

Effects of different deficit irrigation on sugar accumulation of pineapple during development

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Abstract: The potted pineapple cultivar 'Comte de paris' was used to study the influence of deficit irrigation on fruit sugar accumulation in greenhouse during the fruit enlargement period. The study included a control (normal irrigation) and two treatment groups, moderate deficit (50% of the control irrigation) and severe deficit (25% of the control irrigation). The results indicated that the deficit irrigation significantly decreased the sucrose accumulation. The sucrose content in the fruits of moderate deficit irrigation was the lowest. During the mature period, the deficit irrigation decreased the sucrose phosphate synthase activity (SPS) and increased the sucrose synthase (SS) and neutral invertase (NI). The moderate deficit irrigation significantly improved the acid invertase activity (AI). However, it was inhibited by the severe deficit irrigation. In general, the moderate treatment reduced the SPS activity and enhanced the NI and AI activities, while the severe treatment decreased the SPS and AI activities.

1 Introduction

Sugar content is an important attribute of fruit quality and affects fruit production and economic value. Sugar is synthesized and accumulated in the flesh during fruit growth [2]. In plants, the enzymes that are connected with the accumulation of sucrose are invertase (Ivr), sucrose synthase (SS) and sucrose phosphate synthase (SPS) [4, 5]. Among them, SPS is considered as one of the key enzymes that regulate the sucrose synthesis pathway.

Water irrigation is extremely important cultivating practice in agricultural crop production. However, in pineapple cultivation, farmers only realize the importance to fertilization and neglected the irrigation practice. As there are seasonal precipitation and rainfall distribution imbalance in the southern China, the serious seasonal drought frequently occurs. Dry drought stress seriously affected pineapple plant growth, fruit development and pineapple quality. Therefore, the objective of this study was to evaluate the effects of different deficit levels of irrigation on the sucrose content and enzymes activities related to the sucrose metabolism.

2 Materials and Methods

2.1 Materials

Field-grown pineapples (*A. comosus* cv. Comte de paris) were cultivated in the pineapple Resource Bank of South Subtropical Crops Research Institute. A control group (normal irrigation) and two treatment groups, moderate deficit irrigation (50% of the control irrigation level) and severe deficit irrigation (25% of the control irrigation level) under the same other management conditions such as fertilization, soil management, disease control and pruning were designed to carry out this study. Ninety



fruit samples were selected after the full florescence period from July to September in 2015. The fruits were randomly sampled every 10 days from the 20th day after anthesis (DAA). They were immediately frozen in liquid nitrogen and stored at -80°C before being analyzed

2.2 Determination of sucrose content

For determination of sucrose content, the flesh tissues (10 g) in 80% ethanol was ground and then adjusted to pH 7.0 with 0.1 M NaOH. The mixture was heated for 5 min at 80°C and then obtain supernatant extract. The extract corresponding to 0.5 g fresh weight (FW) was dried in vacuum and re-dissolved in water. The solution was passed through an ion-exchange column (Dowex 50W-X8 and Dowex 1-X8). The eluate (10 μL) was then analyzed by the high-performance liquid chromatography (HPLC) method described in Zhang & Li [6] The HPL system (Shimadzu LC-6A; Kyoto, Japan) as equipped with a RI detector and a SP1010 column (Showa Denko K. K., Tokyo, Japan) with a flow rate of $0.5\text{ mL}\cdot\text{min}^{-1}$.

2.3 The extraction and activity analysis of SPS, SS, NI and AI enzymes

Sucrose-metabolizing enzymes were extracted from the frozen flesh tissue by the method described by Zhang et al. [7]. The flush tissue was homogenized in 10 mL of ice-cold buffer containing 50 $\text{mmol}\cdot\text{L}^{-1}$ Hepes-NaOH (pH 7.5), 0.5 $\text{mmol}\cdot\text{L}^{-1}$ Na-ethylenediaminetetraacetic acid (EDTA), 2.5 $\text{mmol}\cdot\text{L}^{-1}$ DTT, 3 $\text{mmol}\cdot\text{L}^{-1}$ diethyldithiocarbamic acid, 0.5% (w/v) bovine serum albumin (BSA) and 1% (w/v) insoluble polyvinylpyrrolidone (PVP). After centrifugation at 12,000 g for 20 min at 4°C . The supernatant was collected and dialyzed for approximately 20 h in the solution consisting of 25 $\text{mmol}\cdot\text{L}^{-1}$ Hepes-NaOH (pH 7.5) and 0.25 $\text{mmol}\cdot\text{L}^{-1}$ Na-EDTA. The insoluble pellet was washed twice by the medium above and then incubated with shaking for 4 h in ice-cold the medium containing $1\text{ mol}\cdot\text{L}^{-1}$ NaCl.

Invertase activity was assayed in a final volume of 25 mL of the solution containing 0.2 mL of dialyzed enzymatic extraction and 0.8 mL of reaction solution (pH 4.8 or 7.2, $0.1\text{ mol}\cdot\text{L}^{-1}$ Na_2HPO_4 , $0.1\text{ mol}\cdot\text{L}^{-1}$ sodium citrate, $0.1\text{ mol}\cdot\text{L}^{-1}$ sucrose). The activity was measured by the quantity of reducing sugars released in the assay media with dinitrosalicylic acid [3].

SPS activity was assayed by using 0.15 mL of reaction medium and 0.2 mL of enzyme sample. The reaction medium was composed of 50 $\text{mmol}\cdot\text{L}^{-1}$ Mops-NaOH (pH 7.5), 10 mM MgCl_2 , 5 $\text{mmol}\cdot\text{L}^{-1}$ glucose-6-phosphate, 10 $\text{mmol}\cdot\text{L}^{-1}$ fructose-6-phosphate and 5 $\text{mmol}\cdot\text{L}^{-1}$ UDPG. After the mixture was incubated for 30 min at 37°C , the reaction was stopped by adding 0.1 mL 30% (w/v) NaOH and kept in boiling water for 5 min. When cooled to room temperature, 0.5 mL of resorcinol solution (12%, v/v) and 0.5 mL of HCl ($12\text{ mol}\cdot\text{L}^{-1}$) were added into the mixture and held in an 80°C water bath for 10 min. The blank group was obtained by adding the sterile water to the medium containing resorcinol. The procedure for the SS activity assay was identical to that of SPS except for the reaction mixture, where fructose 6-phosphate or glucose 6-phosphate was replaced by contained $10\text{ mmol}\cdot\text{L}^{-1}$ fructose [1].

2.4 Statistical Analysis

All data were analyzed using DPSv3.01 for the variance and SAS9.0 for correlation according to the different experimental requirements.

3 Results and Discussion

3.1 The changes of sucrose contents in the control and different deficit levels of irrigation

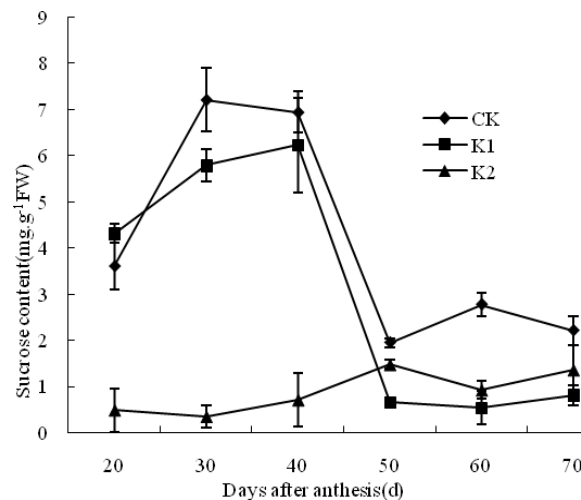


Figure 1. Effects of different water stress on sucrose content in the developing pineapple fruits (CK:control, K1:moderate deficit; K2: severe deficit)

The sucrose content in control was high in the early stage of fruit developing from 20d to 40 days after anthesis (DAA), then decreased rapidly, to reach the lowest value at 50 DAA ($1.94 \text{ mg.g}^{-1}\text{FW}$) (Fig.1). Although the sucrose content was lower than the control during the fruit development (except for 20 DAA), the trend of sucrose content change of moderate deficit treatment was basically consistent with the control. The sucrose content change of severe deficit treatment was completely different from the control with lowest in sucrose content during the first 30 days (lower than $0.48 \text{ mg.g}^{-1}\text{FW}$). Then the higher value was from 30 days to the mature. During the period (50 to 70 DAA), the sucrose content was lower than control and higher than moderate deficit treatment. However the difference between moderate and severe deficit treatments was not significant.

3.2 The effects of the different deficit levels irrigation on SPS activity

The changes of SPS activity of pineapple fruit, at different development stages and different irrigation deficit conditions, are shown in Figure 2. The SPS activity in control decreased during the fruit development to the lowest activity value ($10.56 \mu\text{mol.g}^{-1}.\text{h}^{-1} \text{FW}$) appeared in the 70 days after anthesis. In the moderate deficit treated fruit, the SPS activity during the first 30 days was lower than that from 40 to 70 DAA. The change of severe deficit treatment was in agreement with the control, but slightly fluctuant between 6.2 and $9.2 \mu\text{mol.g}^{-1}.\text{h}^{-1} \text{FW}$. During the development, the SPS activity of control was significantly higher than the moderate or severe deficit treatment. However, no significant difference could be observed between the moderate and severe deficit treatment during the later stages of fruit development. The results showed deficit irrigation reduced the SPS activity and had poor sucrose accumulation.

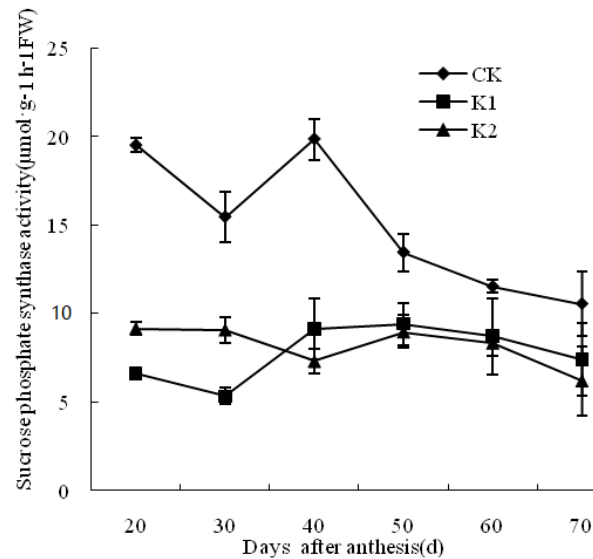


Figure 2. Effects of different water stress on sucrose phosphate synthase (SPS) activity in the developing pineapple fruits (CK: control, K1: moderate deficit; K2: severe deficit)

3.3 The effects of different deficit irrigation levels on SS activity

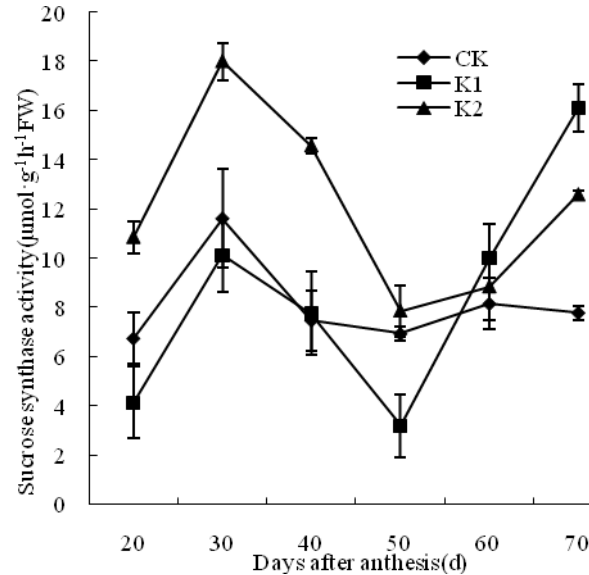


Figure 3. Effects of different water stress on sucrose synthase (SS) activity in the developing pineapple fruits (CK: control, K1: moderate deficit; K2: severe deficit)

SS activity in control fruits rapidly raised up from $6.76 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1} \text{FW}$ during 20 to 30 days after anthesis, then sharply declined to the level of $6.95 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1} \text{FW}$ at 50 days, and presented a slight fluctuation with the range of 50 to 70 days (Fig. 3). The changes of SS activity in the different deficit irrigations were the consistent with the control. Their activities decreased the lowest, then remarkably increased until the harvest. The SS activity of moderate deficit treated ripen fruits had

the highest value with $16.11 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ FW, followed by the severe deficit treated fruits ($12.59 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$, FW), while the SS activity of control was the lowest ($7.80 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$, FW).

3.4 The effects of different deficit irrigation levels on NI activity

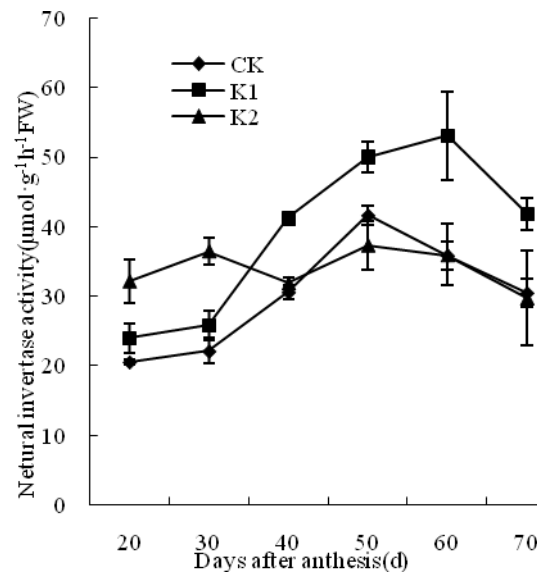


Figure 4. Effects of different water stress on natural invertase(NI) activity in the developing pineapple fruits
(CK:control, K1:moderate deficit; K2: severe deficit)

In the moderate deficit treated and control fruits, their peak times and values of NI activity were different (Fig.4), though both of the tendencies increased from 20 to 60 DAA. In the moderate deficit treatment fruit, peak value ($53.01 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ FW) occurred on the 60 DAA, and then dropped at the maturity time. In the control fruit, NI activity started to go up to the maximum of $41.57 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ FW on the 50 DAA, and declined to the lowest level at harvest time as well. The total activity of moderate deficit ripened fruits was higher than the control. In the severe deficit treatment fruit, the NI activity fluctuated between 29.68 and $37.26 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ FW during the whole development. However, there was no significant difference between the NI activities of control and severe deficit treatment.

3.5 The effects of different deficit irrigation levels on AI activity

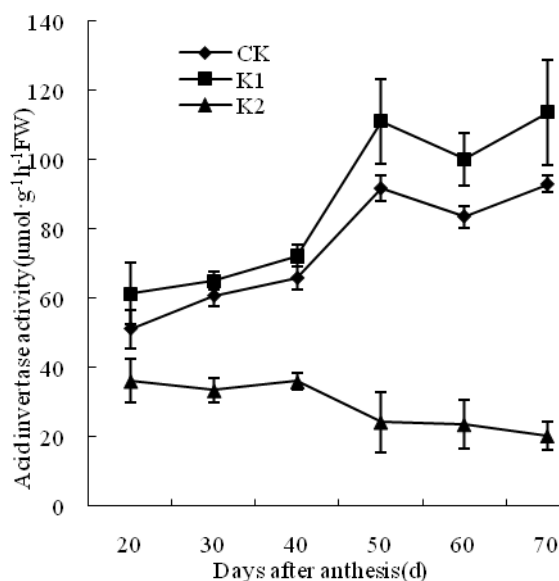


Figure 5. Effects of different water stress on acid invertase(AI) activity in the developing pineapple fruits
(CK:control, K1:moderate deficit; K2: severe deficit)

The AI activity change of control fruit went up during the whole development stage, the highest value was $93.00 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1} \text{FW}$ at harvest (Fig.5). The change of moderate deficit treatment was in accordance with the control, but the activity value was higher than the control from 20DAA to the harvest. At the maturity time, the moderate deficit treatment ($113.64 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1} \text{FW}$) was significant higher than the control ($93.00 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1} \text{FW}$). The AI activity in the severe deficit treated fruit presented the downward trend till the harvest to reach the lowest activity $20.36 \mu\text{mol} \cdot \text{g}^{-1} \cdot \text{h}^{-1} \text{FW}$ at the ripen time.

4 Conclusion

Water deficit stress can significantly low the fruit sucrose content. The moderate deficit treatment had the lowest sucrose content, followed by the severe deficit treatment and control. Different water deficits level had different effect on sucrose metabolism enzymes. The moderate deficit treatment significantly reduced the activity of sucrose phosphate synthase, enhanced the sucrose synthetase activity, neutral invertase and acid invertase activities of ripen fruits, while the severe deficit treatment decreased sucrose phosphate synthase and acid invertase activities. Our study suggested that water stress could change the sugar content in pineapple fruit, through the changes of activities of the enzymes related to sucrose metabolism the influence on the enzymes related to sucrose metabolism.

Acknowledgements

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