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Stress Tolerance in Sugar Beet under Various Drought Stress Cycles

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ABSTRACT

Background: Drought stress is one of the most common and important environmental factors affecting crop growth and production. Sugar Beet (*Beta vulgaris* L.) is a temperate halophyte crop, but it is sensitive to water scarcity, which can reduce its growth and yield.

Methods: To study the performance of tropical sugar beet under water deficit conditions, a pot experiment was conducted in January to May 2021, using a completely randomized design with three replicates. First and second cycles of drought stress were imposed on seven-week-old sugar beet plants (LS-6 tropical variety). The first phase of stress involved with 5, 10, 15 and 20 days of irrigation withholding to 8 treatments, followed by 15 days of normal watering. Then, half of the treatments were subjected to a second cycle of drought by imposing 3 days of stress. Drought effects were studied in terms of growth, physiological, biochemical and yield characteristics.

Result: Water deficit hampered plant growth by reducing leaf area and leaf area index. A marked decrease in relative water content and chlorophyll stability index was also observed. Proline and total soluble carbohydrate content increased compared to the control, while starch content was decreased. However, after 15 days of watering under the second duration of stress, all parameters recovered against control, with a slight effect compared to single-stressed treatments. Yield reduction was observed under severe stress 20 days water withholding, whereas the drought effect was not significant on the harvest index.

Key words: Drought, Harvest index, Irrigation intervals, Proline, Tropical sugar beet, Water scarcity.

INTRODUCTION

Plants under field cultivation face one or more unfavourable environmental conditions. Around 90% of the world's arable lands are affected by several stressors (Rojas, 2020), leading in yield reductions up to 70% loss in major crops (Mantri *et al.,* 2012). Drought, a common abiotic stress, affects approximately 45% of the global cultivated area (Ashraf and Foolad, 2007) and around 35% of Indian agricultural land (Pal *et al.,* 2022). Drought stress alters plant distribution (Ghaffari and Tadayon, 2019), limits crop productivity and stability and affects plant growth, development, physiological and biochemical processes. Limited water supply causes leaf wilting, early maturation reduces leaf area and weight, that alters root length (Jaleel *et al.,* 2009). Furthermore, the reduction in relative water content is a common consequence of water stress in many crop species (Deka *et al.,* 2018). Plants respond to water stress primarily by maintaining water status through increased water uptake and decreased water loss, employing various strategies to overcome drought stress and adaptation. Under limited water availability, crop plants close their stomata, reduce their height, leaf number, leaf area and accumulate more osmolytes. Sugar beet, a root crop accounting for approximately one-quarter of global sugar production (Singh *et al.,* 2018), is also used as fodder and a source of bioethanol. Although it is a temperate crop primarily grown in cold and mediterranean climates, gradually many heat-resistant varieties have been developed for cultivation in tropical and subtropical areas. Sugar beet requires less water than other sugar crops and exhibits moderate drought tolerance, however, limited water supply can hinder its growth, development and quality (Moosavi *et al.,* 2017). This is the part of master research work presented with the objectives to evaluate the sugar beet tolerance ability to various stress durations under water deficit condition during summer.

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MATERIALS AND METHODS

Plant material and experimental details

Tropical sugar beet LS-6 variety seeds were supplied by the Indian Institute of Sugarcane Research (IISR), Lucknow, India and used as planting material for this research. The pot experiment was conducted at the ICAR-CTCRI and FAS, SOA for lab work, Bhubaneswar, Odisha, India, during January to May 2021. Sugar beet seeds were sown in the

poly-pots containing 25 kg of soil each in three replicates. Adequate number of pots per replication was prepared as per the experiment in a completely randomized design. After 7 weeks of normal growth, the plants were subjected to drought stress by withholding water. The treatments were exposed to single stress (1st cycle) of 5, 10, 15 and 20 days durations as in T1, T2, T3 and T4 respectively. After first regime of stress T1, T2, T3 and T4 were re-watered continuously till harvest, while double stress (1st cycle + 2nd cycle) was imposed in T5, T6, T7 and T8 treatments, initially subjected to 5, 10, 15 and 20 days of 1st cycle stress respectively, which were then re-watered for 15 days after completing 1st cycle and then subjected to 2nd cycle of 3 days stress by withholding water supply in the pots where, control plants were watered daily.

Growth parameters

The number of fully expanded healthy green leaves was counted in the field for each treatment. Total plant leaf area was measured using a leaf area meter and the leaf area index was calculated by following formula:

$$\text{Leaf area index} = \frac{\text{Plant leaf area}}{\text{Ground area}}$$

Root to shoot ratio

The fresh weight of the root and shoot of each sample plant was measured separately to calculate the root to shoot ratio.

Physiological parameters

Relative water content (RWC%)

To measure the relative water content in percentage, the fresh weight of five fully expanded healthy leaves from each plant was recorded. These leaves were then submerged in water for 30 minutes to determine their turgid weight before being placed in a hot air oven at 80°C for drying to measure the dry weight. Relative water content percentage (RWC%) was calculated using the formula:

$$\text{RWC\%} = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

Chlorophyll stability index (CSI)%

Chlorophyll stability index was calculated by taking fresh leaf samples. Two sets of test tubes containing 100 mg of leaf sample in water were prepared. Half of each set was placed in an 85°C water bath and the other half was kept at normal conditions for 30 minutes. Both were then macerated with 80% ethanol and the mixture was centrifuged. The optical density (OD) of the supernatant was measured at 652 nm using a spectrophotometer for both treated and untreated samples. The Chlorophyll Stability Index (%) was calculated using the formula:

$$\text{CSI\%} = \frac{\text{Chlorophyll content in treated sample}}{\text{Chlorophyll content in untreated sample}} \times 100$$

Biochemical analysis

Proline

Proline determination was obtained by using ninhydrin method (Bates *et al.,* 1973). About 100 mg of fresh, fully expanded leaves were macerated with 3% sulfo-salicylic acid. Then it was centrifuged for 15 minutes. A mixture of 2 ml each of the supernatant, ninhydrin and glacial acetic acid was placed in a boiling water bath for 1 hour. Then, it was separated with 4 ml toluene to measure the optical density at 520 nm. The amount of proline in the sample was calculated in µg/g fresh weight and then the percentage increase or decrease over the control was also calculated for each treatment using the formula:

Proline % increase or decrease over control =

$$\frac{\text{Proline content in treatment} - \text{Proline content in control}}{\text{Proline content in control}} \times 100$$

Total soluble carbohydrate

Total carbohydrate percentage in leaves was determined by using anthrone method (Scott and Melvin, 1953). About 100 mg of fresh sample were hydrolysed in a boiling water bath for three hours with 2.5 N HCl and neutralized with solid sodium carbonate after cooling. The volume was then adjusted to 100 ml. Then a mixture of sample extract, distilled water and anthrone reagent prepared and incubated in boiling water bath before cooling it to measure the optical density of the green solution at 630 nm. The percentage of total soluble carbohydrate in the sample was calculated to express TSC% increase or decrease over control using the formula below.

TSC % increase or decrease over control =

$$\frac{\text{TSC content in treatment - TSC content in control}}{\text{TSC content in control}} \times 100$$

Starch

Starch content was determined from dry leaves using the anthrone method (Hedge and Hofreiter, 1962). Oven-dried residue of previously extracted sugar was used for starch extraction. A mixture of 2.5 ml starch extract and 5 ml of anthrone reagent was taken and incubated in boiling water bath, then cooled in an ice bath to measure the absorbance at 630 nm and the amount of starch was calculated from glucose as standard and the percentage increase or decrease in starch was calculated for each treatment by following formula:

Starch % increase or decrease over control =

$$\frac{\text{Starch content in treatment} - \text{Starch content in control}}{\text{Starch content in control}} \times 100$$

Yield parameters

Biological yield was determined by measuring the weight of the whole harvested plants and economic yield by

measuring the tuber weight. The harvest index (HI) was calculated for each treatment using the formula:

$$HI = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

Statistical analysis

One-way analysis of variance (CRD) was performed using SPSS v 26. Statistically significant differences between treatments were indicated at P<0.05. During stressful periods, each treatment was analysed and compared with its respective controls. Fisher's t-test (P<0.05) was applied to study their significance level.

RESULTS AND DISCUSSION

Growth parameters

Reduction in plant growth was observed at p<0.05 as the number of leaves per plant was reduced to half compared to the control after 20 days of withholding irrigation, 7.33 leaves/plant in T4 to 14.67 leaves/plant in control (Fig 1). Moreover, a significant reduction in the photosynthetic area in terms of leaf area per plant was observed 1235.11 cm^2 after 5 days of stress and 433.59 cm^2 exposed to 20 days stress against the respective control plants, which were 1971.42 cm^2 and 2650.01 cm^2, respectively (Table 1). This decline in leaf area resulted due to decrease in plant growth under severe stress. Leaf area index also reduced which was reached minimum value of 0.61 at 20 days long stress duration (Table 1). With gradual increase of drought duration in sugar beet plants, 15 days and 20 days of water withholding caused a significant reduction in leaf number, leaf area and LAI under first cycle of stress against respective controls. Unlike other growth parameters, the root: shoot ratio increased by increasing drought duration showed more biomass accumulation and reaching 0.37 in 20 days stress treated plants by the end of 1st duration of drought stress (Fig 2). Drought impact was somehow managed and recovered in 10 days of water stressed plants than other treatments, the result was more pronounced in double stress of 3 days after 15 days re-watering, same trend of results observed for all growth parameters, where the plants were able to obtain more growth and the results showed recovery in the previous parameters. The number of leaves per plant increased compared to the first cycle of stress. The differences between treatments to that of control were less 13.67 at T5 and 14 at T8 where as in control 16.00 and 17.00 leaves per plant, respectively. Similarly, plant leaf area and leaf area index were observed to recover during the second stress period in plants under double drought stress cycles, where leaf area was 1045.97 cm^2 at T8 with LAI 1.480 in contrast to its control 1981.43 cm^2 and 2.80 respectively. On the other hand, the increment in foliar growth resulted a decrease in the root-to-shoot ratio in the plants after 15 days of watering with 3 days of 2nd cycle of stress, reaching its maximum at T6 was 0.33. The reduction in sugar beet growth under drought stress has been reported previously (Hamed and Emara, 2019), which was explained a significant reduction in leaf area index as a result of increasing water scarcity also match with current results of this investigation, indicated a reduction in leaf area and leaf area index under moderate to severe drought under 14-days of irrigation intervals (Mehanna *et al.,* 2020, Majeed *et al.,* 2016). When water availability is limited due to drought, root: Shoot ratio increases because low water potentials cause more reduction in shoots than roots growth, presented the same in the sugar beet (Wu and Cosgrove, 2000; Khorshid *et al.,* 2018).

Table 1: Leaf area, leaf area index, relative water content and chlorophyll stability index in sugar beet leaves under different stress cycles.

Treatments		LA cm^2	LAI	RWC%	CSI%
Single stress cycle	Control	1971.42±11.88	2.79±0.02	85.58±0.02	84.58±0.05
	T1	1235.11±15.07	1.75±0.02	81.18±0.03	82.15±0.03
	Control	2187.81±14.93	3.09±0.02	85.85±0.03	84.19±0.02
	T2	1164.73±13.25	1.65±0.02	79.27±0.03	80.79±0.05
	Control	2998.09±18.76	4.24±0.03	86.19±0.03	83.82±0.02
	T3	864.04±9.14	1.22±0.01	61.91±0.03	77.67±0.02
	Control	2650.01±17.48	3.75±0.02	86.73±0.04	81.85±0.04
	T4	433.59±10.02	0.61±0.01	51.29±0.04	71.58±0.04
Double stress cycles	Control	2523.07±13.82	3.57±0.02	84.75±0.03	82.32±0.03
	T5	1274.44±12.92	1.80±0.02	80.76±0.02	81.74±0.04
	Control	2638.38±10.18	3.73±0.01	84.16±0.02	82.22±0.04
	T6	1548.77±11.32	2.19±0.02	80.36±0.06	80.23±0.04
	Control	2294.22±15.17	3.25±0.02	83.87±0.03	81.84±0.03
	T7	1126.54±33.53	1.59±0.05	79.77±0.02	78.90±0.06
	Control	1981.43±6.43	2.80±0.01	82.86±0.03	81.20±0.02
	T8	1045.97±24.42	1.48±0.03	79.25±0.02	77.16±0.03

All values in the table are averages of 9 plants from 3 replicates (n=9), ± standard errors and significant according to Fisher's t-test (P<0.05).

Physiological parameters

In terms of chlorophyll stability index (CSI) %, the observation showed a gradual decrease with increasing drought duration, up to 71.58% at 20 days of stress in T4. This somehow recovered after 15 days of re-watering and reached 77.16% in T8. On the other hand, sugar beet plants showed a significant reduction in relative water content under water deficit conditions, from 81.18% at 5 days of stress to 51.29% at 20 days of stress, against the respective controls 85.58% and 86.73%. Imposing 2nd stress of 3 days water withholding did not cause further reduction in RWC, but the effect of the recovery period was clear in managing the water balance in the plants, as the results showed a slight increment in leaf RWC% as 79.25% for T8 compared to the control, 82.86% (Table 1). Under water deficit condition green pigments were reduced and photosynthetic activity also declined in terms of chlorophyll total (Loho *et al.,* 2022), which was expressed in CSI%. Previous researches indicated a decrease in RWC after 7 to 9 days of stress (Wedeking *et al.,* 2018). In the present study, RWC% gradually declined as the drought period increased but maximum decrease in RWC% in leaf was visible at longer drought duration. Reports explained that higher RWC% in leaves as an important characteristics of stress tolerance in plants (Shaw *et al.,* 2002, Kebede *et al.,* 2020).

Biochemical parameters

The results showed a significant accumulation of proline over the control in sugar beet leaves during the drought period, which increased with the duration of stress from 63.14% at 5 days to 276.36% at 20 days of single stress (Fig 3). Proline regulated ROS (hydroxyl radicles) to evoke resistance in plants under prolonged drought stress (Rana *et al.,* 2017). After the recovery period of rewatering, the crop tolerated the second stress duration showing less proline accumulation compared to first stress cycle (Leufen *et al.,* 2016). In this study it was observed that proline accumulation in sugar beet leaves was higher under long duration stress of 20 days, has been reported by many researchers (Loho *et al.,* 2022).This increase in proline, combined with a gradual increase in total soluble carbohydrates in leaves upto 15 days of stress (92.78%)

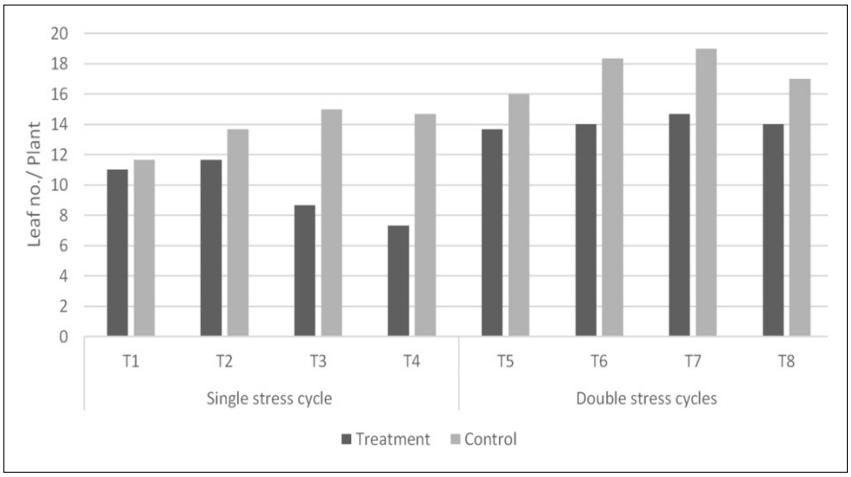


Fig 1: Leaf number of sugar beet under different stress cycles against control.

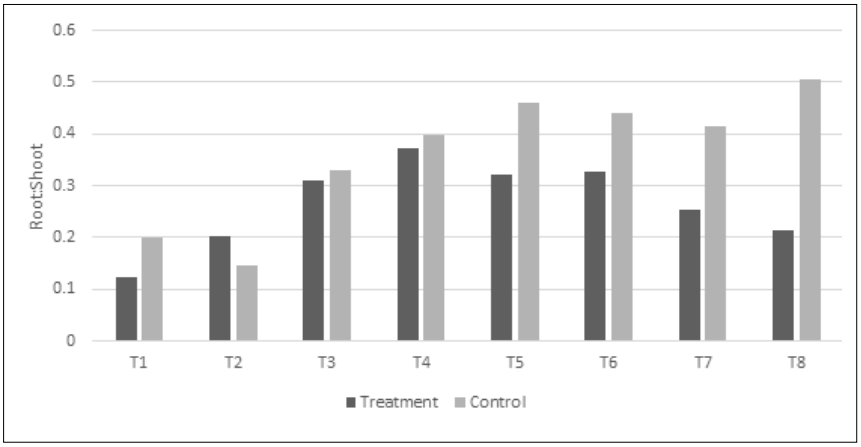


Fig 2: Root: shoot of sugar beet under different stress cycles.

over respective control, then declined with prolonged stress exposure, reaching 24.55% at 20 days stress, explaining its utilization to overcome severe stress. The same trend was observed after the second stress cycle with rewatering (Fig 4). Plants can overcome the adverse effect of water stress by accumulating more TSC in their leaves (Rana *et al.,* 2017). The current results are matched with a strong correlation with previous researches explained under drought stress (Ghaffari *et al.,* 2019). More decrease in leaf starch% was resulted at 20 days of stress, also reflected in second round of stress (-1.61%) even after re-irrigation. The stress effect was recovered after irrigation

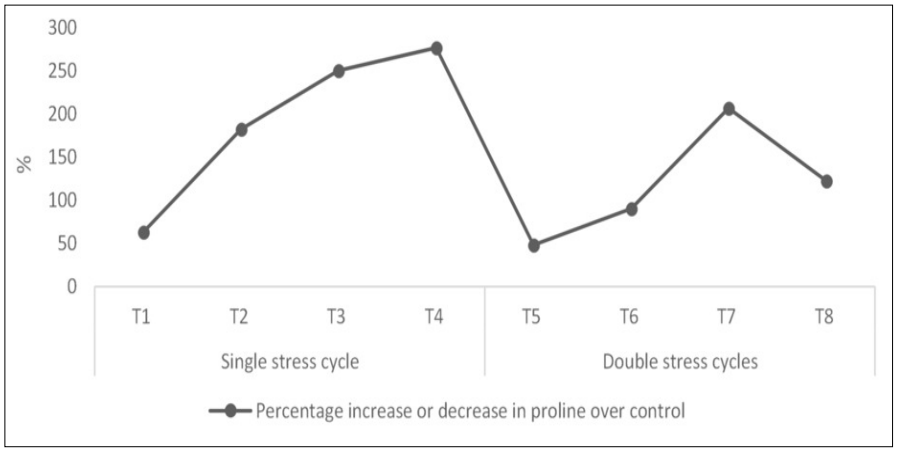


Fig 3: Percentage increase or decrease in proline content in sugar beet leaves over control under different stress cycles.

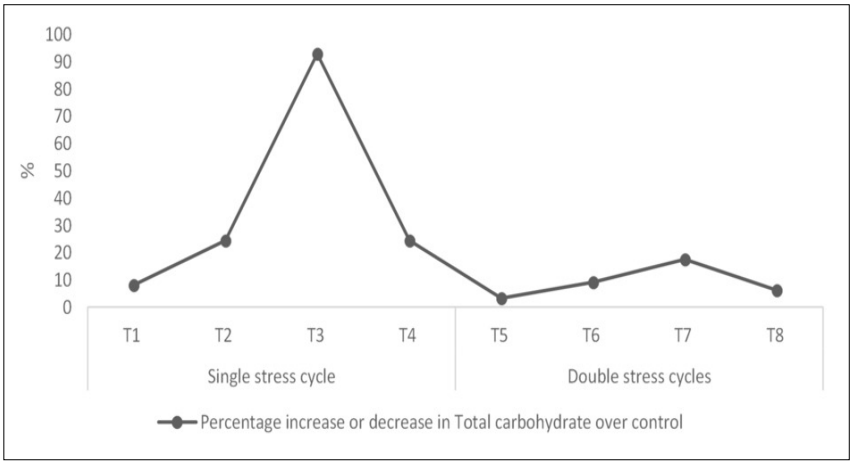


Fig 4: Percentage increase or decrease in total carbohydrate content in leaves over control during stress.

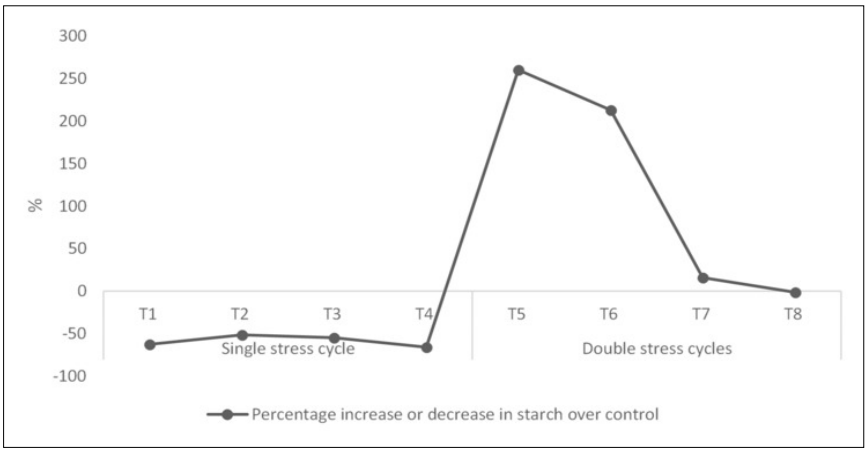


Fig 5: Percentage increase or decrease in starch content in sugar beet leaves over control under different stress cycles.

in T5, T6 and T7, where short period of 3 days water withholding showing least effect (Fig 5). Exhibiting the tolerance activity, primary starch accumulation and its mobilization in the leaves are the most valuable factors within plant responses (Thalmann and Santelia, 2017). When plants growing older, starch accumulation is gradually declined in the leaves under long stress periods and the evidence showing its utilization towards metabolic and cellular adjustments under stress (Wedeking *et al.,* 2018), which was observed in the present experiment during T7 and T8.

In case of sugar beet roots an increase in total soluble carbohydrates deposit also resulted after 15 days of re-watering. In root at harvest, reduction in TSC% over control was observed under long exposure (15 and 20 days) to drought stress along with summer climatic temperature. More accumulation of TSC% was observed in T6, T7 and T8 as 126.98%, 106.36% and 21.53% respectively, showing its growth maturity and stress recovery over control (Fig 6). The starch% in root tubers reduced against control in all types of stress durations. Percentage increased in starch was less decreased in roots of 10 days (T2) stressed plants (-3.30%) and the same also resulted in 2nd phase of stressed plants in T6 (-1.32%) than other treatments exhibiting their tolerance against drought durations (Fig 7). Sugar beet has no such specific self-regulatory mechanism to enhance sugar accumulation in their roots which was observed in the total sugar accumulation and starch content reduction under long duration of drought stress as 15 and 20 days of dryness, even observed at harvest, but is affected by external stimuli based on climatic factors to a great extent (Petkeviciene, 2009).

Yield

More than 50% of yield reduction was observed in both biological and economic yield of 20 days stressed plants (18.65 t/ha and 13.92 t/ha) compared to control (37.23 t/ha and 27.65 t/ha) respectively. After 15 days of re-watering with 3-days stress, the 10days stressed plants were managed their economic yield to 26.304 t/ha compared to the control 27.65 t/ha. Harvest index from treatments was

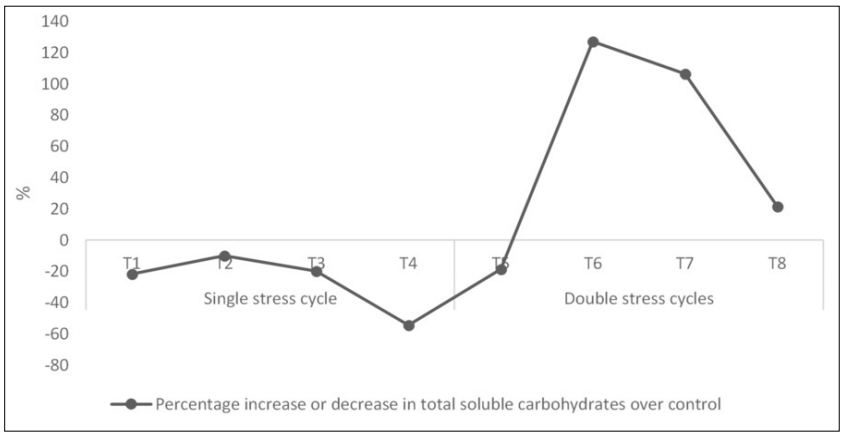
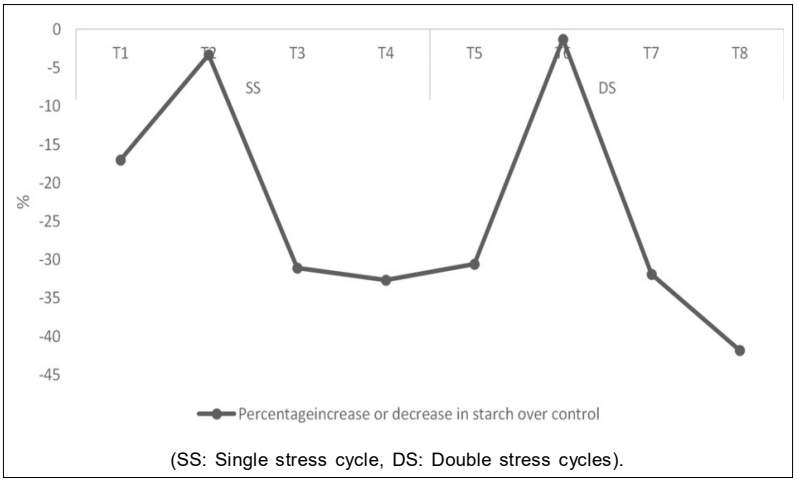


Fig 6: Percentage increase or decrease in total soluble carbohydrates over control in sugar beet tubers at harvest.



(SS: Single stress cycle, DS: Double stress cycles).

Fig 7: Percentage increase or decrease in starch over control in sugar beet tubers at harvest.

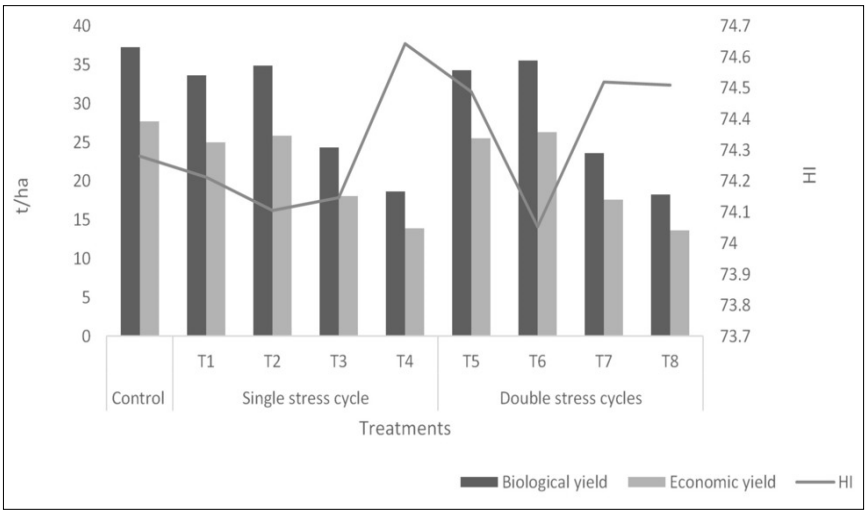


Fig 8: Biological yield, economic yield and harvest index (HI) of sugar beet at harvest.

mostly coincide with normal watering plants and non-significantly increased after re-watering (Fig 8). The depletion in sugar beet yield under drought conditions has been noted in previous findings (Mehanna *et al.,* 2020). The results implies that sugar beet plants had the ability to manage their biomass accumulation under drought stress. Economic Yield and quality of the product is the complex set of interactions, expressed during crop growth and development due to genetic, agronomic and environmental factors which influence sugar beet yield (Brar *et al.,* 2015). Temperature more than 30°C retards yield and quality of sugar beet root (Singh *et al.,* 2019), which was observed throughout the experimental period and reflected in yield results at harvest with respective duration of drought regimes.

CONCLUSION

Imposing drought stress on sugar beet plants restricted their growth, affecting plant water relations, pigment stability and yield. However, the plants coped with water scarcity by accumulating more osmolytes, such as proline and carbohydrates and using the accumulated starch as an energy source to withstand the condition. Long duration stress affected sugar beet performances and these harmful effects reduced after 15-days recovery period of normal watering, leading to less significant effects from the shorter period of second drought duration, where the plants largely recovered and returned to normal conditions in the studied parameters. The tropical sugar beet LS-6 variety can be considered as moderately tolerant to water deficit, even in summer condition, showing good performance under drought up to 10 days of withheld irrigation and exhibiting the ability to recover after rewatering.

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Disclaimers

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Conflict of interest

The authors declare that there are no conflicts of interest. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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