



RESEARCH ARTICLE

Foliar supplements improve physio-biochemical responses and root Brix in sugar beet under water deficit condition

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Abstract

Sugar beet is a major sugar crop, it is a moderate drought tolerant plant however, high dehydration levels or prolonged drought exposure durations can affect its growth and physiology. Two seasons of study were conducted in a split plot design during winter-summer under Odisha conditions, to evaluate the effect of foliar supplements (nano silicon 1 mM, humic acid 300 ppm, jasmonic acid 5 μ M, salicylic acid 100 ppm, boric acid 1000 ppm, kaolin 5 % and chitosan 200 ppm) in tropical sugar beet LS-6 variety, growing under 3 irrigation intervals as 3 days (Normal watering -NW), 7 days (S_1) and 14 days (S_2) of water withholding on its physio-biochemical changes and the root Brix. The effect of water deficit stress on physiological processes was observed with the decline of relative water content (RWC %) and the membrane stability index (MSI %) up to 25.33 % and 23.38 % respectively, after 4 cycles of severe stress (S_2). In response to water scarcity, leaf chlorophyll and protein content decreased, while proline, sugars and ascorbic acid content in the leaves increased. Similarly, root Brix increased with the severity of stress. The application of various foliar treatments regulated the physiological processes by stabilising the water balance in the stressed leaves and strengthening the cell membrane. Moreover, these treatments protected the chlorophyll and protein content by inhibiting their destruction under stress condition and boosted the accumulation of proline, total soluble carbohydrate and ascorbic acid amount in the leaves. Humic acid was recorded as the most effective foliar treatment in case of chlorophyll retention. Nano silicon proved as efficient protective supplement for membrane stability and water content. Salicylic acid also induced more accumulation of ascorbic acid in the leaves with an increment of 41.79 % under S_2 after 4th spray. In contrast, boron was the most effective treatment to maintain root Brix with 10.52 % increment against stress control when subjected to severe stress (S_2) of 2 months.

Keywords: drought; membrane stability index; proline; relative water content; total soluble carbohydrate; tropical sugar beet

Introduction

Sugar beet (*Beta vulgaris* L.) is a temperate halophyte sugar crop that belongs to the Chenopodiaceae family. It is a multi-use crop, serving as the second most important source of white sugar in the world after sugar cane. Tropical sugar beet is a moderately drought-tolerant crop (1), suitable for tropical conditions. However, exposing it to severe stress or the prolongation of a water deficit period hampers its physiological processes, which disturbs the biochemical and water balance in the leaves and disarranges sucrose accumulation in the roots.

Drought is a major challenging abiotic stress for agriculture, the continuous changes in rainfall patterns and the frequency of dry periods highlight the severity of drought stress nowadays. The rapid spreading of drought increases the affected areas in the world, which is expected to exceed 50 % of the planet by 2050, this threatens agricultural sustainability and aggravates the food security issue. Limited water supply significantly affects plant growth, impairs their physiological, morphological and biochemical traits (2). As a normal survival behaviour, plants respond to a deficit in water availability by adjusting their physiological and metabolic processes to withstand these unfavourable environments. However, after a certain limit, plants may lose their tolerance to overcome such drought effects.

When plants are subjected to stress, besides their natural survival behaviour, plant resistance could be enhanced by exogenous foliar treatments without disturbing soil health. Foliar spray with various supplements, such as stress hormones, minerals and antitranspirants, is mentioned as a promising approach for mitigating abnormal circumstances. Though these treatments are biostimulants in various ways but selected on the basis of their action under stress and according to the type of crop taken for the research. Chitosan (Chi) is a chitin derivative and has proven its efficacy as a safe elicitor and an antitranspirant, stimulating water stress tolerance in plants by activating the defence mechanisms against oxidative damage and protecting the plants (3). Using nanoparticles in agriculture is a new strategy which took its importance from the advanced role of nano-molecules in comparison with their bulk materials. Silicon, especially in its nano form (N-Si), is a beneficial element for plants in drought condition. Nano silicon is considered as an effective supplement to rejuvenate plants under less water availability (4) by strengthening the cell walls, increasing structural stability and overall plant tolerance. Humic acid enhances drought tolerance in the plants through generating a mild stress in the leaves (5), which leads to activate defence mechanisms and stimulate plant growth. Kaolin is an inert, non-toxic antitranspirant and has a role in

alleviating the risk of abiotic stress by forming a protective layer on the leaves that can reduce the canopy temperature and the effect of heat, which supports the plant to withstand unfavourable conditions, maintains water status and physiological processes under such challenges (6). Jasmonic acid (JA) biosynthesis increases in the plants under limited water availability (7). Reports indicated that jasmonate accumulation increased due to activation of the jasmonate defence pathway with the exogenous application of JA and its derivatives, which enhances plant resistance under water scarcity conditions. Salicylic acid (SA) is a crucial signalling molecule in plants, enhancing stress tolerance through boosting antioxidant defence, maintaining cell integrity and regulating stomatal mechanisms as stress responses (8). It stimulates osmolyte synthesis and involves in various physiological survival mechanisms. Boron (B) plays a critical role as an essential micronutrient for plant growth and development, it has been reported to show stress mitigation effects by maintaining cell membrane stability, stomatal regulation, improving root growth, sugar transportation and correcting the physiological disorders in stressed plants (9).

The pursuit for more sustainable agriculture under such erratic global weather and rising drought risk needs more studies on the way to enhance plants tolerance and production. With this objective and due to lack of research findings on mitigation strategies in tropical sugar beet varieties under drought stress condition, this study aimed to check the effectiveness of various exogenous supplements on the physio-biochemical performance and sucrose accumulation in term of Brix percentage in tropical sugar beet LS-6 variety under different irrigation intervals during winter-summer condition of Odisha.

Materials and Methods

The research was conducted over two consecutive years (2022–23 and 2023–24) following winter-summer climatic conditions of Odisha in the research farm of Faculty of Agricultural Sciences, Siksha 'O' Anusandhan, deemed to be University. The weather data during the experimental period in 1st and 2nd year recorded as the total accumulated rainfall of (114.0 mm and 65.4 mm), the maximum-minimum range of temperature noted as (15.2–37.5 °C and 16–43.5 °C) and relative humidity in that period reported as (31–96% and 37–98%) respectively.

The experiment was laid out in a split-plot design with three replications using tropical sugar beet (LS-6) as a planting material, planted in 50 x 20 cm spacing in the field size of 8 m² subplot. Main plots were assigned with 3 levels of stress treatment NW (normal watering, 3-day irrigation intervals), S₁ (moderate stress, 7-days irrigation intervals) and S₂ (severe stress, 14-days irrigation intervals). The foliar supplements applied as T₁ control (water spray), T₂ nano silicon (N-Si) 1 mM (10), T₃ humic acid (HA) 300 ppm (11), T₄ jasmonic acid (JA) 5 µM (12), T₅ salicylic acid (SA) 100 ppm (11), T₆ boric acid (B) 1000 ppm (13), T₇ kaolin (KA) 5% (14) and T₈ chitosan (Chi) 200 ppm (15), were allocated in the subplots.

The plants were allowed to grow for 50 days of normal growth (50 days after sowing (DAS)) before imposition of drought stress. Drought stress cycles applied for 2 months counting 8 cycles of S₁ and 4 cycles of S₂ and irrigation was given as per the irrigation schedule for NW, S₁ and S₂ using surface irrigation. The first foliar spray was given before stress application as a preparatory and the

later sprays (2nd, 3rd and 4th) were continued up to 2 months of stress in 15-days interval. Foliar treatments consisted of 2 L of solution applied to every subplot in the afternoon by using sprayer as per the specific spray schedule. Precautions were taken to ensure that rainfall did not interfere with the experiment.

To study the treatment effect against stress, samples were collected after 4 days of foliar spray before irrigation. From every treatment plot, 3 plants per each replication were taken for analysis. The biochemical and physiological analysis were done using fresh or dry materials according to the standard methods used.

Physiological analysis

To measure the relative water content (RWC) in plants, the fresh weight (F (wt)), turgid weight (T(wt)) and dry weight (D (wt)) of a selected number of healthy leaves were measured and RWC % was calculated using Eq. 1:

$$\text{RWC (\%)} = \frac{F(\text{wt}) - D(\text{wt})}{T(\text{wt}) - D(\text{wt})} \times 100 \quad (\text{Eq. 1})$$

Membrane stability index (MSI %) was evaluated according to the standard method (16) and the value was calculated using Eq. 2:

$$\text{MSI (\%)} = \left[1 - \left(\frac{EC_{40}}{EC_{100}} \right) \right] \times 100 \quad (\text{Eq. 2})$$

Biochemical analysis

The total chlorophyll content following the procedure (17). Fresh leaf sample, 100 mg, was ground using 80 % acetone and the optical density (OD) of supernatant was measured at 652 nm. Total chlorophyll content was calculated as mg g⁻¹ of fresh leaf. To determine the proline content in sugar beet fresh leaves, the sampled leaves were processed using ninhydrine method (18) and proline content was determined as µg g⁻¹ using a proline standard curve. Total soluble carbohydrates (TSC) in fresh leaf samples were determined by using the anthrone method, following the standard procedure and expressed as mg.g⁻¹ using a glucose standard curve (19). Dry leaf samples were used to determine protein content and the amount of protein accumulated in the sample was calculated as mg g⁻¹ using a Bovine serum albumin (BSA) standard curve (20). The titration method was used for ascorbic acid (AsA) determination in dry leaves (21) and calculated using Eq. 3:

Amount of ascorbic acid mg/ 100 g =

$$[(0.5/V_1) \times (V_2/5) \times (V/\text{wt})] \times 100 \quad (\text{Eq. 3})$$

Root Brix

The Brix (sucrose) percentage of fresh sugar beet roots were measured using a digital refractometer.

Experimental soil

The seeds were sown in sandy-loam soil, soil texture and chemical properties are presented in Table 1. The organic carbon (OC), pH, electrical conductivity (EC) and NPK content of the experimental

Table 1. Soil texture and chemical properties of experimental soil

Soil texture	Sandy-loam
PH	6.02
EC (µs)	327.6
OC (%)	0.729
N (Kg ha ⁻¹)	385.869
P (Kg ha ⁻¹)	412.16
K (Kg ha ⁻¹)	168.784

soil were analysed according to standard soil procedures.

Statistical analysis

Statistical analysis for a split plot design was conducted using SPSS v26 at a probability level $p \leq 0.05$. The analysis of variance across three replications with 3 levels of water stress (S) and 8 foliar treatments (T) was performed on homogeneous average data from 2 years. The comparison of means was done through ANOVA test followed by the least significant difference (LSD) and Duncan tests. LSD test was done for 2nd, 3rd and 4th spray separately while Duncan test was conducted for stress groups NW, S₁ and S₂ separately after each spray to analyse the efficiency of treatments in each stress level.

Results and Discussion

Relative water content

The data presented in Table 2, is indicated a significant reduction in leaf relative water content (RWC) percent at $p \leq 0.05$, observed in untreated stressed plants after 4th cycle of water withholding from 7 and 14 days recorded up to 13.07 % and 23.38 % of decrease in water content respectively against normal control plants. Relative water content percentage decreased with a lowest value of 68.22 % in S₁ and 60.13 % in S₂ recorded after 8 weeks of prolonged stress cycles in comparison with 78.48 % in normal watering (NW). Foliar treatments significantly increased the relative water content in the leaves over respective stress controls. The RWC % in NW plants increased from 2nd to 3rd spray then declined after 4th spray, while in S₁ and S₂ the relative water content decreased gradually from 2nd to 3rd to 4th spray irrespective of all foliar treatments. All 7 treatments are effective but N-Si, HA and JA showed the most effective recovery action to retain more water in the leaves under long stress cycles. The percentage of increase reached up to 31.96 % and 29.68 % in S₂ under N-Si (T₂) and HA (T₃) spray, where as 20.20 % and 19.70 % in S₁

with JA (T₄) and N-Si application respectively increased after 4th spray over respective stress controls. Under moderate and severe stress Chi also maintained water balance efficiently along with other foliar treatments.

Membrane stability index

The results in Table 3 clearly showed that membrane stability index (MSI) reduced continuously when stress period increased to 8 weeks of exposure. The MSI % of decrease in S₁ and S₂ after 4th spray was severely reduced by 10.31 % and 25.33 % against normal irrigated plants (NW). The rate of reduction was more acute in S₂ (14 days) over S₁ (7 days) resulted a significant reduction of 16.75 % MSI in the leaf tissue. Low MSI % was recorded in S₂ and S₁ as 51.28 % and 61.60 % respectively in comparison with NW 68.68 % after 4th spray of drought stress cycles. All foliar treatments N-Si, HA, JA, SA, B, KA and Chi significantly increased MSI % against their stress controls ($p \leq 0.05$). N-Si and Chi retained the highest membrane stability of 36.08 % and 34.63 % respectively after 4th spray than other treatments.

Total chlorophyll content

A significant degradation in total chlorophyll content at 652 nm was observed in sugar beet leaves in response to water stress (Fig. 1). More than 50 % (52.42 %) reduction in total chlorophyll content occurred in plants subjected to severe S₂ and 43.14 % in moderate S₁, showing more stress effect against NW plants after 3rd spray. Repeated foliar applications significantly increased the stability of sugar beet leaf chlorophyll content ($p \leq 0.05$) over the non-treated stress controls across all levels of drought conditions. Chlorophyll content increased in 2nd spray to 3rd spray (80 DAS), declined after 4th spray (95 DAS) but the retention was significantly higher under foliar treatment in all stressed types against their respective non-treated stress controls (T₁), although overall decline

Table 2. The effect of various foliar treatments and different irrigation intervals on RWC % in sugar beet

Foliar treatments	After 2 nd spray			After 3 rd spray			After 4 th spray		
				Irrigation intervals					
	NW	S ₁	S ₂	NW	S ₁	S ₂	NW	S ₁	S ₂
T ₁	85.12 ^b	81.20 ^b	79.78 ^c	86.23 ^b	77.36 ^c	70.36 ^d	78.48 ^d	68.22 ^d	60.13 ^f
T ₂	90.07 ^a	88.26 ^a	86.83 ^a	90.48 ^{ab}	85.71 ^a	84.65 ^a	88.95 ^a	81.66 ^a	79.35 ^a
T ₃	90.25 ^a	87.89 ^a	84.59 ^{ab}	91.12 ^{ab}	85.38 ^a	84.26 ^a	87.63 ^a	81.08 ^a	77.98 ^a
T ₄	89.00 ^{ab}	86.62 ^a	85.71 ^{ab}	89.29 ^{ab}	84.92 ^a	82.91 ^{ab}	82.50 ^{bcd}	82.00 ^a	73.86 ^{bc}
T ₅	87.19 ^{ab}	84.96 ^{ab}	83.93 ^{ab}	87.73 ^{ab}	83.41 ^{ab}	81.11 ^{ab}	85.85 ^{abc}	80.74 ^{ab}	71.63 ^{cd}
T ₆	90.62 ^a	83.53 ^{ab}	82.22 ^{bc}	91.81 ^a	80.55 ^{bc}	78.44 ^{bc}	86.25 ^{ab}	77.71 ^{bc}	70.19 ^{de}
T ₇	89.31 ^{ab}	85.21 ^{ab}	86.16 ^{ab}	89.98 ^{ab}	82.81 ^{ab}	75.79 ^c	86.48 ^{ab}	75.96 ^c	68.91 ^e
T ₈	88.53 ^{ab}	88.36 ^a	87.07 ^a	88.97 ^{ab}	86.19 ^a	83.69 ^a	81.68 ^{cd}	79.29 ^{ab}	74.57 ^b
LSD (0.05)	S	T	S*T	S	T	S*T	S	T	S*T
	1.67	2.23	3.85	0.97	2.34	4.05	1.08	1.75	3.02

LSD: least significant differences ($p \leq 0.05$) ($n = 3$), different letters indicate Duncan's multiple range test at 0.05 level.

Table 3. The effect of various foliar treatments and different irrigation intervals on MSI % in sugar beet

Foliar treatments	After 2 nd spray			After 3 rd spray			After 4 th spray		
				Irrigation intervals					
	NW	S ₁	S ₂	NW	S ₁	S ₂	NW	S ₁	S ₂
T ₁	80.55 ^d	74.06 ^c	69.53 ^d	79.85 ^c	70.25 ^c	63.53 ^e	68.68 ^d	61.60 ^d	51.28 ^e
T ₂	86.81 ^a	87.98 ^a	79.82 ^a	85.44 ^{ab}	80.12 ^a	76.51 ^a	79.58 ^a	73.11 ^a	69.78 ^a
T ₃	85.96 ^{ab}	82.84 ^b	71.69 ^{cd}	82.79 ^{abc}	71.10 ^c	69.22 ^c	76.29 ^{abc}	66.52 ^c	61.38 ^{cd}
T ₄	81.79 ^{bcd}	84.65 ^{ab}	74.46 ^b	80.95 ^{bc}	74.56 ^{bc}	72.01 ^b	73.71 ^c	68.99 ^{abc}	65.10 ^b
T ₅	81.45 ^{cd}	84.91 ^{ab}	73.88 ^{bc}	80.35 ^{bc}	73.91 ^c	71.65 ^b	73.12 ^c	67.15 ^{bc}	63.21 ^{bc}
T ₆	83.96 ^{abcd}	88.10 ^a	77.64 ^a	82.11 ^{abc}	78.44 ^{ab}	72.55 ^b	75.66 ^{bc}	70.62 ^{abc}	65.27 ^b
T ₇	87.02 ^a	88.31 ^a	78.27 ^a	86.90 ^a	79.25 ^a	65.46 ^d	79.69 ^a	70.81 ^{abc}	58.44 ^d
T ₈	85.11 ^{abc}	87.86 ^a	79.05 ^a	83.77 ^{abc}	79.77 ^a	75.82 ^a	78.87 ^{ab}	71.33 ^{ab}	69.04 ^a
LSD (0.05)	S	T	S*T	S	T	S*T	S	T	S*T
	0.82	1.96	3.39	1.58	2.02	3.49	0.96	1.95	3.38

LSD: least significant differences ($p \leq 0.05$) ($n = 3$), different letters indicate Duncan's multiple range test at 0.05 level.

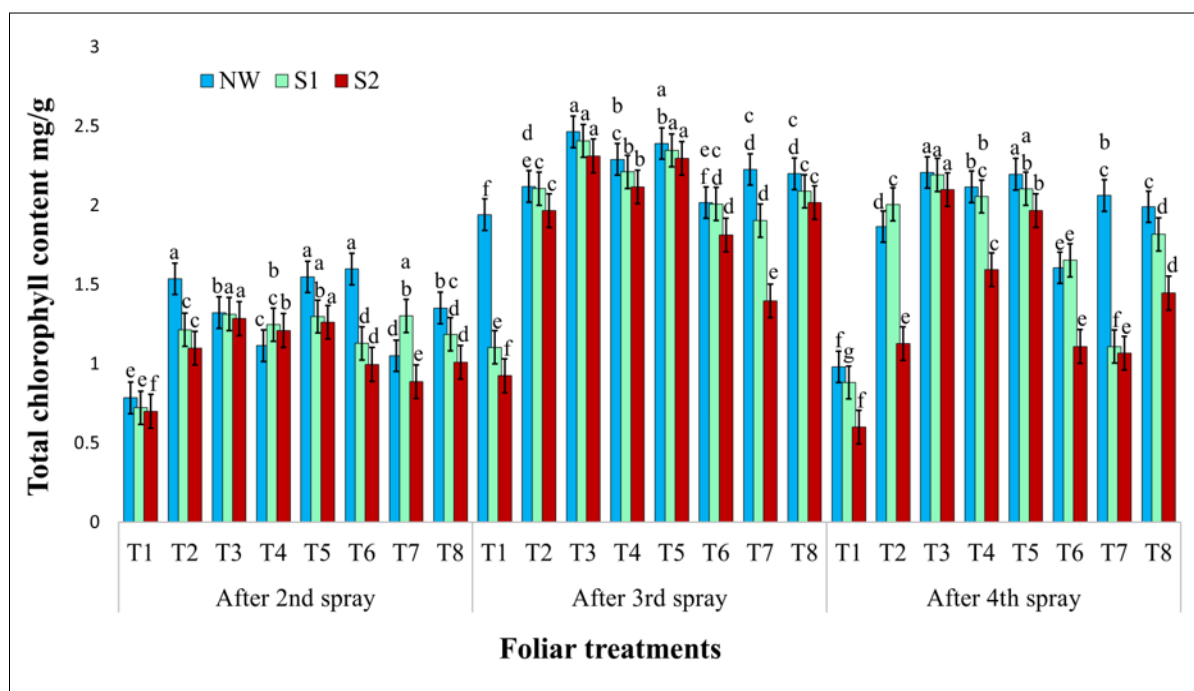


Fig. 1. The effect of various foliar treatments and irrigation intervals on total chlorophyll content in sugar beet leaves. Error bars express as means $\pm$ SE of 3 replicates. Different letters indicate Duncan's multiple range test at 0.05 level.

was observed in comparison with normal irrigated (NW) plants. All seven effective foliar treatments increased tolerance against chlorophyll degradation subjected to 8 weeks of water deficit cycles after every round of spray against respective stress controls, which is clearly visible in Fig. 1. Supplementation with HA and SA were more effective with an increment percentage up to 148.70 %, 249.67 % with HA in S_1 and S_2 respectively and 138.82 %, 227.67 % with SA in S_1 and S_2 respectively from their stress controls after 4th spray. In this context, kaolin and boron application showed less effectiveness to overcome stress compared to other JA, N-Si and Chi treatments, recorded as 1.108 mg g⁻¹ and 1.652 mg g⁻¹ respectively at 7 days of moderate stress (S_1) and under 14 days (S_2) of prolonged stress resulted 1.066 mg g⁻¹ and 1.108 mg g⁻¹ total chlorophyll respectively after 4th spray (8th week of stress) over non-treated stress control plants.

Proline content

Decreasing the availability of water to the plants by withholding the irrigation for 7 and 14 days encouraged the accumulation of proline in the stressed leaves, which increased with the repetitions of drought cycles up to 2 months. Proline content reached its peak value of 9.78 μ g g⁻¹ in T_1 after 4 cycles of severe stress (S_2) and 4.87 μ g g⁻¹ in S_1 at the end of stress period. Proline accumulation increased according to the degree of stress imposition, after every spray, $S_2 > S_1$ even under treatment. Application of foliar treatments significantly boosted proline content at $p \leq 0.05$ in the sugar beet leaves under both stress conditions S_1 and S_2 after 2nd and 3rd spray, whereas these treatments appeared to restrict proline accumulation when the plants were sprayed with N-Si, HA, B and KA under both moderate and severe stress after 4th spray and a nonsignificant increment was recorded in other treatments like SA, JA and Chi over stress control under 14 days of irrigation intervals (S_2). The percentage of proline increment was less under JA (0.41 %) compared to Chi (0.82 %) and SA (1.53 %) over control. The highest percentage of increment in proline content was found 204.51 % recorded with SA application against stress control (T_1) under S_2 condition after 2nd spray in comparison to 3rd and 4th spray (Fig. 2).

Total soluble carbohydrate content:

The response of total soluble carbohydrate (TSC) during the stress period is summarised in Fig. 3. The TSC content raised in the leaves when the stress severity increased continuously after 2nd to 3rd spray days, the highest value was obtained as 29.04 mg g⁻¹ in NW, 30.45 mg g⁻¹ in S_1 and 35.56 mg g⁻¹ in S_2 but declined after 4th spray to 21.68 mg g⁻¹, 14.89 mg g⁻¹ and 12.34 mg g⁻¹ in case of NW, S_1 and S_2 respectively. The percentage of increase at $p \leq 0.05$ was significantly declined to 31.32 % (untreated S_1) and 43.08 % (untreated S_2) against respective NW untreated control plants after 4th spray. Application of treatments like N-Si, HA, JA, SA, B, KA and Chi, were encouraged to increase TSC content under stress and normal watering plants. The TSC accumulation was higher in all cases of S_2 than S_1 and less in NW treated or untreated plants. The most suitable foliar treatment was observed in SA and Chitosan spray that enhanced TSC up to 541.98 % and 516.77 % respectively over stress control at the end of 14 days stress cycles after 4th spray, where KA and B resulted in the lowest increment of 58.00 mg g⁻¹ (370.02 %) and 59.78 mg g⁻¹ (384.44 %) over control (Fig. 3). N-Si increased TSC content efficiently in moderate S_1 and HA resulted more TSC in S_2 compared to other treatments.

Protein content

Imposing drought stress on sugar beet, the amount of protein in the leaves significantly reduced when the drought stress increased from 7 days (S_1) to 14 days (S_2) duration, explained in Fig. 4. The highest level of soluble protein recorded in S_1 and S_2 were 81.06 mg g⁻¹ and 62.68 mg g⁻¹ after 30 days (3rd spray) of repeated drought cycles and declined to 70.20 mg g⁻¹, 52.29 mg g⁻¹ respectively compared with normal watering plants (84.79 mg g⁻¹ to 74.90 mg g⁻¹) when stress was applied for prolonged stress cycles. Application of various foliar supplements enhanced protein levels in the plants under all types of irrigation intervals against respective controls. It was observed that the protein content significantly increased after 2nd spray to 3rd spray period, then decreased after 4th spray in all plants under control as well as in foliar treatments. The application of N-Si, JA and SA

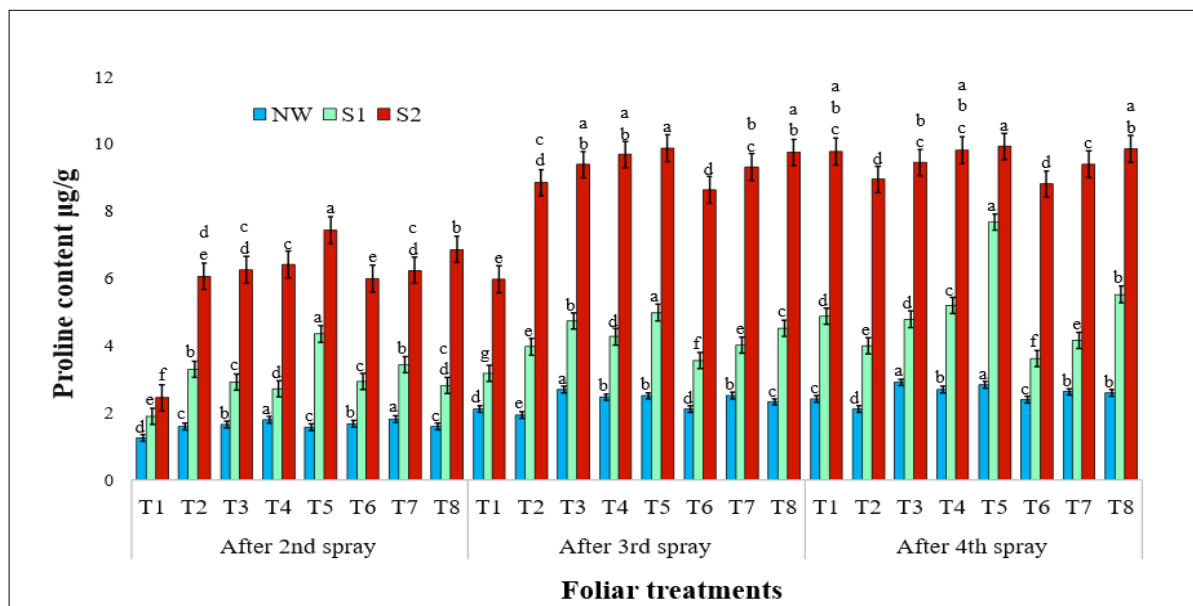


Fig. 2. The effect of various foliar treatments and irrigation intervals on proline content in sugar beet leaves. Error bars express as means $\pm$ SE of 3 replicates. Different letters indicate Duncan's multiple range test at 0.05 level.

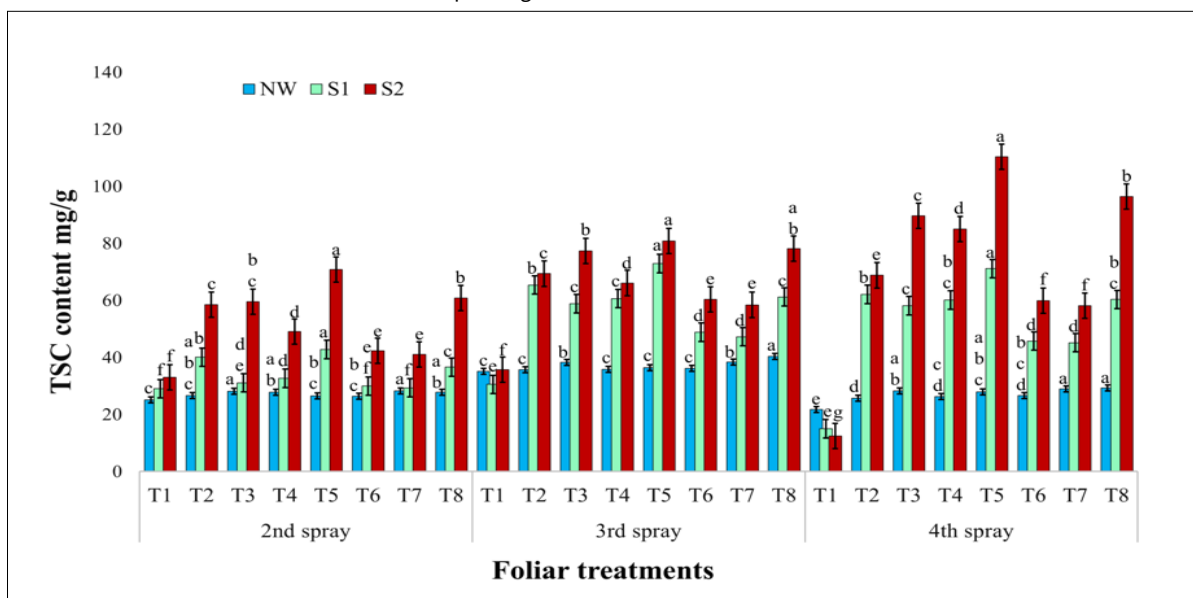


Fig. 3. The effect of various foliar treatments and irrigation intervals on TSC content in sugar beet leaves. Error bars are expressed as means $\pm$ SE of 3 replicates. Different letters indicate Duncan's multiple range test at 0.05 level.

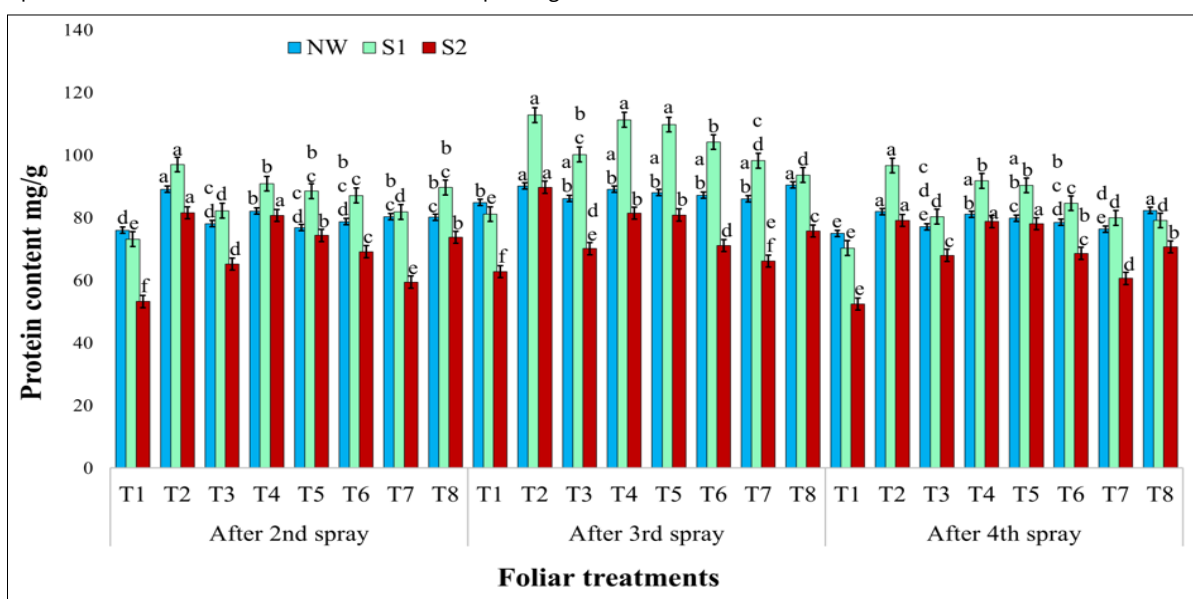


Fig. 4. The effect of various foliar treatments and irrigation intervals on protein content in sugar beet leaves. Error bars are expressed as means $\pm$ SE of 3 replicates. Different letters indicate Duncan's multiple range test at 0.05 level.

marked as most promising treatments with the highest value of protein accumulation at $p \leq 0.05$ was increased 37.58 %, 30.58 % and 28.52 % in S_1 while in S_2 situation protein increment in the leaves reached up to 51.18 %, 50.47 % and 49.17% respectively to that of non-treated stress control plants after 4th foliar spray drought stress cycles (Fig. 4).

Ascorbic acid content

More accumulation of Ascorbic acid (AsA) in sugar beet leaves was observed with the increasing irrigation intervals from NW to S_1 and S_2 , as shown in Fig. 5. The highest accumulation of ascorbic acid content reached to 9.60 mg/100 g and 11.82 mg/100 g in the leaves of untreated stress control plants after 4th spray subjected to S_1 and S_2 drought cycles respectively. Increase in stress duration influenced more accumulation of AsA in the sugar beet leaves ($S_2 > S_1$) after every cycle. Foliar spray induced more ascorbic acid accumulation as per drought length gradually in S_1 till 4th cycle but decreased from 3rd to 4th spray in S_2 . Foliar spray with different treatments like N-Si, HA, JA, SA, B, KA and Chi, significantly increased ascorbic acid amount at $p \leq 0.05$ in all 3 stress levels (NW, S_1 and S_2) compared with untreated controls, presented in Fig. 5. The SA was effective in severe stress, while JA was effective in moderate stress after 3rd and 4th spray. JA, HA and SA recorded 57.29 %, 55.73 % and 43.44 % of AsA increment in S_1 respectively, whereas 41.79 %, 40.86 % and 34.35 % of more AsA accumulation observed in S_2 with SA, HA and JA application respectively after 4th spray against stress control plants. Spraying the plants with SA resulted in the highest increment percentage of ascorbic acid content in the sugar beet leaves as 71.61 % (18.74 mg/100 g) in S_2 and JA with 58.10 % (13.17 mg/100 g) in S_1 after 3rd spray against control plants among all treatments.

Root Brix (sucrose)

The data in Table 4 showed that the elongation of irrigation intervals from 7 to 14 days resulted a significant increment ($p \leq 0.05$) in Brix percentage recorded in the sugar beet roots throughout the experimental period. The percentage of increase in sucrose accumulation was 2.11 % and 4.94 % in S_1 and S_2 respectively over NW untreated plants after 4th spray of stress cycles. The enhancement of Brix value was higher in long stressed plants S_2 (19.18 %) than moderate S_1 (18.80 %) in comparison with NW (18.26 %) recorded after 3rd spray but later declined in both cases to 18.91 % (S_2) and 18.40 % (S_1) Brix in roots observed after 4th spray of drought stress. More sucrose accumulation was seen in the roots subjected to severe and prolonged stress cycles compared with normal watering plants. Application of various foliar supplements resulted further accumulation of sucrose in the root and significantly increased Brix percentage under all irrigation levels at $p \leq 0.05$ and

according to stress severity ($S_2 > S_1$) against stress control plants (T_1). Brix (%) value, majorly contained sugar was decreased when plants were exposed to stress from 3rd spray to 4th spray days in all stress levels (NW, S_1 and S_2) under foliar treatments. Boric acid and humic acid showed a promising effect in terms of increasing Brix up to 20.35 % and 20.12 % in S_1 respectively and 20.90 % and 20.69 % in S_2 respectively after 2 months of drought application. N-Si looked impressive under both S_1 and S_2 after every spray. On the other hand, in comparison with other treatments after 4th spray, JA and chitosan observed effective under moderate stress (S_1) than severe stress (S_2), resulted $S_2 < S_1$ with a Brix percentage of 19.54 % and 19.26 % in S_1 respectively, while decreased to 19.30 % and 19.11 % Brix in S_2 respectively at the end of drought period (after 4th spray) (Table 4).

Drought stress is an environmental condition, where plants face a shortage of water in the soil, restricting natural physiology of the plants and triggering many physiological and biochemical changes for survival in such harsh situation through adjusting their internal cellular metabolism. In this investigation, the physiological indicators like RWC % and MSI % are prominently affected by dehydration because of less water available in the soil. The water-storing ability in the plants is also reduced with the depletion of soil water, especially under severe stress. The RWC % in sugar beet leaves significantly reduced under drought stress when the stress duration increased from moderate to severe. The decline in plant water status was more visible when the stress was for a prolonged period in cycles along with intermittent watering. Water deficit induced more generation of harmful oxidants as reactive oxygen species (ROS), usually resulting in cellular oxidative damage in the leaves. Due to ROS production in severe and prolonged stress, membrane stability (MSI %) in the leaves continuously declined and promoted leakage of electrolytes in the leaf cells (22). Moreover, the stress affected a considerable decrease in the chlorophyll content throughout the stress period after every foliar spray in both moderate (S_1) and severe (S_2) water deficit condition compared to normal watering plants (NW), which may have been due to excess ROS and Chlorophyllase accumulation that created a situation that affecting photosynthetic rate and plant growth performance. This result was similar to the previous findings (23), in that the degradation in chlorophyll content occurred when irrigation intervals increased up to 10 days of water withholding. In some cases, chlorophyll content increased even under unfavourable drought stress condition which was observed previously in potato plants (24), the same was visible in this experiment, where total chlorophyll content significantly increased from 2nd to 3rd spray indicated plant adaptation to resume more photosynthetic activity to accelerate root growth in the first phase of drought situation, but

Table 4. The effect of various foliar treatments and different irrigation intervals on Brix % in sugar beet

Foliar treatments	After 2 nd spray			After 3 rd spray			After 4 th spray		
	Irrigation intervals			Irrigation intervals			Irrigation intervals		
	NW	S_1	S_2	NW	S_1	S_2	NW	S_1	S_2
T_1	13.55 ^d	14.32 ^e	15.16 ^e	18.26 ^d	18.80 ^d	19.18 ^c	18.02 ^c	18.40 ^d	18.91 ^d
T_2	14.82 ^{ab}	16.03 ^{ab}	17.22 ^a	20.31 ^a	21.00 ^a	21.16 ^a	19.67 ^a	19.96 ^{abc}	20.48 ^{ab}
T_3	14.51 ^{abc}	15.64 ^{abc}	16.11 ^{bc}	19.56 ^{bc}	20.52 ^{ab}	21.23 ^a	18.61 ^{bc}	20.12 ^{ab}	20.69 ^a
T_4	14.96 ^{ab}	15.13 ^{cd}	15.99 ^{cd}	20.18 ^{ab}	20.38 ^{ab}	20.25 ^{ab}	19.44 ^a	19.54 ^{abc}	19.30 ^{cd}
T_5	15.10 ^a	15.39 ^{bcd}	16.73 ^{ab}	19.85 ^{abc}	19.99 ^{bc}	20.78 ^{ab}	19.07 ^{ab}	19.84 ^{abc}	19.92 ^{bc}
T_6	13.98 ^{cd}	15.92 ^{ab}	17.14 ^a	20.09 ^{ab}	20.88 ^a	21.29 ^a	19.24 ^{ab}	20.35 ^a	20.90 ^a
T_7	14.13 ^{bcd}	14.84 ^{de}	16.37 ^{bc}	19.32 ^c	19.64 ^c	20.36 ^{ab}	18.55 ^{bc}	19.07 ^{cd}	19.64 ^{cd}
T_8	15.01 ^a	16.23 ^a	15.35 ^{de}	19.71 ^{abc}	20.14 ^{bc}	19.91 ^{bc}	18.93 ^{ab}	19.26 ^{bcd}	19.11 ^d
LSD	S	T	S*T	S	T	S*T	S	T	S*T
(0.05)	0.19	0.39	0.68	0.29	0.42	0.73	0.32	0.40	0.70

LSD: Least significant differences ($p \leq 0.05$) ($n = 3$), Different letters indicate Duncan's multiple range test at 0.05 level.

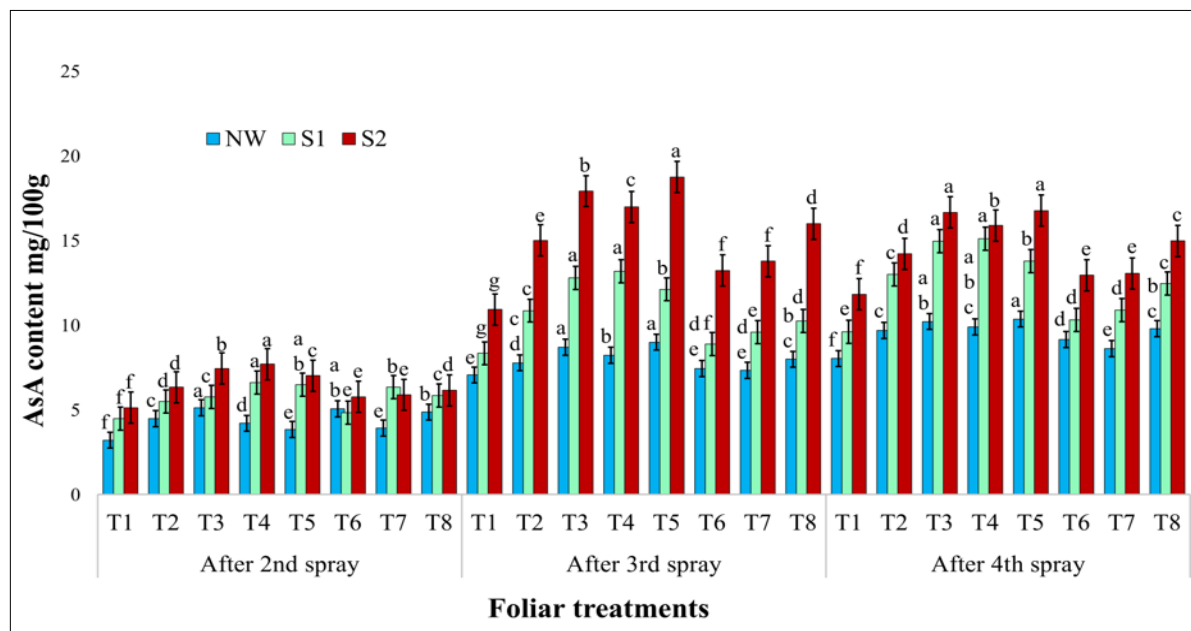


Fig. 5. The effect of various foliar treatments and irrigation intervals on AsA content in sugar beet leaves. Error bars express as means $\pm$ SE of 3 replicates. Different letters indicate Duncan's multiple range test at 0.05 level.

later declined perhaps due to long stress effect and maturity of the crop. Proline is an amino acid acts as a stress indicator in plants that contributes with other osmolytes as sugars in osmotic adjustment and maintain proper osmotic potential to protect plants under dehydration. Proline and soluble sugars are major osmolytes increased in tropical sugar beet leaves under drought condition, have been reported previously (25). Unlike the continuous increase in proline, the results exhibited a decline in TSC content with the prolongation of the stress period, which explained its metabolic use to overcome drought. Protein content declined in the sugar beet leaves with the increase in stress severity from moderate to severe ($S_2 < S_1$) over NW, resulted due to protein denaturation and the inhibition of protein biosynthesis under prolonged drought cycles (26). While stress exposure increased from 30 days (after 2nd spray) to 45 days of stress (after 3rd spray) protein content increased in the leaves which was consistent with the findings of previous study, explained that there was initiation of stress protein accumulation and activating more antioxidant enzymes, increased the protein content in the leaves during stress (24), Protein accumulation reduced after 60 days of stress (after 4th spray) with intermittent watering, may be the result of extra protein utilisation towards metabolic activity and developing resistance against long drought exposure. Normal watering sugar beet plants showed normal growth enhancement over stressed plants and protein reduction indicated the maturity of the crop. Plants try to adapt to abnormal environmental conditions by activating their inner defence mechanisms through stimulating the production and accumulation of antioxidant molecules as ascorbic acid, which has an important role in ROS detoxification, contributing to both enzymatic and non-enzymatic defence systems (27). In this study, ascorbic acid accumulation significantly increased in NW plants as a result of normal plant growth metabolism and neutralising internal free radicle production, whereas maximum AsA accumulation in S_1 and S_2 after every spray presented its stress severity and drought length, indicating attenuation of excess harmful ROS generation to prevent oxidative cell damage. Brix indicates total soluble solids, primarily sucrose in sugar beet roots, which is increased with drought duration 7 days (S_1) to 14 days (S_2) and stress length (2nd to 3rd spray) than

normally grown plants (NW). This increase in Brix may be due to the reduction in root moisture content, which boosts the accumulation of sugar (28), or the mobilisation of storage forms of sugar, such as starch and the upregulation of sucrose transport genes under insufficient soil water. After exposing the plant up to 2 months of drought (after 4th spray), less Brix was observed in the roots. The downregulation of sucrose transport genes may explain this decline under a long period of drought stress cycles, which was also reported previously in sweet potato (29). Besides that, at the end of the experimental period after 4th spray, an increment in the summer temperature was observed, which might coincide with the report that temperature also slowed down the process of sugar translocation from leaves to roots (30).

As per the experimental results, spraying the plants with various exogenous supplements help the plants to adjust and overcome the adverse effects of drought in tropical sugar beet. Foliar spray is considered as an effective strategy to mitigate drought stress, stimulate physiological processes and manage the insufficient nutrient uptake under drought condition (31). Although all the treatments used in this investigation were effective in the mitigation of stress effects and modulating the physiological and biochemical changes in sugar beet leaves and roots, however, it behaves differently in each studied parameter according to its nature and contribution in physiological and metabolic processes in the plant. The findings from this study suggested that N-Si application significantly enhanced maximum RWC % in moderate to severe stress, indicating the recovery of membrane damage under prolonged drought period (4). This might be due to the ability of N-Si to maintain turgor pressure in the stressed plants through promoting water uptake and translocation (32). The impact of HA was maintained consistency in RWC % in S_1 and S_2 covering 4sprays, this might be due to its involvement in osmotic adjustment through facilitating the absorption of ions and the accumulation of compatible solutes, enhancing water uptake and promoting root growth (5). Under moderate and severe stress, Chi interacted with ABA signalling pathways and reduced water loss by promoting stomatal closure, hence showing its effectiveness to overcome stress impact by maintaining water balance in leaf cells (33). As per

previous study JA was promising in enhancing relative water content, improving the moisture balance in the leaves. This was similar to the present finding, as JA actively increased RWC % by influencing stomatal activity to minimize transpirational water loss, was more pronounced under moderate stress than severe stress (12).

The results showed that N-Si application significantly enhanced MSI % to maximum in moderate to severe stress among all foliar treatments used, indicating the recovery of membrane damage under prolonged drought period. This was similar to previous reports, where Nano-Silicon significantly preserved membrane integrity by minimizing electrolytic leakage, interacting with cell wall proteins and increasing its thickness with reducing ROS and MDA in the leaves (32), which reflected more membrane stability index. Another convincing foliar treatment, Chitosan, an antitranspirant enhanced MSI % in sugar beet leaves significantly due to its ability to cross-link cell wall proteins and reverse the damage effect by increasing the resistance against water deficit stress (3). All foliar treatments used in the study were accelerators having their protecting and scavenging roles when applied in normal watering plants as well as stressed plants (S_1 & S_2) than nontreated plants. In the present study, the impact of humic acid (HA) maintained consistency in chlorophyll content in both moderate and prolonged stress that covered 4sprays, may be the reason explaining the involvement of humic acid in chlorophyll biosynthesis and stimulating the activity of endogenous plant hormones to prevent leaf chlorophyll degradation, resulting in high chlorophyll amount in sugar beet leaves. These results were supported with previous research findings, where HA, a biostimulant, protected chlorophyll integrity by scavenging excess ROS accumulation under drought stress (11), entered in auxin and cytokine biosynthesis to delay leaf senescence. From the results, Salicylic acid (SA), a novel plant growth regulator, appeared promising under 7 days to 14 days of prolonged drought period (8 weeks), enhancing leaf chlorophyll content, may be due to alleviating the toxic effect of ROS in the leaves, regulating plant growth and increasing photosynthetic activity in response to abiotic stress (34). Jasmonic acid, N-Si and Chi were also considered as effective in preserving biochemical efficiency and signalling internal stress response. When comparing with other treatments, though B was effective to some extent, it played a completely different biological mechanism, where foliar application helps to support normal physiological functions but shows less efficiency under stress. Boron showed synergetic effect with nitrogen metabolism and protein synthesis, which may have resulted in a positive impact in chlorophyll breakdown (13), the same was resulted under long moderate stress than severe S_2 . While Kaolin, an antitranspirant film sprayed on leaves, lowered leaf surface temperature and maintained water balance to prevent chlorophyll degradation, but less involved in plant processes and metabolism, which may explain its less effectiveness to retain chlorophyll in comparison with other supplements. Accumulation of osmolytes like TSC and proline directly protect cell membranes and maintain turgor pressure even under severe water deficits. Salicylic acid showed most pronouncing in mitigation of drought effect by accumulating more proline in both moderate (S_1) and severe stress (S_2) in sugar beet. The result of the present study showed that SA induced significantly more accumulation of osmolyte under moderate stress where, at severe prolonged stress (S_2), SA activated plant defence system to synthesise high proline content to prevent tissue death than plant normally produced (NW). These findings are

consistent with the studies showing that as a signalling molecule, SA protects plant from drought-induced tissue damage and osmotic stress by maintaining cell turgor, scavenging harmful ROS and improving photosynthesis capacity (35). Foliar spray of chitosan elevated proline accumulation in the leaf, boosting drought tolerance by maintaining cell integrity, osmotic balance in the cell and detoxifying ROS to nullify stress effect (36). Similar findings reported in this study, where chitosan increased proline accumulation in sugar beet leaves under both moderate and severe stress after every foliar spray. Foliar application of JA induced more proline accumulation in both moderate and severe stress cycles with intermittent watering after 2nd and 3rd spray, highlighted with the previous reports that the signalling molecule JA may be upregulated genes involved in proline biosynthesis led to rapid proline accumulation and reduced oxidative cell damage. Proline level generally high to alleviate prolonged stress, JA application prevents over-accumulation of proline and maintaining the cell protein integrity. When plant's natural defence exhausted, the novel hormone JA activity shifted to more protective and survival rather than plant growth (37). Similar result was obtained in this study where, increment percentage of proline with JA application was less under prolonged stress (S_2 after 4th spray) than moderate stress. As per the results, proline accumulation in the leaf tissue was reduced by N-Si, HA, B and KA after 4th foliar spray, these results might be explained as N-Si has a catabolic inhibition (38), so it can convert proline to its analogue form Si-proline under drought stress. These findings were supported by previous researches (39), indicating that boron can affect various physiological processes and relieve drought stress with less amount of proline accumulation. While KA, an antitranspirant protects the plants from severe dehydration by restricting water loss and reducing the oxidative stress (40), that may be preventing excessive proline production to increase cellular osmolarity. Similarly, HA application strengthens drought resistance in plants, resulting a reduction in the accumulation of proline (41). Though TSC decreased after 3rd to 4th spray expressing crop maturity and long stress treatment, the result indicated that foliar spray with SA significantly increased TSC content in the sugar beet leaves, perhaps mitigated the negative effects of drought stress. This may be related to previous findings that salicylic acid performed a defence pathway, an important signalling pathway which is stimulated by exogenous SA application which directly affects carbohydrate metabolism in the plant by regulating important enzyme activities, such as sucrose synthase and amylase (42). Exogenous SA helps to enhance breakdown of starch into soluble sugars, critically involved with osmotic adjustment and preserving structural integrity to resume growth (43). In addition, chitosan accumulated more TSC under severe stress which reported previously as effective supplement for overcoming drought stress and enhancing TSC accumulation (44). JA also enhances the enzyme activity involved in sucrose and soluble sugar biosynthesis (43). In this study, N-Si showed convincing in mitigating moderate stress S_1 , while HA induced more TSC accumulation in prolonged stress S_2 . As reported that Nano silicon and HA both synergistically upregulating accumulation of total soluble sugar by promoting the activity of enzymes involved in sugar metabolism, enhanced osmotic adjustment and photosynthetic efficiency under water deficit stress (45). The major protective mechanism by N-Si foliar treatment in response to drought stress is increasing soluble proteins and specific defence related enzymes in N-Si treated plants under water deficit stress, which was observed under S_1 and S_2 conditions throughout the experiment. Foliar

application of N-Si is highly effective, penetrates rapidly into the leaves due to its nano size enhances plant growth and stress tolerance, similar results recorded in S_1 and S_2 as well as NW plants. Under stress condition, N-Si mitigated the stress damage by enhancing nitrogen metabolism and upregulating more protein accumulation over non-stressed normal watering plants (46). These findings also showed a promising enhancement of protein accumulation when the plants sprayed with JA induced high levels of soluble proteins might be related to its mechanism to increase the production of stress-responsive proteins such as dehydrins (37). Another result showed that foliar application of SA, has been reported for its role in regulating protein synthesis under less water availability condition (27). Ascorbic acid boosts plant natural stress tolerance but its accumulation decreased at prolonged stress in S_2 after 4th spray, explained decrease in biosynthesis and rapid depletion of existing ascorbic acid reserves in the leaf tissue (47). Salicylic acid and JA are important phytohormones in the plant signalling and defence pathways responsible for accumulation of antioxidant molecule as AsA (48), which was clearly observed in this experiment. When exogenously applied, these hormones induced more ascorbate accumulation in the leaves alleviating the stress effect under moderate, severe and prolonged drought cycles. Foliar application of HA initiated endogenous accumulation of ascorbic acid in the leaf which synergistically enhanced plant's natural defence system that activates JA and SA signalling pathways (5), the findings may be related to this study that foliar humic acid significantly alleviated the negative effects of plant stress and stimulated antioxidant defence system by increasing AsA content in the sugar beet leaves in S_1 and S_2 compared to untreated plants under prolonged water stress. The high values of Brix % in the sugar beet root resulted in this study, under boric acid (B) application as an essential nutrient, can be explained by its ability to form a complex with sucrose for long-distance sugar transportation in the plants (49). The promising effects of HA on sugar beet growth and stress mitigation affects the accumulation of more sucrose in the tubers making it as a positive supplement that increases Brix % under water scarcity. Among all treatments, N-Si and SA also increased Brix % in S_1 and S_2 over non-treated plants. From the previous research reports, these treatments highlighted as potential biostimulants to protect leaf photosynthetic machinery and improves cellular activity (4, 50). In contrast to other treatments, the effect of jasmonic acid and chitosan on increasing the root Brix %, was more effective under moderate stress than severe stress, this may be due to the epinastic effect of endogenous JA on leaves growth and pigments (7) and that induced by foliar application JA and Chi (3).

Conclusion

Imposition of drought stress by elongation of irrigation intervals disturbed the sugar beet physiological processes and biochemical accumulation. Stress application in cycles with intermittent watering ameliorated drought effect and recovery of plants, could be a suitable approach to future scenario of water depletion situation. Foliar supplements are highly effective and widely involved in plant stress response but their function in tropical sugar beet is less studied. These treatments significantly manage abiotic stresses, regulating plant functions by stimulating antioxidant defences without interfering soil health. For enhancement of sugar beet tolerance and minimising the stress effect, foliar spraying with nano-

silicon, humic acid, jasmonic acid, salicylic acid, boron, kaolin and chitosan seemed promising differently in protecting cell pigments, maintaining water balance, managing redox homeostasis, increasing osmolyte and antioxidant production in the plants. Growing sugar beet crop by using less water and the use of foliar treatments could be helped the farmers to manage overall crop yield and sugar yield (Brix) in certain unfavourable drought situations. However, the molecular mechanisms of these treatments to improve crop response, still need more research in different climatic conditions and with different varieties.

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Authors' contributions

LNL carried out lab work, data analysis and manuscript preparation, review, visualisation and revision. NJ performed conceptualisation, review, analysis, editing and revision. Both authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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