

## Physiological Response of Drip-Irrigated Sugar Beet to Water Deficit During the Sugar Accumulation Stage: Postprint

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### Abstract

Under drip irrigation conditions, three soil water treatments were established with lower limits of soil water content in the 0–40 cm layer at 70%, 50%, and 30% of field capacity during the sugar accumulation stage of sugar beet. The physiological responses of sugar beet before and after rewatering were analyzed in terms of leaf photosynthetic characteristics, water stress index, recovery degree, yield, and sugar yield to determine the maximum tolerable lower limit of water deficit during the sugar accumulation stage. The results showed that the sugar beet yield and sugar yield under the 30% field capacity treatment were significantly higher than those under the 70% and 50% field capacity treatments, increasing by 51.34% and 51.47% compared with the 70% field capacity treatment, and by 36.72% and 39.48% compared with the 50% field capacity treatment. Before rewatering, the net photosynthetic rate of sugar beet leaves under the 30% field capacity treatment was significantly lower than that of the other treatments; after rewatering, the differences in leaf net photosynthetic rate among treatments decreased over time, while intercellular CO<sub>2</sub> concentration showed an opposite trend. When soil water content decreased to the predetermined lower limit, changes in leaf proline and soluble sugar contents were the most sensitive and were positively correlated with the degree of water deficit. After rewatering, positive compensation effects were observed in leaf cell membrane permeability, antioxidant defense system, and osmotic adjustment substances, manifested as decreased malondialdehyde content, enhanced antioxidant enzyme activities, and increased proline and soluble sugar contents that regulate osmotic adjustment. Therefore, during the sugar accumulation stage, supplemental irrigation when soil water content decreases to 30% of field capacity can compensate to some extent for the negative effects of water deficit on sugar beet, achieving the goal of water-saving, high-yield, and high-quality sugar beet production under drip irrigation in arid regions.

## Full Text

# Physiological Response of Sugar Beet (*Beta vulgaris* L.) to Water Deficit at Sugar Accumulation Stage Under Drip Irrigation

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## Abstract

Understanding the effects of water deficit on the physiological characteristics of sugar beet is essential for determining the minimum soil water content during the sugar accumulation stage. A field experiment was conducted to study changes in leaf photosynthetic characteristics, water stress index, recovery degree, yield, and technological sugar yield under drought resistance and rehydration cultivation. The study included three soil water content levels in the 0–40 cm depth –70% of field capacity (T1), 50% of field capacity (T2), and 30% of field capacity (T3). The results showed that yield and technological sugar yield under T3 were significantly higher than those under T1 and T2 by 51.34% and 51.47%, and 36.72% and 39.48%, respectively. Leaf proline and soluble sugar contents were sensitive to water deficit before rehydration and were positively correlated with the degree of water deficit. Rehydration after water stress showed positive compensation effects on membrane permeability, antioxidant enzyme activities, and osmotic adjustment substance contents of sugar beet. Enhancements were observed in leaf malondialdehyde content, peroxidase and catalase enzyme activities, and proline and soluble sugar contents, which favored increased osmotic adjustment. Therefore, prompt supplemental irrigation when soil water content dropped to 30% of field capacity during the sugar accumulation stage had a compensation effect on sugar beet under water deficit cultivation and helped achieve high yield and quality of sugar beet under drip irrigation in arid areas.

**Keywords:** Sugar beet; Water deficit; Rehydration; Compensation effect; Physiological response; Water stress index; Yield

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## Introduction

The arid northwest region of China is an important agricultural production area where water shortage has become a critical bottleneck severely affecting crop yield and quality. Moderate water deficit during non-water-sensitive periods of crop growth can achieve yield and biomass levels comparable to or even exceeding those under full irrigation, thereby attaining the dual goals of high yield and water savings. Previous studies have shown that the sugar accumulation stage exhibits decreased sensitivity to water, and excessive water at this stage

can reduce sugar beet sugar content [1-2]. Meanwhile, water deficit affects various physiological processes related to crop yield formation to different degrees [3-4]. When crops experience water stress, membrane lipid peroxidation is exacerbated or initiated, leading to damage to biological membranes, disruption of material and energy exchange as well as information transfer, thereby affecting plant physiological and biochemical metabolism and growth and development processes [5-6]. Osmotic adjustment capability is an important characteristic of plant stress resistance and forms the basis for compensation effects under adverse conditions [7]. The antioxidant system can actively adapt and regulate to maintain normal plant physiological functions and is closely related to plant stress resistance [8-9]. Additionally, plant gas exchange parameters such as photosynthesis, stomatal conductance, and transpiration are also sensitive to water stress [10-11]. Previous research has indicated that under moderate water deficit (50% field capacity) during the sugar beet leaf cluster growth stage, the enzymatic reactive oxygen scavenging system shows the most sensitive response in CAT [12].

Currently, high-yield sugar beet fields under drip irrigation are emerging continuously in Xinjiang at the cost of high water and fertilizer input. How to leverage the unique local climatic advantages and water-saving drip irrigation technology to produce more high-quality white sugar while reducing water input—improving quality and efficiency—is an urgent problem facing sugar beet production in arid regions. Therefore, this study selected the non-water-sensitive period of sugar beet for experimentation, utilizing the crop's strong drought resistance characteristics to analyze from a physiological and biochemical perspective the effects of water deficit and rehydration during the sugar accumulation stage on drip-irrigated sugar beet yield, and to determine the lower limit of water control during this growth period, providing a reference for further improving water use efficiency of drip-irrigated sugar beet in arid regions.

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### 1.1 Study Area Description

The experiment was conducted from April to October in 2014 and 2015 at the experimental station of the Agronomy College, Shihezi University, Xinjiang (45°19 N, 86°03 E) at an elevation of 450.8 m. The site has a typical temperate continental climate with an average annual temperature of 7.5–8.2 °C, sunshine duration of 2,318–2,732 h, frost-free period of 147–191 d, annual rainfall of 180–270 mm, and annual evaporation of 1,000–1,500 mm. The experimental area soil is irrigated gray desert soil with heavy loam texture. The topsoil contained total nitrogen 0.89 g · kg<sup>-1</sup>, available phosphorus 0.022 g · kg<sup>-1</sup>, available potassium 0.249 g · kg<sup>-1</sup>, and alkali-hydrolyzable nitrogen 0.058 g · kg<sup>-1</sup>, with pH 7.3 and soil organic matter 13.25 g · kg<sup>-1</sup>. The topsoil bulk density was 1.6 g · cm<sup>-3</sup>, field capacity was 19% (mass water content), and groundwater depth was greater than 5 m.

## 1.2 Experimental Design

The experimental material was sugar beet (*Beta vulgaris* L.) cultivar ‘Beta356’. During the sugar accumulation stage (105–130 days after seedling emergence), three soil water control lower limits were established: mild deficit (70% field capacity, T1), moderate deficit (50% field capacity, T2), and severe deficit (30% field capacity, T3). When measured soil water content fell to the set range, the plot was irrigated to saturated water content. Throughout the experiment, there was no difference in emergence irrigation among the three treatments. No irrigation was applied during the seedling stage (seedling hardening). All three treatments began irrigation 40 days after emergence. Between 40 and 105 days after emergence, the soil water lower limit for all three treatments was maintained at 70% field capacity (i.e., when the three treatments dropped to 70% field capacity, irrigation was applied to saturated water content, and this process was repeated). When sugar beet entered the sugar accumulation stage (105–130 days after seedling emergence), the three levels of water deficit were initiated, and rehydration to saturated water content was applied after reaching the deficit lower limit. Harvest and yield measurement occurred after 130 days.

Irrigation amount was determined by the irrigation quota calculation formula [13], expressed as:

$$H\rho\beta\beta$$

where  $m$  is irrigation amount (mm);  $b$  is soil bulk density in the planned wetting layer during that period ( $\text{g} \cdot \text{cm}^{-3}$ );  $H$  is planned wetting layer depth (cm);  $i$  is target water content (field capacity multiplied by target relative water content). The planned wetting layer depth in this experiment was 40 cm;  $j$  is soil water content before irrigation. Irrigation amount was recorded by water meter. Since it was difficult to precisely control the irrigation lower limit in practice, each treatment was assigned a 5% range value (percentage of field capacity).

Sugar beet was planted under mulched drip irrigation with a row spacing configuration of 20 cm  $\times$  50 cm. The drip tape configuration followed the “one tube for two rows” pattern, i.e., one lateral controlled two rows of sugar beet. Plot size was 4 m  $\times$  12 m with a 1 m isolation zone between plots, arranged in a randomized block design with three replications. To ensure emergence, all plots were irrigated to soil saturated water content after sowing. Treatments began through controlled irrigation amounts 105 days after emergence, and the sugar accumulation stage ended 130 days after emergence.

## 1.3 Test Items and Methods

During the sugar accumulation stage, soil moisture at 20 cm, 40 cm, and 60 cm depths was monitored daily from 9:00–10:00 AM using Watermark sensors (Irrometer Company, Riverside, CA). These values reflect soil water content

status, with a measurement range of 0 to  $-2$  bar (0 to  $-200$  kPa), where 0 bar indicates saturated soil water content and  $-2$  bar indicates extreme drought.

At 0 h, 24 h, 48 h, and 72 h after rehydration in each treatment, fully expanded sugar beet leaves were selected from each replication. Gas exchange parameters were measured from 10:00 AM-12:00 PM using a Li-6400 (Li-Cor Inc., Lincoln, USA) portable photosynthesis system, followed by destructive sampling. Corresponding leaves and underground taproots were cut with scissors. One portion of the material was immediately taken back to the laboratory for determination of malondialdehyde (MDA) content and electrical conductivity. Another portion was rapidly wrapped in aluminum foil and placed in a liquid nitrogen tank for analysis of peroxidase (POD) and catalase (CAT) activities, as well as proline (Pro) and soluble sugar contents. Yield and sugar content were measured after the root expansion stage ended 130 days after emergence.

Sugar yield = single plant yield  $\times$  sugar content  $\times$  theoretical plants per hectare  $\times$  emergence rate (2)

Physiological indices were determined using methods referenced in [14-15]: MDA content by the thiobarbituric acid method, electrical conductivity by conductivity meter, POD activity by the guaiacol method, CAT activity by the ultraviolet absorption method, Pro content by the sulfosalicylic acid method, and soluble sugar by the anthrone colorimetric method.

The water stress index (WSI) reflects the degree of deviation of each index from the control under stress conditions and can be used to indicate the effect of water stress on seedlings. The calculation formula is as follows [16]:

$$WSI = \frac{W_{xs}}{X_{ck}}$$

where WSI (water stress index) is the water stress index for a particular parameter under water stress,  $W_{xs}$  is the measured value of a particular parameter at a certain growth stage under water stress, and  $X_{ck}$  is the measured value of the corresponding parameter in the control. If a parameter change is negatively correlated with plant drought resistance, the reciprocal of  $X_{ws}/X_{ck}$  is used. WSI values range from 0 to 1, with larger values indicating greater impact of water stress on that parameter.

Recovery degree ( $Rd$ ) refers to the closeness of each parameter's recovery to the control after water stress relief. It is calculated using the following formula [16]:

$$Rd = \frac{X_r}{X_{CK}}$$

where  $Rd$  is the recovery degree of a particular parameter after water stress relief,  $X_r$  is the measured value of a particular parameter after water stress relief, and

$XCK$  is the measured value of the corresponding parameter in the control (70% field capacity treatment). If a parameter change is negatively correlated with plant drought resistance, the reciprocal of  $Xr/XCK$  is used.  $Rd$  ranges from 0 to 1, with larger values indicating higher closeness to the control value and better recovery after stress relief.

#### 1.4 Data Processing

Data were statistically analyzed using SPSS 12.0 software. One-way ANOVA and least significant difference (LSD) tests were used to compare differences among different data groups ( $P < 0.05$ ). Figures were created using Origin 8.5 software.

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#### 2.1 Soil Water Potential Distribution in the Cultivated Layer Under Different Water Treatments

During the sugar accumulation stage (105–130 days after seedling emergence) in the two-year experiment, the average soil water potential in the 0–40 cm soil layer ranged from  $-199$  to  $-5$  kPa. The fluctuation amplitude was smaller under the mild water deficit treatment (T1), moderate under T2, and severe under T3. Soil water potential increased slightly with soil depth. Compared with the 20–40 cm soil layer, soil water potential decreased in the 0–20 cm layer and increased slightly in the 40–60 cm layer, which may be related to the placement of the Watermark sensors (Fig. 1 [Figure 1: see original paper]).

#### 2.2 Effects of Water Treatments on Gas Exchange Parameters of Drip-Irrigated Sugar Beet

Gas exchange parameters of sugar beet varied under different soil water conditions (Fig. 2 [Figure 2: see original paper]). Leaf net photosynthetic rate, stomatal conductance, intercellular  $CO_2$  concentration, and transpiration rate in T3 treatment were significantly lower than those in T1 and T2 treatments both before and after irrigation, possibly due to irreversible leaf damage caused by excessively low soil water content under T3. The leaf gas exchange parameters of T1 and T2 treatments showed different patterns before and after irrigation in the two-year experiment. In 2014, before irrigation, leaf net photosynthetic rate and transpiration rate showed  $T2 > T1$ , while stomatal conductance and intercellular  $CO_2$  concentration showed  $T1 > T2$ . After irrigation, leaf net photosynthetic rate, stomatal conductance, and transpiration rate showed  $T1 > T2$ , while intercellular  $CO_2$  concentration showed  $T2 > T1$ . In 2015, before irrigation, leaf net photosynthetic rate and stomatal conductance showed  $T1 > T2$ , while transpiration rate and intercellular  $CO_2$  concentration showed  $T2 > T1$ . After irrigation, leaf net photosynthetic rate, stomatal conductance, and transpiration rate showed  $T2 > T1$ , while intercellular  $CO_2$  concentration showed  $T1 > T2$ . Although the leaf gas exchange parameters of T1 and T2 treatments differed

before and after irrigation between the two years, leaf net photosynthetic rate and intercellular CO<sub>2</sub> concentration consistently showed opposite trends, indicating that leaf growth in T2 treatment may have been limited by non-stomatal factors [17].

### **2.3 Comparison of Water Stress Index and Recovery Degree of Drip-Irrigated Sugar Beet Under Different Water Treatments**

Water stress indices and recovery degrees of sugar beet leaves and taproots varied under different soil water conditions (Table 1 ). Compared with mild water deficit, moderate water deficit caused greater stress on malondialdehyde content, relative electrical conductivity, peroxidase activity, and proline content in both leaves and taproots, but less stress on catalase activity. Severe water deficit caused the greatest stress on proline and soluble sugar contents in leaves and taproots, but the least stress on catalase activity. After rehydration, all physiological indices recovered to varying degrees, with different recovery levels between leaves and taproots. Relative electrical conductivity and peroxidase showed higher recovery degrees in leaves than in taproots, while catalase, proline, and soluble sugar showed lower recovery degrees in leaves than in taproots. Recovery degrees of leaf malondialdehyde, relative electrical conductivity, peroxidase, and proline were greater than 1, indicating that positive compensation effects were produced in cell membrane permeability, antioxidant defense systems, and osmotic adjustment substances of sugar beet leaves after rehydration.

### **2.4 Variance Analysis of Yield Traits of Drip-Irrigated Sugar Beet Under Different Water Treatments**

Two-year yield variance analysis showed (Table 2 ) that sugar beet root yield and sugar yield differed significantly among the three soil water conditions, increasing as the soil water control lower limit decreased, following the pattern T3>T2>T1. Root yield and sugar yield under T3 treatment increased by 51.34% and 51.47% compared with T1, and by 36.72% and 39.48% compared with T2. Differences in root sugar content among treatments were not significant, with two-year averages showing T3>T2>T1.

During the sugar accumulation stage, sugar beet root and leaf growth nearly ceases, and dry matter transfers from aboveground parts to the root, accumulating as sucrose in the root. Therefore, water demand is lower than during the rapid leaf cluster growth and root expansion stages, and excessive water actually reduces sugar beet sugar content [18]. Research by Hou Zhen' an et al. [1] showed that adequate water supply during the rapid leaf cluster growth stage followed by limited water supply in the later stage can increase sugar beet root sugar content and total sugar yield while improving water use efficiency. Chen Yanyun et al. [17] pointed out that different irrigation timing and amounts have different effects on sugar beet yield and quality; full irrigation increases yield but reduces sugar content, and the later the irrigation timing and the higher the frequency, the more sugar content decreases. The results of this study showed

that the 30% field capacity treatment increased sugar beet yield and sugar yield by 51.34% and 51.47% compared with 70% field capacity, and by 36.72% and 39.48% compared with 50% field capacity. Therefore, supplemental irrigation when soil water content drops to 30% of field capacity during the sugar beet sugar accumulation stage can achieve the goals of water saving, high yield, and high quality.

Plants often respond to water stress through a series of physiological and ecological changes [19-20], such as alterations in growth rate and biomass allocation patterns, water potential balance, transpiration rate, and enzyme activities [19,20-23]. Sugar beet during the leaf cluster growth stage can adapt to water stress by regulating physiological and biochemical changes including photosynthetic rate, cell membrane permeability, protective enzyme activities, and osmotic substances [12]. The results of this study showed that proline and soluble sugar water stress indices were more sensitive to water deficit during the sugar accumulation stage, and recovery degrees of leaf malondialdehyde, relative electrical conductivity, peroxidase, and proline were greater than 1 after rehydration, indicating that positive compensation effects were produced in leaf cell membrane permeability, antioxidant defense systems, and osmotic adjustment substances after rehydration following mild, moderate, and severe water deficit during the sugar accumulation stage.

Water deficit affects crop photosynthetic physiological processes, causing decreases in net photosynthetic rate and transpiration rate, reduction in chlorophyll content, and accumulation of reactive oxygen species, hydrogen peroxide, and hydroxyl radicals, which damage photosynthetic membranes and subsequently affect various photosynthetic processes. Some studies have shown that rehydration after water deficit has a super-compensation effect on crop photosynthetic rate and super-compensation accumulation of photosynthetic products, facilitating their transport and distribution [24]. Leaf net photosynthetic rate, transpiration rate, stomatal conductance, and yield all decrease with increasing drought stress severity [25]. The results of this study showed that leaf net photosynthetic rate of sugar beet decreased significantly under severe water deficit. Net photosynthetic rate gradually decreased with increasing rehydration time, and intercellular CO<sub>2</sub> concentration showed opposite trends to other parameters among treatments, suggesting that leaf metabolic processes may have been blocked after water stress. Further work is needed to elucidate the relationship between soil water and sugar beet leaf growth and root sugar accumulation from the perspectives of leaf stomatal number, stomatal morphology, chloroplast anatomical structure, and ultrastructure.

Sugar beet can tolerate water deficit to 30% field capacity during the sugar accumulation stage. This treatment produced the highest sugar beet yield and sugar yield, increasing by 51.34% and 51.47% compared with 70% field capacity, and by 36.72% and 39.48% compared with 50% field capacity. Proline and soluble sugar contents in sugar beet physiological processes were most responsive to water deficit and were positively correlated with water deficit degree, making

them suitable indicators for monitoring water deficit status in sugar beet. After rehydration, positive compensation effects were produced in leaf cell membrane permeability, antioxidant defense systems, and osmotic adjustment substances of sugar beet. Therefore, supplemental irrigation when soil water content drops to 30% of field capacity during the sugar accumulation stage can achieve the goals of water saving, high yield, and high quality for drip-irrigated sugar beet in arid regions.

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