

Evaluation of Yield and Morphophysiological Characteristics of Sugar Beet in Response to Putrescine and Spermidine Application under Drought Stress*

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Abstract

Drought stress poses a major global challenge for sugar beet crops. Applying polyamines (PAs) may offer a promising solution by enhancing the drought resistance of these plants. With the main objective to explore how foliar treatment with PAs impacts various qualitative and morphophysiological traits, a three-replicate split-plot experiment based on a randomized complete block design was conducted during 2020-2021 to evaluate how polyamine foliar applications affect sugar beets root yield, quality parameters, and morphophysiological characteristics under water-limited conditions. The main plots consisted of three irrigation levels (100% (I1), 75% (I2), and 50% (I3) of water requirement), while sub-plots received foliar treatments of putrescine (PUT) and spermidine (SPD) at concentrations of 0.5 and 1 millimolar for each of them, with distilled water serving as the control. In the study, polyamines were applied to sugar beet leaves in 3 three stages: at 8 leaves, 12-16 leaves, and 20-24 leaves. Statistical analysis revealed that irrigation treatments, polyamine foliar applications, and their interactions had significant effect on root yield (RY), α -amino-N content, leaf area index (LAI), total plant dry weight (TPDW), leaf greenness (SPAD), relative water content (RWC), and water use efficiency (WUE). Additionally, white sugar yield (WSY) and Proline content showed significant responses to these treatments. Applying 1 millimolar PUT at irrigation level I2 increased LAI, TPDW, RWC, SPAD, RY, and WSY by 21.1, 28.3, 21.8, 35.3, 27.7, and 47.5 percent, respectively, in comparison to the untreated control group, While PUT 1 millimolar treatment at irrigation level I3 led to a 62% increase in Proline. In general, foliar application with the tested polyamines significantly improved the morphophysiological, quantitative, and qualitative properties. Notably, when polyamines were administered under mild stress conditions, WSY of sugar beet increased, while also achieving water savings.

Keywords: Drought stress, Osmotic adjustment, Proline, Root yield, White sugar yield

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1. Introduction

Sugar beet (*Beta vulgaris* L.) serves as a vital industrial crop, primarily for sugar production, while also providing value as animal feed and an energy source (Duraisamy et al., 2017). Global cultivation spans roughly 4.3 million hectares (FAO, 2023). However, its high water requirements under irrigation pose challenges for expansion in water-scarce semi-arid areas such as Iran (Fabeiro et al., 2003; Hosseinpour et al., 2023). Despite these challenges, sugar beet remains a significant crop in Iran, with around 132 thousand hectares under cultivation and a production of about 8 million tons (Feizi et al., 2017; Azaryar et al., 2022). The long vegetative growth stage, deep root system, and osmotic adjustment capability are important characteristics of sugar beet, contributing to its better tolerance to water scarcity compared to cereals (Koocheki et al., 1996).

Among environmental stresses, drought represents the most critical challenge (El-Sabagh et al., 2019), significantly impacting both yield quantity and quality across various crops, with sugar beet being particularly vulnerable due to disrupted physiological and metabolic functions (Arshadi et al., 2024; Ramazi et al., 2024). Insufficient water availability leads to biochemical changes that produce reactive oxygen species (ROS), causing damage to cellular components. (Sohag et al., 2020; Medrano-Macias et al., 2022). Plants react to this oxidative stress by enhancing their production of protective osmolytes, including Proline, sorbitol, phenol, and ascorbic acid - to maintain cellular homeostasis (Sharma et al., 2019). For sugar beet cultivation specifically, water deficit conditions not only diminish root production but also elevate root impurity levels, ultimately leading to reduced white sugar yields (Zare-Abyaneh et al., 2017; Ebmeyer et al., 2021).

Agricultural researchers have extensively documented the vital role of proper farming practices in reducing drought stress impacts (Farazi et al., 2018; Soltanbeigi, 2019). Among various water conservation strategies, regulated deficit irrigation (RDI) has emerged as a particularly effective approach (Chai et al., 2014; Chai et al., 2016). Research has also highlighted polyamines' potential in improving water use efficiency and drought resistance across various plant species (Aslam et al., 2016).

In the context of increasing global warming, understanding the relationship between polyamines (PAs) and water stress has become increasingly relevant (Shao et al., 2022). Research has particularly focused on how polyamines contribute to water deficit tolerance (Ebeed et al., 2017). Studies indicate that applying optimal concentrations of exogenous polyamines enhances stress tolerance and crop quality by stimulating physiological processes and increasing osmotic compounds like soluble sugars, amino acids, and Proline (Sánchez-Rodríguez et al., 2016; Mohammadi et al., 2018).

Polyamines are small aliphatic amine compounds that contain nitrogenous bases with amino groups. These compounds are essential for normal plant development and enhance stress tolerance (Thomas et al., 2020; Tsaniklidis et al., 2020; Hasan et al., 2021). Their diverse configurations of amine groups allow them to perform multiple functions, such as cell signaling, membrane stabilization, and cellular proliferation (Apel and Hirt, 2004; Rangan et al., 2014; Sequera-Mutiozabal et al., 2016). Higher plants primarily contain three types of polyamines: putrescine (PUT), Spermidine (SPD), and Spermine (SPM), which can exist in either free or bound forms (Jangra et al., 2022; Gholami et al., 2023).

Applying plant hormones externally has been shown to increase tolerance in plants against various abiotic stresses, such as drought (Basra et al., 1997; Nayyar and Chander, 2004; Tyagi et al., 2022). These compounds influence DNA, RNA, and protein synthesis, enhance growth and development, slow aging, and boost disease resistance (Kaur-Sawhney et al., 2003). They also protect cellular membranes from oxidative damage through free radical scavenging (Besford et al., 1993). Studies in wheat and maize have shown that PAs enhance water deficit tolerance by increasing endogenous PA levels (Ebeed et al., 2017; Kutlu Caliskan et al., 2017). This increase in PA content can affect the growth of the nucleus and membrane-bound organelles (Vondrakova et al., 2015).

Water retention under drought conditions represents a crucial plant response (Xu et al., 2022). putrescine pretreatment aids in maintaining water content and photosynthetic efficiency, indicating its role in enhancing osmotic stress tolerance (Kotakis et al., 2014). Similarly, spermidine treatment increases gibberellin levels and strengthens plant defense mechanisms by enhancing antioxidant enzyme activity and transcription processes, particularly regarding ascorbic acid, thus protecting photosynthetic machinery during drought stress in white

clover (Li et al., 2014). In sugar beet specifically, applying 0.9 millimolar PUT significantly improved growth rate recovery (214%), leaf area (40.20%), and leaf relative water content (36.8%) (Islam et al., 2022).

Putrescine and spermidine have been shown to significantly enhance the drought tolerance of tomato plants (Montesinos-Pereira et al., 2014). spermidine notably improved grain filling in wheat by mitigating the adverse effects of drought, while putrescine exhibited the opposite effect (Liu et al., 2016). The impact of polyamine application varies depending on the plant type and growing conditions. For instance, under salt stress, putrescine did not significantly affect the root or shoot growth of sugar beet, whereas spermidine resulted in a notable reduction in shoot growth, without affecting root growth (Hajiboland and Ebrahimi, 2013). In contrast, the application of spermidine improved growth in tobacco plants under saline conditions (Hajiboland and Ebrahimi, 2011).

In another study, a concentration of 1 millimolar spermidine significantly reduced Proline content and helped maintain chlorophyll levels in plants under stress; however, higher concentrations were either ineffective or detrimental (Toupchi Khosrowshahi et al., 2020). Results indicated that foliar application of polyamines at concentrations ranging from 0.5 to 1 millimolar progressively improved physiological traits and wheat yield, while higher doses (1.5 millimolar) resulted in a decline of these traits (Wasaya et al., 2023).

The findings suggest that polyamines can have varying effects depending on plant type, the specific stress condition, and concentration levels (Khan et al., 2018). Consequently, further research is necessary to understand how polyamines can be effectively used to enhance stress tolerance in plants. Given the diverse outcomes of experiments with exogenous polyamines, it remains unclear why different types of polyamines perform distinct roles in promoting stress tolerance. Therefore, a pertinent question arises: Does the foliar application of two types of polyamines (putrescine and spermidine) at various concentrations (0.5 and 1.0 mM) enhance drought tolerance in sugar beet plants under water deficit conditions?

Given the economic importance of sugar beet in sugar production and the challenges posed by climate change and increasing scarcity of water resources, especially in arid and semi-arid regions, finding solutions to enhance and maintain the yield of this crop under water scarcity conditions is essential (Cheng, 2017; Muir, 2022; Chaffai et al., 2024). Therefore, this study was conducted to explore methods to improve and maintain sugar beet yield under limited water resources by investigating the effects of low irrigation and foliar application of two types of polyamines, spermidine and putrescine, on morphophysiological traits, water use efficiency, and sugar and root performance in sugar beet.

2. Materials and Methods

2.1. Materials

This experiment was carried out at the Dasht Gol Agricultural Complex, located in a semi-arid climate 75 km northeast of Torbat-Heydarieh in the Khorasan Razavi Province, Iran (35°N, 59°04E). It was done during two crop years. The average meteorological data, maximum temperature 28.3 and 29.1 °C, minimum temperature 13.7 and 14.2 °C, relative humidity 35.1 and 35.8% and pan evaporation of 9.4 and 9.6 mm per day during the growing season at the field site for 2020 and 2021, respectively. The soil analysis results at a depth of 30 centimeters indicate that the experimental site features a fine-loamy, mixed (calcareous), mesic Aridic Haplustoll (Abbaszadeh et al., 2019). (Table 1).

Table 1. Physical and chemical qualities of soil of the experiment area

Year	EC (dS m ⁻¹)	pH	OC (%)	TNV (%)	Total N (%)	P (mg kg ⁻¹)	K (mg kg ⁻¹)	Clay (%)	Silt (%)	Sand (%)
2020	4.54	7.81	0.61	17.51	0.056	9.37	237.54	43	33	24
2021	4.94	7.83	0.57	18.78	0.053	8.92	214.8	45	33	22

2.2. Methods

The research utilized a split-plot design nested within a randomized complete block design (RCBD) with three replications. Irrigation treatments were established in the main plots at three levels: complete irrigation

meeting 100% of sugar beet water needs (I1), moderate water restriction at 75% (I2), and severe water limitation at 50% (I3). The sub-plots consisted of five foliar treatments: two concentrations each of putrescine (44 mg/L [0.5 millimolar] and 88 mg/L [1 millimolar]) and spermidine (72.5 mg/L [0.5 millimolar] and 145 mg/L [1 millimolar]), along with a control treatment using distilled water application.

The irrigation requirement for sugar beets was determined using the Netwat software, which utilizes the FAO-Penman-Monteith method and 30-year meteorological data for the region. A 90% drip irrigation efficiency was applied, resulting in a total of 12,230 cubic meters of water required, which was then measured using a volumetric counter. The researchers adhered to conventional agricultural management practices throughout the study. Prior to planting, they applied fertilizers at the following rates: 50 kg per hectare of urea (containing 46% nitrogen), 200 kg per hectare of triple superphosphate, and 100 kg per hectare of potassium sulfate. The seeds were sown on April 21, 2020, and April 23, 2021, with the field density of approximately 100,000 plants per hectare. Irrigation for all treatments was done uniformly every five days until the 8-leaf stage, after which irrigation was continuously applied until the end of the growth stages based on the determined levels. Foliar application of polyamines was done in three stages: at 8 leaves, 12-16 leaves, and 20-24 leaves, with a spraying volume of 50 ml per square meter. The control treatment used distilled water instead of PAs.

2.2. Morphophysiological traits

The study evaluated various morphological parameters, including the leaf area index, relative water content, leaf greenness index, total plant dry weight, and Proline concentration.

2.3. Leaf Area Index estimation

To determine the leaf area index (LAI), measurements were taken at 50 days post-planting and then monthly thereafter. In each plot, five plants were chosen at random for leaf area analysis. The Digimizer software was used to photograph and calculate the area of a single leaf from each selected plant. LAI was then derived by taking the leaf area (LA) of an individual plant and dividing it by the plot area (P) that the plant occupied.

$$LAI = \frac{LA}{P} \quad (\text{Eq. 1}).$$

2.4. Relative water content

The method described by Ritchie et al. (1990) was used to measure relative water content. Five samples were taken from the newest fully expanded leaf in each plot. These leaf samples were cut into squares measuring 1 cm², and their fresh weight (FW) was recorded with precision to 0.0001 g. To determine turgid weight (TW), the samples were submerged in distilled water in petri dishes and refrigerated at 4°C for 24 hours. The samples were then dried in an oven at 70°C for 48 hours to measure their dry weight (DW). The relative water content was then determined using the equation:

$$RWC(\%) = \left(\frac{FW - DW}{TW - DW} \right) * 100 \quad (\text{Eq. 2}).$$

This equation considers the difference between fresh and dry weights relative to the difference between turgid and dry weights, expressed as a percentage.

2.5. Leaf greenness index

A SPAD device (SPAD-502 Minolta Co Ltd, Osaka, Japan) was used to assess the chlorophyll reading index on 10 fully expanded leaves, intact leaves in the presence of bright sunlight in each plot (Heydari et al., 2023). The measurements were performed axially (like an organ) on the right side and middle of the leaf. The average value of 6 measurements was the SPAD reading for each plot.

2.6. Total plant dry weight

At the end of the growing season (November 14), five plants from each plot were randomly selected to determine plant dry weight, excluding the two side rows and half a meter from the beginning and end of each planting line. The above-ground parts were oven-dried at 75°C for 48 hours and then weighed (Kandil et al., 2020). To measure root dry weight, approximately 100 grams of root pulp were weighed (wet weight) and then

kept in an autoclave at 105°C for two days, it was weighed again (dry weight), and finally, using the tuber's wet weight, the dry weight percentage was calculated (Babaei et al., 2021; Zhou et al., 2024).

2.7. Proline content

The acid ninhydrin technique outlined by Bates et al. (1973) was employed to measure Proline content. Fresh leaf tissue (0.5 g) was processed by homogenizing it with 3% aqueous Sulfosalicylic acid (10 mL), followed by filtration using Whatman No. 2 filter paper. The analysis continued by combining equal volumes (2 mL each) of the filtered solution, ninhydrin acid, and glacial acetic acid in a test tube. This mixture underwent heat treatment in a 100°C water bath for 1 hour, followed by toluene extraction (4 mL). After cooling to ambient temperature, the solution's absorbance was quantified at 520 nanometers using spectrophotometric analysis.

2.8. Yield and root quality evaluation

Plants were harvested from the two central rows (6 m²) of each plot, excluding border areas, on November 14, 2020, and November 16, 2021, to assess root yield and quality parameters. Shoots and leaves were dried in an oven at 75°C for 48 hours before weighing. Roots were cleaned, washed, cut into pieces, and oven-dried at 75°C for 72 hours, then weighed using a digital scale (0.1 precision). For sugar content and quality analysis, a 25 kg sample was submitted to Fariman Sugar Company's laboratory. The roots were washed using an automatic pulp preparation machine, and the root pulp was prepared with an automatic VENEMA machine, model G2. Then, 26 grams of root pulp were mixed with a 165 ml solution of lead acetate (5%) using a magnetic stirrer and then filtered to determine the sugar beet yield. Sugar concentration was measured Polarimetric using a Saccharimeter (polarimeter) following (Reinfeld et al., 1974). Sodium and potassium levels in roots were analyzed using a flame photometer, Model 405 (Corning, Halstead Essex, UK). Alpha-amino nitrogen (α -amino N) content was quantified colorimetry using a beta analyzer (Model D-3016, England) according to Kant and Kafkafi (2002), (Ghaffari et al., 2019). The white sugar yield (WSY) of the tested samples was calculated using equation 4, and the white sugar content (WSC) was determined from equation 3 (Abdollahiyan Nouqabi et al., 2005; Topak et al., 2016).

$$WSC = SC - (MS + 0.6) \quad (\text{Eq. 3}).$$

$$WSY = WSC * RY \quad (\text{Eq. 4}).$$

Equations (3, 4) define white sugar content (WSC), white sugar yield (WSY), sugar content (SC), molasses sugar percentage (MS), root yield (RY), and sugar losses in the sugar factory, which are equal to 0.6.

2.9. Water use efficiency

Calculation of Water use efficiency was used by the equation provided by Zare Abyaneh et al. (2010), taking into account both sugar and root yields as components of the sugar beet crop.

$$WUE = \frac{Y}{W} \quad (\text{Eq. 5}).$$

Equation (3) states that Y = yield (kg ha⁻¹), W = water consumption (m³ ha⁻¹).

2.10. Statistical analysis

The data analysis was conducted using SAS software version 9.2 (SAS Institute Inc.). Prior to analysis, data were evaluated for variance homogeneity and normal distribution according to Kozak and Piepho's (2018) methods. The analysis employed standard ANOVA techniques, with a combined analysis across both experimental years to assess interaction effects (Casella, 2008). Comparisons of means were performed using Duncan's new multiple range test (DMRT), and visual representations of the results were created using MS Excel.

3. Results and Discussion

3.1. Morphophysiological Parameters of the Plant

Statistical analysis revealed highly significant effects ($P < 0.01$) for irrigation treatments, polyamine (PA) foliar applications, and their interaction on multiple plant parameters, including leaf area index (LAI), total plant

dry weight (TPDW), relative water content (RWC), and leaf greenness index (SPAD), as shown in *Table 2*. When comparing the mean data, it was observed that the highest RWC, LAI, TPDW, and SPAD (10.4, 11.3, 12.6, and 19.2% respectively) were associated with the I1 irrigation treatment and 1 millimolar putrescine foliar application (PUT 1 millimolar), which significantly differed from other treatments. The lowest LAI, TPDW, RWC, and SPAD (11.5, 17.9, 13.3, and 26.7% respectively) were observed with the I3 irrigation treatment and without foliar application, showing significant differences with all treatments (*Table 3*). Under mild stress conditions (I2 treatment), PUT 1 millimolar foliar application increased LAI, TPDW, RWC, and SPAD by 21.1, 28.3, 21.8, and 35.3% respectively, compared to the control treatment. These results indicate that despite drought stress decreasing LAI, TPDW, RWC, and SPAD, the foliar application of PUT.1 millimolar significantly increased drought resistance and reduced the negative effects of stress.

Research has demonstrated that polyamines (PAs) enhance plant growth under drought stress in various species including sesame (Gholipour et al., 2020), wheat (Balkan et al., 2019), and Mexican marigold (Babaei et al., 2021). PAs help plants cope with drought by regulating osmosis and neutralizing harmful reactive oxygen species (Aziz et al., 1999; Farooq et al., 2009). While drought typically disrupts plant physiology and weakens antioxidant defenses, applying PAs externally helps plants produce protective compounds like Proline and sugars (Chen et al., 2019), ultimately preserving normal function (Islam et al., 2021).

In this study, applying 1 millimolar putrescine improved several key metrics in sugar beet, including leaf area index, plant dry weight, and relative water content (RWC), and chlorophyll levels. Similar benefits have been observed in drought-stressed maize (Todorov et al., 1998) and wheat (Jahanbakhsh Godehkahriz et al., 2022). Put's protective effects likely stem from its involvement in hormone signaling, chlorophyll preservation, antioxidant activation, and osmotic regulation (Islam et al., 2020; Sohag et al., 2020; Nazim et al., 2024).

PUT application enhances photosynthesis by boosting chlorophyll content (Liu et al., 2018), leading to better sugar beet growth. Studies on Mexican marigold showed PUT application countered drought effects by increasing antioxidant and Proline levels (Arasteh et al., 2020). Similarly, treating sugar beet with L-ornithine (a PA precursor) improved root development under drought, particularly in sandy soils (Ali Hussein et al., 2019). Recent wheat studies (Wasaya et al., 2023) demonstrate that PAs help maintain root growth during drought by improving potassium uptake and water retention. The increased relative water content from PUT application appears linked to improved membrane function and higher free PUT levels in plant tissues (Ola et al., 2019; Abbaspour et al., 2020). Comparable enhancements in plant growth patterns have been documented in studies of sesame (Gholipour et al., 2020), wheat (Balkan et al., 2019), and *Thymus vulgaris* (Abd Elbar et al., 2019) under drought conditions. These beneficial responses can be attributed to polyamines' (PAs) capacity to regulate osmosis and neutralize reactive oxygen species (Aziz et al., 1999; Farooq et al., 2009). While drought disrupts morphophysiological processes and suppresses antioxidant enzyme activity, external PA application effectively modulates physiological processes and stimulates the production of osmotic regulators like Proline, soluble sugars, and amino acids (Chen et al., 2019), protecting plants from stress damage and restoring normal function (Islam et al., 2021).

Our findings demonstrated that applying 1 millimolar putrescine (PUT) externally enhanced key metrics in sugar beet, including leaf area index, total plant dry weight, relative water content, and chlorophyll content (SPAD). Similar positive effects of PUT foliar application on plant dry weight have been observed in drought-stressed maize (Todorov et al., 1998) and wheat (Jahanbakhsh Godehkahriz et al., 2022). PAs have been extensively shown to mitigate various abiotic stresses in plants (Zeid et al., 2014; Ola et al., 2019). Put's effectiveness appears to stem from its role in hormone signaling, chlorophyll protection, antioxidant enzyme enhancement, and osmotic regulation (Islam et al., 2020; Sohag et al., 2020; Nazim et al., 2024).

External PUT application improves photosynthetic performance by enhancing chlorophyll content (Liu et al., 2018), thereby promoting sugar beet growth. In Mexican marigold, PUT foliar application significantly reduced drought stress damage, with drought intensity correlating with increased antioxidant activity and Proline levels, despite decreased leaf water content (Arasteh et al., 2020). Similarly, foliar application of L-ornithine (a PA precursor) effectively mitigated drought stress effects on sugar beet root and shoot development, particularly in sandy soils (Ali Hussein et al., 2019; Lakde et al., 2024). Wasaya et al. (2023) found that PA application in wheat reduced drought's impact on root development by enhancing potassium accumulation and root water

content, while PUT application improved relative water content and leaf parameters, supporting our findings. The observed increase in relative water content following PUT application can be explained by improved free putrescine levels and membrane characteristics (Kotakis et al., 2014; Ola et al., 2019; Abbaspour et al., 2020).

Table 2. Results of the analysis of variance (ANOVA) for the effects of low irrigation and foliar application of polyamines on various traits of sugar beet, including root yield (RY), leaf area index (LAI), total plant dry weight (TPDW), Proline content (PRO), relative water content (RWC), leaf greenness index (SPAD), white sugar yield (WSY), α -amino nitrogen (α -N), root potassium (R K), and root sodium (R Na).

S.O.V	df	RY	LAI	TPDW	PRO	RWC	SPAD	WSY	α - N	R K	R Na
Year (Y)	1	127.6**	5.56 **	103.2 ns	13.91 **	73.12 **	54.5 ns	17.54**	0.195**	2.301**	1.169**
Block	4	3.13 ns	0.009	21.45	0.11	2.23	7.47	0.045 ns	0.006 ns	0.004 ns	0.003 ns
Irrigation(I)	2	2910.1**	20.57 **	3256 **	132.3 **	7281 **	2613 **	49.45**	0.014*	0.410**	0.113**
Y \times I	2	100.04**	0.09 **	0.08 ns	0.14 **	0.09 ns	0.104 ns	0.491 ns	0.001 ns	0.003 ns	0.019*
Error a	8	9.57	0.006	7.25	0.002	2.61	0.61	0.174	0.002	0.012	0.004
Polyamine(P)	4	144.1**	0.83 **	1018 **	14.87 **	187.3 **	182.8 **	13.94**	1.196**	3.243**	0.056**
P \times I	8	42.57**	0.07 **	78.65 **	10.89 *	18.28 **	11.28 **	0.605*	0.004**	0.018 ns	0.001 ns
Error b	48	3.77	0.006	11.83	0.007	2.328	1.023	0.130	0.007	0.027	0.001
CV (%)		10.78	10.25	10.45	9.45	9.24	9.68	10.79	9.41	9.25	9.54

*, ** and ns: significant at 1% and 5% level and not significant respectively

3.2. Proline Content

Statistical analysis revealed that both irrigation treatments and foliar applications significantly influenced

Table 3. Data and comparison of means of the interaction effects of drought stress and foliar application of polyamines on RY, RWC, LAI, TPDW, PRO, SPAD, WSY and α -amino nitrogen (α - N)

Treatments	RY (t ha ⁻¹)	RWC (%)	LAI	TPDW (gr)	PRO (μ mole g ⁻¹ DW)	SPAD	WSY (t ha ⁻¹)	α - N (mmol 100 g ⁻¹)
PUT 1	54.93 a	83.59 a	4.42 a	179.13 a	8.12 d	49.02 a	7.71 a	1.78 ef
PUT 0.5	51.03 b	79.97 bc	4.22 b	171.35 bc	6.62 f	44.75 bc	6.92 bc	1.84 sf
I ₁ SPD 1	52.68 ab	81.46 b	4.29 b	174.56 b	6.33 f	45.58 b	7.23 ab	1.8 d-f
SPD 0.5	51.59 b	78.42 c	4.13 c	168.01 c	5.69 g	43.89 c	6.67 c-e	1.9 bc
Control	45.99 c	75.70 d	3.97 d	159.13 d	4.43 h	41.14 e	5.08 fg	2.43 a
PUT 1	47.65 c	74.61 d	4.08 c	159.81 d	10.20 c	48.00 a	7.36 ab	1.75 f
PUT 0.5	45.00 cd	71.21 e	3.76 e	152.50 e	7.80 e	39.60 d	6.50 c-e	1.85 c-e
I ₂ SPD 1	42.00 e	67.58 f	3.86 e	144.68 f	8.27 d	40.46 ef	6.26 de	1.81 d-f
SPD 0.5	42.92 de	66.04 f	3.49 f	141.38 f	6.59 f	42.48 f	6.00 e	1.88 b-d
Control	37.38 f	61.61 g	3.18 g	131.53 g	5.90 g	35.49 g	4.97 h	2.45 a
PUT 1	34.12 g	51.56 h	2.77 h	112.43 h	12.62 a	29.86 h	4.89 g	1.8 d-f
PUT 0.5	31.29 gh	49.22 i	2.59 i	105.22 i	10.39 c	27.61 i	4.35 h	1.88 b-d
I ₃ SPD 1	32.14 gh	49.52 i	2.60 i	105.85 i	12.02 b	27.77 i	4.46 f	1.84 c-f
SPD 0.5	31.06 h	48.37 i	2.54 i	103.39 i	9.18 d	27.13 i	4.13 h	1.94 b
Control	29.54 h	46.25 j	2.35 j	99.22 j	7.56 e	23.57 j	3.40 i	2.48 a

Different letters in the columns indicate significant differences at $p \leq 0.05$, according to Duncan's multiple range test. I₁, I₂ and I₃, representing 100%, 75% and 50% of water requirement, respectively. PUT0.5 and PUT1 putrescine at rates of 44 and 88 mg L⁻¹, SPD0.5 and SPD1 spermidine at rates of 72.5 and 145mg L⁻¹, respectively, Control distilled water.

Proline content ($P < 0.01$), with a significant interaction effect between these factors ($P < 0.05$), as documented in *Table 2*. Comparing treatment means showed that the combination of severe water stress (I3) and putrescine (PUT) foliar application produced the highest Proline concentration ($12.62 \mu\text{mol}$ per fresh leaf weight), which was significantly higher than all other treatment combinations. At this level of irrigation, foliar application of 1 millimolar PUT improved Proline (62%) compared to control conditions. The lowest Proline amount ($4.43 \mu\text{mole g}^{-1}$ DW) was observed in level of irrigation I1 without foliar application, indicating a statistically significant difference from the other foliar application treatments at this irrigation level (*Table 3*). Additionally, at the I2 irrigation level, the PUT 1 millimolar foliar application increase Proline (78%) compared of treatment with control, showing a statistically significant difference from other treatments.

Analysis of polyamine (PA) treatments across irrigation levels showed that putrescine (PUT) 1 mM treatment under severe water stress (I3) resulted in the highest Proline content. During drought stress, plants experience disrupted reactive oxygen species (ROS) homeostasis, leading to oxidative damage (Szabados et al., 2010). In response, plants accumulate Proline as a protective mechanism (Hosseini-fard et al., 2022), with higher Proline levels indicating successful drought adaptation (Da Silva Folli-Pereira et al., 2016; Sohag et al., 2020) and helping maintain cellular osmotic potential (Shikari et al., 2022).

This Proline accumulation pattern aligns with previous findings in chickpea under drought conditions (Mafakheri et al., 2010). Similarly, wheat studies showed that external PUT application enhanced both Proline levels and sugar accumulation under drought stress (Ebeed et al., 2017; Heydari et al., 2023). Research by Kubis et al. (2014) on cucumber demonstrated that foliar PA application reduced membrane damage while promoting Proline accumulation. These results support Ozturk and Demir's (2003) suggestion that external PUT application helps plants manage osmotic stress by modifying osmolyte concentrations.

3.3. Root Impurities

Analysis of variance demonstrated highly significant effects ($P < 0.01$) of both irrigation regimes and polyamine (PA) treatments on root impurity parameters, including α -amino nitrogen (α -N), potassium (K), and sodium (Na). Additionally, a highly significant interaction ($P < 0.01$) was observed between irrigation levels and PA treatments specifically for α -amino nitrogen content, as shown in *Table 2*.

Analysis of the mean data revealed that the highest levels of K and Na among different irrigation treatments were associated with I2 and I3 treatments (5.37 and 2.81 milliequivalents per 100 grams of root pulp, respectively), while the lowest amounts of root K and Na were found in I3 and I1 treatments, averaging 5.14 and 2.60 milliequivalents per 100 grams, respectively (*Table 4*). The lowest amount of α -N (1.75 milliequivalents per 100 grams of root pulp) was recorded at the I2 irrigation level with PUT 1 application, while the highest α -N (2.48 tons per hectare) was observed at the I3 irrigation level without foliar application (*Table 3*).

Under severe drought stress (I3), foliar application of PUT 1 treatment resulted in a 38% decrease in α -amino nitrogen (α -N) compared to the control. Similarly, under mild drought (I2), PUT 1 application led to about a 40% reduction in α -N (*Table 3*). The study found that α -N increased with drought stress intensity, while polyamines (PAs) application decreased it. Kheirkhah et al. (2016) reported that foliar application of 1 mM salicylic acid under drought stress reduced α -N in sugar beet by 34%. Sanli et al. (2023) showed that amino acid significantly lowered α -N in sugar beet.

Research by Baradaran Firoozabadi et al. (2004) and Armand et al. (2015) demonstrated that water stress conditions enhance the production and movement of nitrogen compounds as a mechanism to regulate root osmotic pressure. In the current study, drought stress led to elevated root sodium (Na) levels, with moderate (I2) and severe (I3) water stress treatments resulting in 4% and 12% increases in Na content respectively, compared to fully irrigated conditions. These findings align with previous studies by Ober (2001), Ghamarnia et al. (2012), and Ru et al. (2023), who also observed increased root impurities under drought conditions.

While PAs reduced root impurities like Na and α -N, 1 millimolar PUT treatment increased root potassium (K) by 22% compared to the control. Rasovsky et al. (2022) found that humic acids and amino acids increased root K by about 16% in sugar beet. K behaved differently from Na and α -N, with PAs causing an increase. Reduced water availability significantly increased root K, suggesting increased K storage for osmosis regulation under stress (Nabizadeh and Fotohi, 2011). Kafi et al. (2011) suggested that drought stress increases K and Na uptake

for osmotic regulation, with uptake depending on environmental availability. Ober (2001) noted that under drought stress, Na uptake decreases due to K's ability to replace it.

PAs were found to limit the invasion of Na ions into the plasma membrane (Shabala et al., 2007). Another investigation showed polyamines reduced sodium uptake and improved nutrient homeostasis in mung bean (Nahar et al., 2016). The external application of putrescine (PUT) was found to decrease the accumulation of sodium (Na) in the root system of a rice variety that is sensitive to salt, according to studies by Assaha et al. (2017) and (Hasanuzzaman et al., 2019).

Table 4. Data and comparison of means of the main effects of drought stress and polyamines applications on Root content of K and Root content of Na

Treatments	Drought stress			Polyamine				
	I1	I2	I3	PUT 1	PUT 0.5	SPD 1	SPD 0.5	Control
R K(Mmol/100g)	5.33 a	5.37 a	5.14 b	5.61 a	5.38 b	5.45 ab	5.38 b	4.59 c
R Na(Mmol/100g)	2.60 b	2.71 b	2.91 a	2.64 d	2.70 bc	2.69 cd	2.74 b	2.81 a

Different letters in the columns indicate significant differences at $p \leq 0.05$, according to Duncan's multiple range test. I₁, I₂ and I₃, representing 100%, 75% and 50% of water requirement, respectively. PUT0.5 and PUT1 putrescine at rates of 44 and 88 mg L⁻¹, SPD0.5 and SPD1 spermidine at rates of 72.5 and 145mg L⁻¹, respectively, Control distilled water.

3.4. Root and White Sugar Yield

The lowest RY (29.54 tons per hectare) was observed at the I3 irrigation level without PAs foliar application, while the highest RY (54.93 tons per hectare) was achieved at the I1 irrigation level with PUT 1 mM application. Under drought stress conditions (I2 and I3), the application of PUT 1 treatment to leaves resulted in approximately 27% and 15% increases in root yield (RY) compared to the control treatment, as shown in Table 3. This indicates that polyamines (PAs) help mitigate the negative effects of water deficit on root yield (RY). The study found that PUT treatment had a more significant effect than SPD, possibly due to PAs osmosis-regulating capabilities, as noted by Liu et al. (2000), Chen et al. (2019) and Gholizadeh et al. (2022).

The research also demonstrated that PAs can mitigate the adverse effects of drought stress, which typically reduces leaf area, chlorophyll content, and photosynthesis rate, ultimately affecting RY by decreasing assimilated production. These findings are supported by another study showing that PAs effectively increased root biomass in thyme (Abd Elbar et al., 2019). PUT, in particular, is thought to aid plants in adapting to drought stress due to its protective role, leading to a significant increase in sugar beet RY, as reported by Christos et al. (2014) and Apostolova (2023).

In summary, PAs were found to enhance shoot growth, increase the production of photosynthetic materials, and consequently boost RY. This aligns with the results of another study, which demonstrated that PUT significantly increased grain yield in safflower (*Carthamus tinctorius* L.) (Fathi Amirkhiz et al., 2021).

Increasing the irrigation level and applying PAs led to an increase in WSY. The lowest WSY (3.4 tons per hectare) was obtained with the I3 irrigation level and no foliar application treatment, while the highest WSY (7.7 tons per hectare) was achieved with the I1 irrigation level and PUT 1 millimolar application (Table 3). The white sugar yield (WSY) showed a decline as stress levels increased, aligning with the results reported by Ebrahimi Pak et al. (2008). While mild drought stress did not have a significant impact on WSY, severe drought stress led to a substantial decrease in plant WSY. The absence of a significant reduction in WSY under mild drought stress conditions can likely be attributed to the positive influence that mild drought stress has on the percentage of extractable sugar, a phenomenon that has been documented in numerous previous studies, including the work of Kiyamaz and Ertek (2015).

The 1 mM treatment was the most effective in the I2 irrigation, resulting in an increase in white sugar yield (WSY) of nearly 48% compared to the control treatment (Table 3). The differences in WSY among the polyamines (PAs) treatments may be due to variations in extractable sugar percentages and root yield (RY).

Utilizing PAs in plants enhances dry matter production by increasing photosynthetic pigments and maintaining leaf greenness (Zhang et al., 2010; Abbaspour et al., 2020). The observed rise in WSY appears to be partially attributed to the effects of polyamines on the quantitative and qualitative traits of sugar beets, especially under water stress situations. It is important to note that WSY is influenced by both root weight and the amount of extractable sugar.

Studies conducted by S anche-Rodr guez et al. (2016), Mohammadi et al. (2018), and Chen et al. (2019) have demonstrated that applying putrescine (PUT) to plant leaves enhances physiological functions and promotes the synthesis of osmotic regulators such as free amino acids, soluble sugars, and Proline. According to Islam et al. (2021), this mechanism helps plants overcome drought-induced reductions in biomass while improving both the quality and quantity of their bioactive compounds.

In wheat studies, Liu et al. (2016), Ebeed et al. (2017), and Khalid et al. (2022) found that external application of PUT led to soluble sugar accumulation. Moreover, applying PUT and spermidine (SPD) not only mitigated drought stress's negative effects but also improved grain filling and wheat protein content.

3.5. Water Use Efficiency

Statistical analysis showed that water use efficiency (WUE) was significantly influenced ($P < 0.01$) by both polyamine (PA) foliar sprays and their interaction with reduced irrigation treatments. putrescine (PUT) demonstrated superior performance among the tested PAs, yielding the highest WUE improvements regardless of irrigation level.

The study of the interaction between water stress and PAs showed that when irrigation was reduced from 100% to 75% of the water requirement, combined with an increase in 1 millimolar PUT concentration, there was a 12% improvement in WUE for root yield and a 20% increase in WUE for sugar yield, compared to the control.

Furthermore, when water consumption was reduced from 75% to 50% of the water requirement, it resulted in a 10% increase in WUE for root yield and a 5% improvement in WUE for sugar yield. These results are illustrated in Figure 1 of the study.

Polyamine (PA) effects on water use efficiency (WUE) were most pronounced under mild water stress conditions. The application of putrescine (PUT) at 1 millimolar concentration achieved the highest WUE across both experimental years, particularly under drought conditions. This treatment resulted in a notable 29% improvement in WUE for root yield compared to untreated plants at the I3 irrigation level.

Studies have shown that external PA application enhances drought tolerance through multiple mechanisms, including preventing lipid peroxidation, improving WUE, and regulating plant metabolism (Sagor et al., 2013; Heydari et al., 2023). Supporting evidence comes from research on bean plants, where foliar putrescine spermidine (SPD) application increased WUE (Torabian et al., 2018). Similarly, in okra, while water deficit reduced WUE, the combined application of Humic Acid and PUT significantly improved water use efficiency (Barzegar et al., 2016).

The enhanced water use efficiency (WUE) clearly results from putrescine (PUT) unique ability to boost essential plant physiological processes, particularly photosynthesis and the dynamics of source-sink relationships. As highlighted by Emadi et al. (2013) and Ghahremani et al. (2020), these findings underscore the effectiveness of applying plant growth regulators, especially PUT, as a strategic and viable solution for significantly improving crop WUE in the face of water deficit situation.

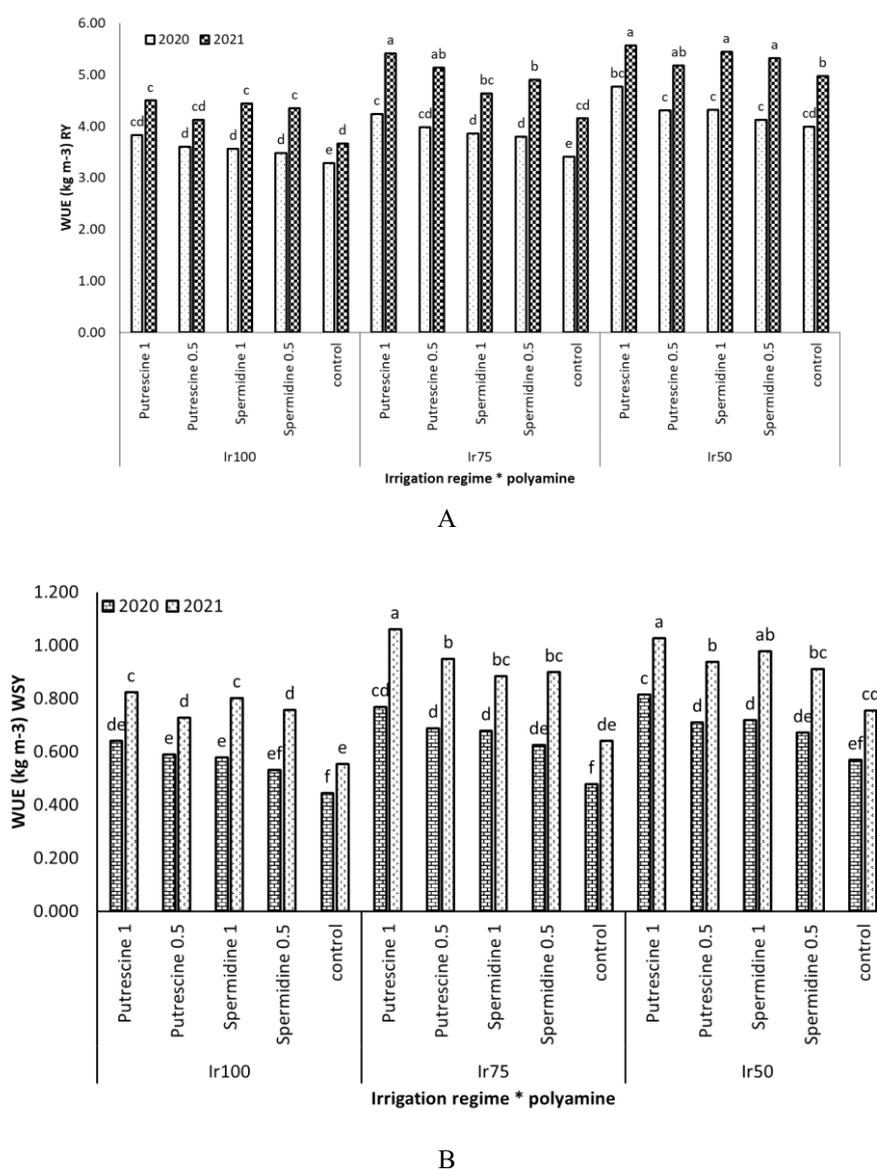


Figure 1. Water use efficiency (WUE) of sugar beet in response to various irrigation regimes and polyamine treatments during the 2020 and 2021 growing seasons. The figure is divided into two parts: **A:** Illustrates the WUE for root yield **B:** Shows the WUE for sugar yield. The irrigation regimes are denoted as follows: I₁, I₂, and I₃ correspond to 100%, 75%, and 50% of crop evapotranspiration, respectively. A control treatment using tap water is also included. The figure employs bar charts, with distinct letters above the bars signifying statistically significant differences at a *p*-value of 0.05 or less, as determined by Duncan's multiple range test

4. Conclusions

The two-year study revealed that drought stress had a negative impact on the growth and yield of sugar beet. However, the external application of putrescine and spermidine significantly increased root and white sugar yield, which was consistent with those of improvements in morphophysiological traits such as leaf area index, total plant dry weight, and leaf greenness index. The use of polyamines, known to be effective in stress signaling pathways, appears to induce plant defense responses. Their application contributed the higher root and white sugar yield by increasing leaf relative water content and Proline under stressful conditions.

Under moderate water deficit (75% water requirement), applying putrescine at one millimolar concentration through foliar spraying effectively enhanced white sugar yield by decreasing sodium and α -amino nitrogen levels. This finding presents a practical solution for sugar beet cultivation in arid and semi-arid regions, where water

scarcity often compromises crop productivity. The foliar application of putrescine at this concentration shows promise in enhancing growth parameters, yield components, and water use efficiency.

These results demonstrate the practical value of polyamines, especially putrescine, as an agricultural tool for managing drought stress in sugar beet production. This approach offers farmers a viable method to strengthen their crops' drought resilience, maintaining both yield quantity and quality under water-limited conditions. The research findings are particularly relevant for agricultural management in drought-prone areas, providing an effective strategy to optimize sugar beet production under challenging environmental conditions.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Concept: Sadrabadi, R.; Design: Sadrabadi, R., Abbaszadeh, M., Bagherzadeh, A., Salari, A.; Data Collection or Processing: Abbaszadeh, M.; Statistical Analyses: Hoseini Mazinani, M.; Literature Search: Abbaszadeh, M., Hoseini Mazinani, M.; Writing, Review and Editing: Sadrabadi, R., Abbaszadeh, M., Bagherzadeh, A., Salari, A., Hoseini Mazinani, M.

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