). Although the KSWE treatments did not elicit any change in leaf length under WW condition, however, 10 and 15% concentrations elicited beneficial response under MS and SS conditions, respectively. The corresponding GBCh concentrations were at par with the above treatments for leaf length. Leaf width was not influenced by the any of the foliar treatments under any of the stress conditions, compared to their respective controls.

Chlorophyll index (CI) was measured at 32, 60, and 86 (at harvest) DAS. At 32 DAS, 10% KSWE treatments showed maximum improvement in CI under WW and MS conditions compared to their respective control, which was at par with 15% Eq-GBCh treatments in their respective groups. In SS condition, 15% KSWE recorded maximum CI and was at par with 15% Eq-GBCh treated plants. At 60 DAS, the foliar treatments did not influence CI compared to its control under WW condition. However, 10% KSWE under MS and 15% KSWE under SS conditions recorded the maximum CI and were superior to their respective controls. At 86 DAS, 10% KSWE recorded significantly higher CI under WW and MS conditions compared to their controls, while 15% KSWE recorded the highest CI under SS condition (Table 3).

Estimation of chlorophyll *a* and *b*, total chlorophyll, and carotenoids revealed that they were significantly influenced by soil moisture regimes, foliar treatments, as well as their interaction. Among the KSWE levels, 10% concentration was found optimum towards significantly increasing the concentration of pigments over control under WW and MS conditions. On the other hand, 15% KSWE apparently was the optimum treatment that best enhanced the content of all of these pigments significantly over control under SS condition.

The photosynthetic rate at 32 DAS varied due to the main effects, viz., foliar spray and stress conditions, but their interaction was not significant (data not shown). At 72 DAS, the highest photosynthetic rate (14.9 μmol CO_2 m^{-2} s^{-1}) was obtained under 10% KSWE compared to only 10.5 μmol CO_2 m^{-2} s^{-1} under control and the interaction between the two main effects was also found significant. Under WW and MW conditions, 10% KSWE was significantly superior to control with respect to photosynthetic rate. Under SS condition, 15% KSWE, found to be at par with 15% Eq-GBCh, was superior to its control. Whereas no significant influence of the main effect foliar sprays on F_v/F_m was apparent at 32 DAS (data not shown), F_v/F_m values were significantly influenced by both the main effects and their interaction at 72 DAS. The highest F_v/F_m under WW condition was recorded in the treatment receiving 10% KSWE which was also at par with 10% Eq-GBCh, both of which were superior to its control in that soil moisture regime. Under MS and SS conditions, Eq-GBCh at the rate of 10 and 15%, respectively, recorded the highest F_v/F_m ratio, which was, however, at par with each other and significantly superior to the controls of all the stress categories. Under MS and SS conditions, 10% Eq-GBCh was statistically equivalent to 15% KSWE with respect to F_v/F_m (Table 3).

Yield parameters

Data on cob length (without husk) revealed no significant change due to foliar treatments compared to control under WW conditions, while the significant change was observed in the length of grain fill on the cob by application of 10% KSWE. In MS condition, only 10% KSWE among the different foliar treatments was found to significantly enhance the total cob length as well as the fill length, which were 25.2 and 25.5% higher than its control, respectively. Under SS condition, no change in cob length, as well as grain fill length, was apparent due to any of the foliar treatments. There was no significant effect of the foliar treatments on 100-seed weight, whereas it varied significantly due to different stress levels, recording decreasing boldness with increase in stress level. The interaction was found significant for this parameter. A conspicuous observation was found under SS condition, wherein the 100-seed weight was found significantly higher at 15% KSWE compared to its control. The interaction between the two main effects was found to be significant in case of number of seeds per cob, and it was found that under both WW and MS conditions, 10% KSWE was superior to their respective controls, recording 8.3 and 17.1% higher values, respectively (Table 4).

The fresh green cob weight of maize differed due to both the main effects and their interaction. Whereas 10% KSWE yielded the highest cob weight under WW and MS conditions, 15% KSWE yielded the highest under SS condition and the yields obtained under these treatments were significantly higher than their respective controls in each of their soil moisture regimes, recording increases of 15, 37.2, and 56.8%, respectively. Under WW and MS conditions, increasing the concentration of KSWE to 15% significantly decreased the green cob weight over its lower concentration of 10%; however, the cob yields were still significantly higher than their respective controls. The highest green cob yield at 10% KSWE was associated with highest cob diameter under WW condition, while the highest green cob yield at 10% KSWE was associated with the highest green cob length under MS condition. Notably, under well-watered and moderate stressed group, 10% KSWE was found at par with 15% Eq-GBCh treatment for green cob weight and length (Table 4).

The seed yield varied significantly due to different levels of stress as well as foliar treatments. Their interaction was also found significant. Ten percent KSWE recorded the maximum grain yield under WW and MS conditions, while 15% KSWE recorded the maximum under the SS condition. All of these treatments were significantly higher over their respective

Table 4 Yield and yield attributes of maize crop as affected by different foliar treatments in all three water regimes

Water regimes	Treatments	Cob parameters with husk			Cob parameters without husk			100 seed weight (g)	No. of seeds	Yield (g plant^{-1})
		Weight (g)	Length (cm)	Diameter (mm)	Full length (cm)	Grain fill length (cm)	No. of seed rows			
Well-watered (WW)	Control	160.3^{c}	22.5^{cd}	46.8^{c}	16.3^{ab}	13.0^{b}	14.0^{a}	10.6^{ab}	347.7^{b}	36.7^{b}
	10% KSWE	184.2^{a}	24.3^{bc}	50.8^{a}	16.6^{ab}	15.6^{a}	13.3^{ab}	11.2^{a}	377.0^{a}	42.2^{a}
	15% KSWE	173.3^{b}	26.6^{a}	49.2^{ab}	17.1^{a}	13.2^{b}	14.0^{a}	11.0^{a}	342.0^{b}	37.7^{b}
	10% Eq-GBCh	163.8^{c}	25^{ab}	49.3^{ab}	15.3^{abc}	11.6^{b}	13.0^{abc}	11.0^{a}	347.7^{b}	38.3^{b}
	15% Eq-GBCh	177.3^{ab}	23.5^{bc}	48.2^{bc}	15.8^{abc}	13.2^{b}	11.3^{abc}	10.7^{ab}	337.7^{b}	36.0^{b}
Moderately stressed (MS)	Control	77.8^{f}	17.4^{f}	41.4^{de}	12.3^{de}	9.8^{c}	13.0^{abc}	9.1^{cd}	246.3^{d}	22.5^{d}
	10% KSWE	107.0^{d}	20.8^{de}	43.1^{d}	15.4^{abc}	12.3^{b}	12.7^{abc}	9.8^{bc}	288.0^{c}	28.3^{c}
	15% KSWE	93.7^{e}	19.5^{e}	41.2^{de}	10.4^{efg}	9.6^{c}	10.7^{abc}	8.6^{d}	238.0^{d}	20.3^{e}
	10% Eq-GBCh	86.2^{f}	18.9^{ef}	39.7^{ef}	13.7^{cd}	8.9^{c}	12.7^{abc}	9.5^{c}	260.7^{d}	24.7^{d}
	15% Eq-GBCh	101.0^{d}	21.0^{de}	41.2^{de}	14.2^{bcd}	9.5^{c}	13.7^{ab}	9.9^{bc}	239.0^{d}	23.7^{d}
Severely stressed (SS)	Control	50.8^{h}	14.8^{g}	38.4^{fg}	9.7^{fg}	8.3^{c}	9.3^{bc}	7.2^{e}	155.0^{e}	11.2^{g}
	10% KSWE	49.7^{h}	15.0^{g}	37.3^{g}	9.4^{g}	8.2^{c}	8.7^{c}	7.0^{e}	94.7^{f}	6.7^{h}
	15% KSWE	80.2^{f}	20.4^{de}	41.5^{de}	11.8^{defg}	9.9^{c}	12.0^{abc}	8.4^{d}	176.7^{e}	14.8^{f}
	10% Eq-GBCh	49.0^{h}	19.8^{e}	36.3^{g}	10.4^{efg}	7.7^{c}	9.3^{bc}	6.9^{e}	165.0^{e}	11.3^{g}
	15% Eq-GBCh	70.3^{g}	20.2^{de}	37.2^{g}	12.1^{def}	9.2^{c}	10.7^{abc}	7.5^{e}	148.7^{e}	11.2^{g}
Analysis of variance table										
Water regimes (factor A)		***	***	***	***	***	***	***	***	***
Foliar treatments (factor B)		***	***	***	ns	***	ns	ns	ns	**
Water regimes × foliar treatments (A × B)		***	***	***	***	*	*	***	***	***

Values represented are mean of three independent replicates. Values followed by different superscript letters in the columns are significantly different using Student-Neuman-Keuls test

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

controls in each of their soil moisture regimes and the percent increases were 13.5, 21.7, and 36.4%, respectively, indicating higher grain yield response to KSWE treatments at higher stress levels. Further, the results indicated the requirement of higher concentrations of KSWE with increasing stress levels. Ten percent KSWE was found significantly superior to 10% Eq-GBCh under WW and MS conditions with respect to grain yield, while 15% KSWE was also found significantly superior to 15% Eq-GBCh under SS condition indicating the role of other KSWE constituents as well.

H_2O_2 content, lipid peroxidation level, and antioxidant enzymes

Under SS, both the KSWE concentrations lowered H_2O_2 levels relative to its control, while both the Eq-GBCh concentrations could not do so. Lowest lipid peroxidation level as measured by MDA content was observed in 10% KSWE-treated plants under WW and MS conditions, while the lowest under SS condition was observed in 15% KSWE-treated plants. Fifteen percent Eq-GBCh under WW condition and both 10 and 15% Eq-GBCh under MS and SS conditions also lowered the MDA content, though the diminution was lower than that by KSWE (Table 5).

In WW and MS conditions, the highest catalase activity was found in 10% KSWE-treated plants compared to controls of their respective soil moisture regimes. In contrast, under SS condition, the highest level of catalase activity was observed in 15% KSWE-treated plants which was at par with 15% Eq-GBCh. The APX activity in WW condition was found the highest in 15% Eq-GBCh treated plants, which was however at par with all other foliar treatments within the same stress group except control, which recorded the lowest. Similar to that in case of catalase activity, the highest APX activity was also recorded in 10 and 15% KSWE, respectively, in MS and SS conditions. In case of GR activity, plants in the WW condition did not register any significant change due to the foliar treatments compared with its control, but under MS and SS conditions, 10% KSWE-treated plants showed the highest GR activity compared to their respective controls of each group and the differences were significant (Table 5).

Grain quality

The nutrient content in grains differed for N, P, K, Ca, and Fe due to the two main effects, viz., stress and foliar spray, while their interaction was significant in case of N, K, Ca, Mg, and Fe. No significant effect was found due to either of the factors

Table 5 Antioxidant enzymes, hydrogen peroxide, and lipid peroxidation level of maize crop as affected by different foliar treatments in all three water regimes

Water regimes	Treatments	Catalase (transformed) (units mg^{-1} protein)	APX (units/mg of protein)	GR (units mg^{-1} protein)	H_2O_2 (mM g^{-1} FW)	MDA (nmol g^{-1} FW)
Well-watered (WW)	Control	5336^{e}	0.019^{j}	2.95^{i}	3.64^{c}	6.93^{cd}
	10% KSWE	8111^{d}	0.033^{ij}	4.44^{i}	3.82^{c}	4.55^{fg}
	15% KSWE	7775^{d}	0.047^{i}	4.32^{i}	4.20^{c}	5.32^{ef}
	10% Eq-GBCh	5482^{e}	0.043^{i}	4.33^{i}	3.62^{c}	6.37^{cde}
	15% Eq-GBCh	4748^{e}	0.055^{i}	3.94^{i}	4.16^{c}	5.50^{ef}
Moderately stressed (MS)	Control	$17{,}954^{c}$	0.245^{d}	71.08^{g}	4.88^{c}	8.41^{b}
	10% KSWE	$23{,}427^{b}$	0.388^{a}	87.13^{d}	4.12^{c}	5.18^{ef}
	15% KSWE	$14{,}705^{c}$	0.214^{e}	44.21^{h}	4.44^{c}	6.98^{cd}
	10% Eq-GBCh	$15{,}028^{c}$	0.317^{b}	74.08^{f}	4.70^{c}	6.08^{de}
	15% Eq-GBCh	$15{,}647^{c}$	0.275^{c}	82.26^{e}	3.91^{c}	6.28^{cde}
Severely stressed (SS)	Control	$24{,}569^{b}$	0.111^{h}	84.15^{e}	6.08^{b}	10.28^{a}
	10% KSWE	$29{,}560^{ab}$	0.136^{g}	117.60^{a}	4.67^{c}	7.38^{c}
	15% KSWE	$38{,}015^{a}$	0.219^{e}	108.10^{c}	3.86^{c}	3.98^{g}
	10% Eq-GBCh	$29{,}604^{ab}$	0.165^{f}	111.30^{b}	8.97^{a}	7.47^{c}
	15% Eq-GBCh	$34{,}191^{a}$	0.180^{f}	87.26^{d}	9.42^{a}	6.07^{de}
Analysis of variance table						
Water regimes (factor A)		***	***	***	***	***
Foliar treatments (factor B)		***	***	***	***	***
Water regimes × foliar treatments (A × B)		***	***	***	***	***

Values represented are mean of three independent replicates. Values followed by different superscript letters in the columns are significantly different using Student-Neuman-Keuls test

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

or their interaction with respect to Mn and Zn. Ten percent KSWE recorded significantly higher N compared to its control under WW condition and was at par with 10% Eq-GBCh. Increasing the KSWE to 15% significantly brought down the N and K% in grains compared to that in 10% KSWE, although they were at par with their controls under WW condition. Ten percent Eq-GBCh enhanced the K uptake in grains which was markedly higher than that by 10% KSWE, but this was true only under WW condition. Under MS condition, 10% KSWE was significantly superior to its control with respect to N, K, and Fe but was at par to 10% Eq-GBCh for these nutrients. Under SS condition, N and Fe were significantly increased by 15% KSWE than its control and were at par with 15% Eq-GBCh (Table 6).

The carbohydrate content in grains was enhanced due to KSWE at 10% concentration only under MS condition. The protein content was influenced significantly over their respective controls in all the stress groups, wherein it was found that 10% KSWE was superior under WW and MS conditions while 15% KSWE was superior under SS condition. In all these stress categories, the corresponding KSWE concentration eliciting the best response was at par to its Eq-GBCh counterpart.

Discussion

The detection of the quaternary ammonium compounds in significant amounts in the KSWE (Mondal et al. 2015) prompted this study to assess its potential for alleviating soil moisture stress tolerance as these compounds have known osmotic properties. The concomitant objective of the study was to determine to what extent GBCh present in sap is responsible for eliciting a beneficial response, if at all. Accordingly, the equivalent concentration of exogenous GBCh as present in the KSWE at either 10 or 15% dilution levels was applied. The results confirmed the conjecture that KSWE can ameliorate soil moisture stress and could significantly enhance grain yield of maize over their respective controls not only under normal conditions but also under stress conditions that we defined as moderate and severe.

Differential pattern of response to the foliar treatments (KSWE and Eq-GBCh) under different stress conditions was obtained compared to their respective controls with respect to growth parameters, pigment content, photosynthetic parameters, yield attributes, stress indicators, and nutritional levels in grains. It was also brought out that to elicit a response, a particular threshold of KSWE concentration is needed depending on the severity of stress. It was found that for most of the parameters, 10% KSWE was sufficient to elicit significant beneficial response under WW and MS conditions, while 15% KSWE was required under SS.

Drought stress has been known to reduce leaf RWC in maize (Jun and Junying 1994; Liu and He 1995; Nagy et al. 1995; Bai et al. 2006). Song et al. (1995) reported characteristic higher RWC in maize plants tolerant to drought stress. The root hydraulic conductivity possibly is reduced by

Table 6 Nutrient quality parameters of maize grain as affected by different foliar treatments in all three water regimes

Water regimes	Treatments	Macroelements (%)					Microelements (ppm)			Grain quality parameters (%)	
		N	P	K	Ca	Mg	Fe	Mn	Zn	Carbohydrate	Protein
Well-watered (WW)	Control	1.00^{f}	0.09^{cd}	0.47^{de}	0.018^{ab}	0.061^{b}	14.6^{bcde}	6.0^{a}	15.3^{a}	55.7^{abc}	6.3^{e}
	10% KSWE	1.19^{bcd}	0.10^{abcd}	0.51^{cd}	0.012^{bc}	0.077^{ab}	25.7^{ab}	7.0^{a}	13.6^{a}	61.5^{a}	7.4^{cd}
	15% KSWE	1.05^{ef}	0.09^{d}	0.43^{e}	0.009^{c}	0.069^{ab}	21.2^{abcd}	6.6^{a}	13.5^{a}	54.7^{abc}	6.6^{e}
	10% Eq-GBCh	1.18^{cd}	0.10^{bcd}	0.62^{ab}	0.011^{c}	0.076^{ab}	11.4^{cde}	7.2^{a}	12.0^{a}	53.6^{bcd}	7.4^{cd}
	15% Eq-GBCh	1.27^{bcd}	0.10^{abcd}	0.59^{abc}	0.010^{c}	0.079^{a}	7.7^{e}	7.6^{a}	12.6^{a}	55.8^{abc}	7.9^{bc}
Moderately stressed (MS)	Control	1.14^{de}	0.10^{bcd}	0.52^{cd}	0.021^{a}	0.072^{ab}	12.6^{cde}	5.7^{a}	13.8^{a}	48.7^{cde}	7.1^{d}
	10% KSWE	1.32^{bc}	0.11^{abc}	0.63^{a}	0.010^{c}	0.071^{ab}	32.2^{a}	7.5^{a}	13.1^{a}	57.8^{ab}	8.3^{b}
	15% KSWE	1.20^{bcd}	0.09^{bcd}	0.54^{bcd}	0.008^{c}	0.070^{ab}	13.2^{cde}	5.8^{a}	12.6^{a}	50.4^{bcde}	7.5^{bcd}
	10% Eq-GBCh	1.27^{bcd}	0.10^{bcd}	0.56^{abc}	0.013^{bc}	0.066^{ab}	23.2^{abc}	5.1^{a}	14.8^{a}	56.4^{abc}	7.9^{bc}
	15% Eq-GBCh	1.29^{bc}	0.10^{abcd}	0.59^{abc}	0.012^{bc}	0.065^{ab}	27.5^{a}	7.1^{a}	16.4^{a}	55.3^{abc}	8.1^{bc}
Severely stressed (SS)	Control	1.34^{b}	0.10^{abcd}	0.57^{abc}	0.005^{c}	0.075^{ab}	8.7^{de}	6.1^{a}	13.9^{a}	47.1^{de}	8.4^{b}
	10% KSWE	1.29^{bc}	0.11^{ab}	0.57^{abc}	0.007^{c}	0.075^{ab}	21.3^{abcd}	6.4^{a}	17.0^{a}	44.4^{e}	8.0^{bc}
	15% KSWE	1.43^{a}	0.11^{abcd}	0.54^{bcd}	0.009^{c}	0.072^{ab}	22.8^{abc}	6.6^{a}	17.5^{a}	54.0^{abcd}	9.0^{a}
	10% Eq-GBCh	1.33^{bc}	0.11^{ab}	0.52^{cd}	0.008^{c}	0.068^{ab}	19.9^{abcde}	5.5^{a}	15.0^{a}	51.2^{bcd}	8.3^{b}
	15% Eq-GBCh	1.45^{a}	0.12^{a}	0.58^{abc}	0.007^{c}	0.075^{ab}	12.1^{cde}	5.3^{a}	15.9^{a}	52.2^{bcd}	9.1^{a}
Analysis of variance table											
Water regimes (factor A)		***	***	**	***	ns	**	ns	ns	***	***
Foliar treatments (factor B)		***	***	***	**	ns	***	ns	ns	*	***
Water regimes × foliar treatments (A × B)		***	ns	***	**	**	***	ns	ns	***	***

Values represented are mean of three independent replicates. Values followed by different superscript letters in the columns are significantly different using Student-Neuman-Keuls test

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

structural changes in roots of maize plants under drought stress affecting soil moisture uptake in maize (Liu et al. 2003). Notably, KSWE at 10 and 15% under MS and SS conditions significantly enhanced root volume over their respective controls which might have resulted in enhanced root surface area. This in turn might have enhanced root water uptake eventually translating into higher leaf RWC under stressed conditions. The higher leaf RWC in the stressed plants treated with the foliar treatments is suggestive of greater hydration status, thereby connoting that the treated plants experienced lesser stress. The improved leaf RWC, decreased number of dead leaves connoting inhibition of chlorophyll degradation, and greater leaf length in the present study may be attributed to the presence of GBCh which probably enhanced the volume of free (cytoplasmic) water per unit of cell dry weight and prevented the inhibition of drought-induced cell volume reduction leading to greater cell expansion. This is in accordance with Cayley et al. (1992) who reported the positive effect of GBCh in *Escherichia coli* and Saneoka et al. (1995) in maize under osmotically stressed conditions. Improvement in the contents of photosynthetic pigments (chlorophyll *a*, *b*, carotenoids) might have enhanced photosynthetic rate under WW as well as both the stress levels leading to high biomass and grain yield production.

Among the stress indicators, while catalase activity was significantly highest and MDA content was significantly lowest at 10, 10, and 15% KSWE under WW, MS, and SS conditions, respectively, GR was enhanced significantly compared to their respective controls only under MS and SS condition at 10 and 15% KSWE, respectively. H_2O_2 was however significantly decreased only under SS condition under either of the KSWE concentrations used. The antioxidant machinery in plants including antioxidant enzymes like catalase, GR, peroxidases, as well as other compounds such as carotenoids has been reported to be the principal defense against reactive oxidants formed under stress (Burke and Mahan 1991; Larson 1988). Evidently, application of KSWE to the stressed plants altered these compounds favorably to induce stress tolerance in the plants by increasing antioxidants and carotenoid levels and decreasing hydrogen peroxide and malondialdehyde levels as was also reported by Mansori et al. (2015) by application of seaweed extracts on bean plants.

Notably, the grain yield increase was also associated with significant improvement in N content and consequently protein content in grains of plants treated with 10% KSWE under WW and MS conditions and 15% KSWE under SS conditions. This might be attributed to better N uptake in plants through roots from soil by application of KSWE. Optimal N uptake may also favorably influence in maintaining higher levels of antioxidant enzyme activities under water deficit conditions (Sun et al. 2000) and our results corroborate the same towards increased tolerance to moisture stress in order to decrease yield loss. This is supported by results from another experiment carried out by us on the effect of KSWE at transcriptome level in roots under drought condition, wherein it was found that KSWE increased expression of nitrate transporters and nitrate reductase (Ghosh 2016). The same study also revealed a considerable increase in transcript abundance of genes related to cell wall modification, lipid metabolism, secondary metabolism, amino acid metabolism, and starch synthesis and a decrease in expression of genes related to carbohydrate degradation and carotenoid degradation under stress condition due to KSWE application compared to water sprayed control. The enhancement in root volume due to KSWE application could also be explained as the genes related to auxin signaling were found overrepresented in the transcriptome study. Such physiological and biochemical alterations could have favorably influenced the maize plants under distress in the present study.

Evidently, the bioactive ingredients present in the KSWE trigger different physiological responses differently which in turn are functionally related to the severity of stress and KSWE concentration as well. Conversely, the results implied that there is no set pattern of plant response elicited by KSWE treatment that is common across all stress categories. This is also evident by observations on yield attributes wherein it was found that while cob fill length and number of seeds per cob could be significantly enhanced by KSWE application at 10% under WW and MS condition, the same parameters were not influenced at SS condition even with higher KSWE dosage, when compared to the respective controls in their stress conditions. In contrast, 100-seed weight was influenced only under SS condition using 15% KSWE concentration. Nevertheless, the green cob and the grain yield being the resultant of all the yield attributes were significantly increased than its respective control under each of the water regimes either at KSWE concentration of 10% under WW and MS condition or at a higher concentration of 15% under SS condition.

In several plant species, GB has been reported to stabilize the quaternary structure of enzymes, maintain the complex ordered state of membranes, and protect the photosynthetic machinery by stabilizing the oxygen evolving PSII complex, thereby preventing disassociation of extrinsic polypeptides under abiotic stresses (Papageorgiou and Murata 1995). It is also reported that it stabilizes rubisco enzyme under stress condition and decreases the ROS generation, thus protecting the translational machinery especially that related to D1 protein synthesis (Chen and Murata 2008) which is important for photosynthesis.

While parameters like leaf width, leaf weight, and number of seed rows were not influenced by either KSWE or Eq-GBCh when compared to their respective controls irrespective of stress conditions, parameters like plant height at harvest, dried leaf number at harvest were found significantly influenced equally by 15% KSWE and its Eq-GBCh when compared over control only under SS condition. In the latter case,

it can be inferred that GBCh component of KSWE is responsible for the observed KSWE effect. Under both stress groups, GBCh component of KSWE could be entirely attributed for the observed beneficial response in case of leaf length, chlorophyll *a*, and protein content of grains, while similar interpretation could be made for root volume only in the case when the plants were subjected to either MS or SS. Under SS, compared to control, GBCh invariably significantly influenced vegetative growth parameters (increased root and aboveground plant biomass) through protection to photosynthetic machinery (increased chlorophyll *a*, chlorophyll *b*, photosynthetic rate, and reduced photoinhibition (F_v/F_m)), probably through enhanced ROS-scavenging activity (enhanced catalase, GR, and reduced MDA levels). Increased F_v/F_m ratio in 10 and 15% Eq-GBCh under WW, MS, and SS condition compared to that in their respective controls strongly suggests an alleviatory effect of GB under a stressful condition in which PSII complex along with the presence of GBCh can work better to reduce photo-induced inactivation which predominantly occurs under abiotic stresses like drought. Similar findings have been reported by Ma et al. (2006) in wheat and Rouphael et al. (2017b) in lettuce in which maintenance of high F_v/F_m by exogenous glycine betaine and plant-derived biostimulant application delays photoinhibition, contributing in better functioning of photosynthetic apparatus, which helps in increasing final yield of plants. Our observations on the marked improvement in the growth, pigment, photosynthesis parameters, F_v/F_m ratio, and activity of antioxidant enzymes are in agreement with the findings of these studies and connote towards the significant contribution of GBCh in the KSWE.

When comparing the most optimum concentration of KSWE (10% for WW and MS group and 15% for SS group) and the corresponding Eq-GBCh with respect to the different types of pigments, it can be observed that while KSWE and Eq-GBCh were at par for chlorophyll *a*, KSWE was found to be invariably superior to Eq-GBCh with respect to carotenoid content across all the stress categories and chlorophyll *b* content under WW and MS conditions. Carotenoids like zeaxanthin have been reported for its role in preventing oxidative damage of membranes under abiotic stress (Davison et al. 2002). Under severe stress, KSWE acted incremental over GBCh towards enhancing the yield parameters like 100-seed weight, cob diameter, and green cob weight and the antioxidant enzymes like catalase and GR, eventually improving the grain yield over and above GBCh application. Notably, under SS, KSWE treatment (at 15%) was superior to Eq-GBCh and control with highest GR, and lowest H_2O_2 and MDA levels, indicating that KSWE-treated plants experienced less stress. Enhanced GR activity helps in increasing $NADP^+$ ensuring its availability to accept electrons from the electron transport chain during photosynthesis (Sudhakar et al. 2001) which lessens the formation of $O_2^{\cdot-}$, further preventing the formation of highly reactive ·OH through the Haber-Weiss reaction (Demiral and Türkan 2004), preventing peroxidation of lipid membranes under abiotic stress (Hernandez et al. 2001; Sudhakar et al. 2001). A significant decrease in MDA content with elevated GR activity under stress condition suggests that exogenous application of KSWE can offer protection to the maize plants against moisture stress.

From these results, it is evident that glycine betaine has a prominent role towards eliciting beneficial plant response, but active ingredients other than these quaternary ammonium compounds in the KSWE may also have an incremental role which synergistically acts upon the plants to affect growth and yield improvement upon KSWE application under normal as well as stress conditions. Notably, KSWE contains plant growth regulators like cytokinins (kinetin, zeatin), gibberellic acid, indoleacetic acid, and macronutrient like potassium might complement in alleviating the drought stress. Positive correlation of cytokinins with carotenoid content—the non-enzymatic antioxidant—has been reported by Cortleven et al. (2014). Kinetin can directly scavenge the ROS and downregulate the activity of lipoxygenase, preventing the formation of ROS (Prakash et al. 1990). Besides, they also have been implicated in increasing the rate of photosynthesis (Rouphael et al. 2017a), delayed senescence (Blunden 1977), and better partitioning of photosynthates to plant parts (Stevens and Westwood 1984). The role of cytokinins and potassium in KSWE on maize was also elucidated by Mondal et al. (2015). Further experiments need to be done to evaluate the role of other constituents in KSWE including their synergistic or antagonistic interactions as well.

Conclusion

The experimental results brought out that KSWE can effectively alleviate soil moisture stress and enhance seed yield of maize. KSWE resulted in higher green cob yield that can fetch higher income to the farmers, and the associated greater cob size in terms of length or diameter can additionally fetch a premium price in the market. In general, optimal crop response was obtained at 10% KSWE concentration under WW and MS conditions, while 15% KSWE concentration was found optimal under SS condition. It can be concluded that the crop response varied with a dose of the KSWE and severity of stress. The predominant role of GBCh as a constituent of KSWE towards modulating various physiological and biochemical processes in the plants under normal condition and drought stress was elucidated but the observations on several parameters also pointed towards the role of other active ingredients present in KSWE that synergistically elicit beneficial crop response.

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References

Aebi H (1984) Catalase. Meth Enzym 105:121–126

Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24(1):1–15

Aslam M, Afzal I, Yaseen M, Mubeen M, Shoaib A (2013) Drought stress, its effect on maize production and development of drought tolerance through potassium application. Cercet Agron în Mold 46:99–114

Atzmon N, van Staden J (1994) The effect of seaweed concentrate on the growth of *Pinus pinea* seedlings. New For 53:187–189

Bai L-P, Sui F-G, Ge T-D, Sun Z-H, Y-Y L, Zhou G-S (2006) Effect of soil drought stress on leaf water status, membrane permeability and enzymatic antioxidant system of maize. Pedosphere 16:326–332

Barrs HD, Weatherley PE (1962) A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust J Biol Sci 15:413–428

Beckett RP, van Staden J (1989) The effect of seaweed concentrate on the growth and yield of potassium stressed wheat. Plant Soil 116(1):29–36

Blunden G (1977) Cytokinin activity of seaweed extracts. In: Faulkner DJ, Fenical WH (eds) Marine natural products chemistry. Plenum Press, New York, pp 337–344

Bradford M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem 72:248–254

Burke JJ, Mahan JR (1991) Environmental regulation of cellular protection systems. In: Gausman HW (ed) Plant biochemical regulators. Marcel Dekker, Inc, New York, pp 47–58

Calvo P, Nelson L, Kloepper JW (2014) Agricultural uses of plant biostimulants. Plant Soil 383:3–41

Cayley S, Lewis BA, Record MT (1992) Origins of the osmoprotective properties of betaine and proline in *Escherichia coli* K-12. J Bact 174:1586–1595

CGIAR research programme on maize (2015) Why maize. http://maize.org/why-maize/. Accessed 29 March 2017

Chen THH, Murata N (2002) Enhancement of tolerance of abiotic stress by metabolic engineering of beatines and other compatible solutes. Curr Opin Plant Biol 5:250–257

Chen THH, Murata N (2008) Glycinebetaine: an effective protectant against abiotic stress in plants. Trends Plant Sci 13:499–505

Colla G, Rouphael Y (2015) Biostimulants in horticulture. Sci Hortic 196:1–2

Cortleven A, Nitschke S, Klaumünzer M, AbdElgawad H, Asard H, Grimm B, Riefler M, Schmülling T (2014) A novel protective function for cytokinin in the light stress response is mediated by the ARABIDOPSIS HISTIDINE KINASE2 and ARABIDOPSIS HISTIDINE KINASE3 receptors. Plant Physiol 164:1470–1483

Craigie JS (2011) Seaweed extract stimuli in plant science and agriculture. J Appl Phycol 23:371–393

Davison PA, Hunter CN, Horton P (2002) Overexpression of β-carotene hydroxylase enhances stress tolerance in *Arabidopsis*. Nature 418: 203–206

Demiral T, Türkan I (2004) Does exogenous glycinebetaine affect antioxidative system of rice seedlings under NaCl treatment? J Plant Physiol 161:1089–1100

Edmeades GO, Deutsch JA (1994) Stress tolerance breeding: maize that resists insects, drought, low nitrogen, and acid soils. CIMMYT, Mexico, D.F.

Edwards EA, Rawsthorne S, Mullineaux P (1990) Subcellular distribution of multiple forms of glutathione reductase in leaves of pea (*Pisum sativum* L.) Planta 180:278–284

Elansary HO, Skalicka-Woźniak K, King IW (2016) Enhancing stress growth traits as well as phytochemical and antioxidant contents of *Spiraea* and *Pittosporum* under seaweed extract treatments. Plant Physiol Biochem 105:310–320

Eswaran K, Ghosh PK, Siddhanta AK, et al (2005) Integrated method for production of carrageenan and liquid fertilizer from fresh seaweeds. US. Pat. No. 6,893,479

Ghosh A (2016) Mechanism of improved drought tolerance in maize upon application of seaweed based biostimulant as revealed through comparative transcriptomic analysis by RNA-seq. In: NextGen genomics, biology, bioinformatics and technologies (NGBT) conference. Cochin, India, p 84

Ghosh A, Vijay Anand KG, Seth A (2015) Life cycle impact assessment of seaweed based biostimulant production from onshore cultivated *Kappaphycus alvarezii* (Doty) Doty ex Silva—is it environmentally sustainable? Algal Res 12:513–521

Hernandez JA, Ferrer MA, Jimenez A, Barcelo AR, Sevilla F (2001) Antioxidant systems and $O_2^{\cdot}/H_2O_2$ production in the apoplast of pea leaves. Its relation with salt-induced necrotic lesions in minor veins. Plant Physiol 127:817–831

Hodges DM, Delong JM, Forney CF, Prange RK (1999) Improving the thiobarbituric acid-reacting-subtances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207:604–611

Jayaraj J, Wan A, Rahman M, Punja ZK (2008) Seaweed extract reduces foliar fungal diseases on carrot. Crop Prot 27(10):1360–1366

Jones DB (1941) Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins. US Department of Agriculture, Washington, DC

Jun C, Junying D (1994) Correlation among photosynthesis, lipid peroxidation and ultrastructural changes of mesophyll cells in corn leaves under water stress. Maize Science, China

Khan W, Rayirath UP, Subramanian S, Jithesh MN, Rayorath P, Hodges DM, Critchley AT, Craigie JS, Norrie J, Prithiviraj B (2009) Seaweed extracts as biostimulants of plant growth and development: review. J Plant Growth Regul 67:636–641

Krom M (1980) Spectrophotometric determination of ammonia : a study of a modified berthelot reaction using salicylate and dichloroisocyanurate. Analyst 105:305–316

Kumar P, Rouphael Y, Cardarelli M, Colla G (2017) Vegetable grafting as a tool to improve drought resistance and water use efficiency. Front Plant Sci 8:1130

Larson RA (1988) The antioxidants of higher plants. Phytochemistry 27: 969–978

Layek J, Das A, Ramkrushna GI, Trivedi K, Yesuraj D, Chandramohan M (2015) Seaweed sap: a sustainable way to improve productivity of maize in North-East India. Int J Environ Stud 72:305–315

Liu L, He RT (1995) Effects of water stress on membrane damage and changes of some related enzyme activities in leaf of maize seedling. J Guangzi Agric Univ 14:195–200

Liu WG, Shan L, Deng XP (2003) Effects of soil bulk density on hydraulic conductivity of maize roots under drying and wet conditions. Acta Pedol Sin 40:779–782

Ma QQ, Wang W, Li YH, Li DQ, Zou Q (2006) Alleviation of photoinhibition in drought-stressed wheat (*Triticum aestivum*) by foliar-applied glycinebetaine. J Plant Physiol 163:165–175

Mansori M, Chernane H, Latique S, Benaliat A, Hsissou D, El Kaoua M (2015) Seaweed extract effect on water deficit and antioxidative mechanisms in bean plants (*Phaseolus vulgaris* L.) J Appl Phycol 27:1689–1698

Mansori M, Chernane H, Latique S, Benaliat A (2016) Effect of seaweed extract (*Ulva rigida*) on the water deficit tolerance of *Salvia officinalis* L. J Appl Phycol 28:1363–1370

Martynenko A, Shotton K, Astatkie T, Petrash G, Fowler C, Neily W, Critchley AT (2016) Thermal imaging of soybean response to drought stress : the effect of *Ascophyllum nodosum* seaweed extract. Spring 5(1):1393. https://doi.org/10.1186/s40064-016-3019-2

Miller RO (1998) Handbook of methods for plant analysis. CRC Press, Boca Raton

Mondal D, Sharma M, Maiti P, Prasad K, Meena R, Siddhanta AK, Bhatt P, Ijardar S, Mohandas VP, Ghosh A, Eswaran K, Shah BG, Ghosh PK (2013) Fuel intermediates, agricultural nutrients and pure water from *Kappaphycus alvarezii* seaweed. RSC Adv 3:17989–17997

Mondal D, Ghosh A, Prasad K, Singh S, Bhatt N, Zodape ST, Chaudhary JP, Chaudhari J, Chatterjee PB, Seth A, Ghosh PK (2015) Elimination of gibberellin from *Kappaphycus alvarezii* seaweed sap foliar spray enhances corn stover production without compromising the grain yield advantage. Plant Growth Regul 75: 657–666

Muchow RC (1989) Comparative productivity of maize, sorghum and pearl millet in a semi-arid tropical environment II. Effect of water deficits. F. Crop Res 20:207–219

Nagy Z, Tuba Z, Zsoldos F, Erdei L (1995) CO_2-exchange and water relation responses of sorghum and maize during water and salt stress. J Plant Physiol 145:539–544

Nakano Y, Asada K (1981) Hydrogen-peroxide is scavenged by ascorbate-specific peroxidase in spinach-chloroplasts. Plant Cell Physiol 22:867–880

Nawaz F, Ahmad R, Ashraf MY, Waraich EA, Khan SZ (2015) Effect of selenium foliar spray on physiological and biochemical processes and chemical constituents of wheat under drought stress. Ecotoxicol Environ Saf 113:191–200

Novozamsky I, Houba VJG, van Eck R, van Vark W (1983) A novel digestion technique for multi-element plant analysis. Commun. In Soil Sci. Plant Anal 14:239–248

Papageorgiou GC, Murata N (1995) The unusual stron stabilizing effects of glycine betaine on the structure and function of the oxygen-evolving Photosystem II complex. Photosynth Res 44:243–252

Park E, Jekni Z, Chen THH (2006) Exogenous application of glycinebetaine increases chilling tolerance in tomato plants. Plant Cell Physiol 47:706–714

Prakash TR, Swamy PM, Sugunal P, Reddanna P (1990) Characterization and behaviour of 15-lipoxygenase during peanut cotyledonary senescence. Biochem Biophys Res Commun 172:462–470

Pramanick B, Brahmachari K, Ghosh A, Zodape ST (2014) Effect of seaweed saps on growth and yield improvement of transplanted rice in old alluvial soil of West Bengal. Bangladesh J Bot 43:53–58

Pramanick B, Brahmachari K, Ghosh A, Zodape ST (2016) Effect of seaweed saps derived from two marine algae *Kappaphycus* and *Gracilaria* on growth and yield improvement of blackgram. Ind J Geo-Mar Sci 45:789–794

Ranum P, Peña-Rosas JP, Garcia-Casal MN (2014) Global maize production, utilization, and consumption. Ann N Y Acad Sci 1312:105–112

Rasul G, Chaudhry QZ, Mahmood A, Hyder KW (2011) Effect of temperature rise on crop growth & productivity. Pakistan J Meteorol 8: 53–62

Rathore SS, Chaudhary DR, Boricha GN, Ghosh A, Bhatt BP, Zodape ST, Patolia JS (2009) Effect of seaweed extract on the growth, yield and nutrient uptake of soybean (*Glycine max*) under rainfed conditions. S Afr J Bot 75:351–355

Raverkar KP, Pareek N, Chandra R, Chauhan S, Zodape ST, Ghosh A (2016) Impact of foliar application of seaweed saps on yield, nodulation and nutritional quality in green gram (*Vigna radiata* L). Legume Res 39:315–318

Rouphael Y, De Micco V, Arena C, Raimondi G, Colla G, De Pascale S (2017a) Effect of *Ecklonia maxima* seaweed extract on yield, mineral composition, gas exchange, and leaf anatomy of zucchini squash grown under saline conditions. J Appl Phycol 29:459–470

Rouphael Y, Cardarelli M, Bonini P, Colla G (2017b) Synergistic action of a microbial-based biostimulant and a plant derived-protein hydrolysate enhances lettuce tolerance to alkalinity and salinity. Front Plant Sci 8:131

Saneoka H, Nagasaka C, Hahn DT, Yang WJ, Premachandra GS, Joly RJ, Rhodes D (1995) Salt tolerance of glycinebetaine-deficient and -containing maize lines. Plant Physiol 107:631–638

Searle PL (1984) The Berthelot or Indophenol reaction and its use in the analytical chemistry of nitrogen. Analyst 109:549–568

Singh S, Singh MK, Pal SK, Trivedi K, Yesuraj D, Singh CS, Anand KGV, Chandramohan M, Patidar R, Kubavat D, Zodape ST, Ghosh A (2016) Sustainable enhancement in yield and quality of rain-fed maize through *Gracilaria edulis* and *Kappaphycus alvarezii* seaweed sap. J Appl Phycol 28:2099–2112

Song FB, Dai JY, WB G, Li HY (1995) Effect of water stress on leaf water status in maize. J Jilin Agric Univ 17:5–9

Spann TM, Little HA (2011) Applications of a commercial extract of the brown seaweed *Ascophyllum nodosum* increases drought tolerance in container-grown "hamlin" sweet orange nursery trees. Hortscience 46:577–582

Stevens GA, Westwood MN (1984) Fruit set and cytokinin-like activity in the xylem sap of sweet cherry (*Prunus avium*) as affected by rootstock. Physiol Plant 61:464–468

Sudhakar C, Lakshmi A, Giridarakumar S (2001) Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. Plant Sci 161:613–619

Sun Q, Wang W, Li X, Liang Z, Zhang F (2000) Effect of membrane lipid peroxidation and photosynthesis rate on water stress under different nitrogen level in maize. Acta Agric Boreali-Occidentalis Sinica 10:7–10

Temminghoff EJM, Houba VJG (2004) Plant analysis procedures (second edition), Kluwer, Dordrecht, DOI: https://doi.org/10.1007/978-1-4020-2976-9

Trivedi K, Anand KGV, Kubavat D, Kumar R, Vaghela P, Ghosh A (2017) Crop stage selection is vital to elicit optimal response of maize to seaweed bio-stimulant application. J Appl Phycol 29: 2135–2144

Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Plant Sci 151: 59–66

Xu C, Leskovar DI (2015) Effects of *A . nodosum* seaweed extracts on spinach growth, physiology and nutrition value under drought stress. Sci Hortic (Amsterdam) 183:39–47

Yemm EW, Willis AJ (1954) The estimation of carbohydrates in plant extracts by anthrone. Biochem J 57:508–514

Zhang X, Ervin EH (2004) Cytokinin-containing seaweed and humic acid extracts associated with creeping bentgrass leaf cytokinins and drought resistance. Crop Sci 44:1737–1745