Potassium Fertilization Stimulates Sucrose-to-Starch Conversion and Root Formation in Sweet Potato (Ipomoea batatas (L.) Lam.)

Yang Gao 1,2,†, Zhongzhou Tang 3,†, Houqiang Xia 1,2, Minfei Sheng 1,2, Ming Liu 3, Shenyuan Pan 1,2, Zongyun Li 1,2,,* and Jingran Liu 1,2,†

1 Institute of Integrative Plant Biology, School of Life Sciences, Jiangsu Normal University, Xuzhou 221116, China; 2020170806@jsnu.edu.cn (Y.G.); 2020180451@jsnu.edu.cn (H.X.); 202021410@jsnu.edu.cn (M.S.); pan-shenyuan@jsnu.edu.cn (S.P.)
2 Jiangsu Key Laboratory of Phylogenomics & Comparative Genomics, School of Life Sciences, Jiangsu Normal University, Xuzhou 221116, China
3 Xuzhou Sweetpotato Research Center, Xuzhou 221131, China; zhonghoutang@sina.com (Z.T.); 20171004@jaas.ac.cn (M.L.)
* Correspondence: zongyunli@jsnu.edu.cn (Z.L.); liujingran_66@jsnu.edu.cn (J.L.)
† These authors contributed equally to this paper.

Abstract: A field experiment was established to study sweet potato growth, starch dynamic accumulation, key enzymes and gene transcription in the sucrose-to-starch conversion and their relationships under six K2O rates using Ningzishu 1 (sensitive to low-K) and Xushu 32 (tolerant to low-K). The results indicated that K application significantly improved the biomass accumulation of plant and storage root, although treatments at high levels of K, i.e., 300–375 kg K2O ha−1, significantly decreased plant biomass and storage root yield. Compared with the no-K treatment, K application enhanced the biomass accumulation of plant and storage root by 3–47% and 13–45%, respectively, through promoting the biomass accumulation rate. Additionally, K application also enhanced the photosynthetic capacity of sweet potato. In this study, low stomatal conductance and net photosynthetic rate (Pn) accompanied with decreased intercellular CO2 concentration were observed in the no-K treatment at 35 DAT, indicating that Pn was reduced mainly due to stomatal limitation; at 55 DAT, reduced Pn in the no-K treatment was caused by non-stomatal factors. Compared with the no-K treatment, the content of sucrose, amylose and amylpectin decreased by 9–34%, 9–23% and 6–19%, respectively, but starch accumulation increased by 11–21% under K supply. The activities of sucrose synthetase (SuSy), adenosine-diphosphate-glucose pyrophosphorylase (AGPase), starch synthase (SSS) and the transcription of Susy, AGP, SSS34 and SSS67 were enhanced by K application and had positive relationships with starch accumulation. Therefore, K application promoted starch accumulation and storage root yield through regulating the activities and genes transcription of SuSy, AGPase and SSS in the sucrose-to-starch conversion.

Keywords: sweet potato (Ipomoea batatas (L.) Lam.); potassium fertilization; storage root; biomass production; sucrose-to-starch conversion

1. Introduction

At present, sweet potato (Ipomoea batatas (L.) Lam.), as a typical storage root crop, is the seventh most essential food crop in world, and is mainly used to produce starch and alcohol and to feed animals [1]. The sweet potato storage root (SPSR) yield depends on the number and the fresh weight of SPSR per plant, and is closely related to root growth and development and SPSR differentiation [2]. The differentiation of SPSR begins at 10–20 days after transplanting (DAT). The adventitious roots grew out of the stem base, and differentiate and enlarge into enlarged roots, and the number of SPSR per plant remains basically stable around 35 DAT [3]. For the fresh weight of SPSR per plant, starch is the...
major form of fixed carbon in SPSR, approximately 50–80% of SPSR’s dry biomass [4,5]. Therefore, the dry matter and starch in SPSR influence the sweet potato yield.

Potassium (K) is one of the necessary nutrients, and the most crucial osmotic factor in crops [6,7]. As is well-known, K can improve crop stress resistance by optimizing leaf photosynthesis, root elongation, regulation of stomatal guard cells, assimilate transport from source to sink, protein synthesis and enzyme activation [6–9]. Sweet potato is an important storage crop with higher K requirements for optimum production than other commercial crops [10]. In recent years, much attention has been paid to K fertilizer application in sweet potato production. K application increased chlorophyll content and net photosynthetic rate (Pn) in sweet potato leaves [11] and enhanced the photosynthetic products distribution in SPSR and the expansion rate of SPSR [12]. However, excessive K application caused high K intake in sweet potato and a decrease of the K utilization rate [13]. Although there are some reports on the effect of K application on SPSR yield, the studies on its dynamic accumulation are not perfect.

Starch, as the most key carbohydrate in tuber and root crops, is biosynthesized by the decomposition of sucrose products [14]. The conversion from sucrose to starch is regulated by a number of key enzymes. In detail, sucrose phosphate synthase (SPS, EC 2.4.1.14) is a critical enzyme for synthesizing sucrose, and regulating the distribution and conversion of photosynthate between sucrose and starch; invertase (EC 3.2.1.26) and sucrose synthase (SuSy, EC 2.4.1.13) are the main enzymes for sucrose breakdown and energy provision in tuber and root crops [15]; ADP-glucose pyrophosphorylase (AGPase, EC 2.7.7.27) catalyzes ADP-glucose synthesis, used for the synthesis of starch polymers; soluble starch synthase (SSS, EC 2.4.1.21) is essential for amyllopectin content, used as a starch branching enzyme (SBE, EC 2.4.1.18); lastly, SBE is essential for amyllose content [16,17]. These metabolizing enzymes play crucial roles in the conversion from sucrose to starch and their encoding genes are closely related to starch production [5,17].

There are different opinions on the influence of K application on starch and sugar in SPSR. Some studies believe that K application increased the content of starch and sucrose in SPSR [12], which is related to higher AGPase and SSS [11]; other studies believe that K application has little effect on starch content in SPSR [18,19], but rather that it increased the SPSR yield. There is also a view that low K was conducive to the accumulation of starch and sucrose [20]. Although many studies have reported on the correlation of starch synthesis with related enzyme activities [11,12,19], whether and how K application at basal enhances starch accumulation by regulating sink activity, from the perspective of the related enzymes activities and genes transcription in the conversion from sucrose to starch in SPSR, is still unclear. Therefore, we investigated the activities of SPS, SuSy, AGPase, SSS and SBE and the transcriptional levels of their encoding genes in sweet potato plants treated with K, and their relationship with starch accumulation and storage root yield. Two other possible enzymes for sucrose decomposition in SPSR, acid invertase (AI) and alkaline invertase (AKI), were also detected for the activity changes.

There were significant differences among varieties or lines of sweet potato with regards to the effect of K on the SPSR. After fertilization, some varieties increased amylase content, soluble sugar content and amylase activity, while some varieties decreased them [20–22]. Additionally, the response of K during SPSR development was significantly different between low-K-tolerant varieties and low-K-sensitive varieties [23,24]. For low-K-tolerant cultivars, K deficiency decreased the total root length, total volume, total surface area, mean diameter and root activity slightly, whereas these parameters decreased about 2–3 times for low-K sensitive cultivars, compared with low-K-tolerant cultivars. In this study, two sweet potato cultivars, Ningzishu 1 (sensitive to low-K) and Xushu 32 (tolerant to low-K) [24,25], were used to study the physiological mechanisms of K2O rates effecting SPSR yield. The main objective was to 1) study the development and growth of the SPSR and 2) investigate the regulation of K in the enzyme activities and the transcriptional levels of their coding genes in the conversion from sucrose to starch, and the relationship of these enzymes with leaf K content.
2. Results

2.1. Photosynthetic Parameters in Sweet Potato Leaves under Different K₂O Rates

With the increase of the K₂O rate, leaf Pn, gs and Ci all increased firstly and then decreased, and the peak value was for a treatment with 150–225 kg K₂O ha⁻¹ (Table 1). Compared with the no-K treatment, at 35 DAT, the 75 and 150 kg K₂O ha⁻¹ treatments improved Pn by 0.4–12%, while excessive K₂O slightly decreased Pn. At 55 DAT, the application of 75–375 kg K₂O ha⁻¹ increased Pn by 3–25%. The trends of gs and Ci were similar to that of Pn. The analysis of variance showed that Pn and gs had significant differences between cultivars and K₂O rates, and the CV of Ningzishu 1 among the six K₂O rates was lower than that of Xushu 32.

### Table 1. Effect of K₂O rates on photosynthetic parameters, stomatal limitation and non-stomatal limitation in the functional leaf of sweet potato. Each value represents the mean of four replications.

| Cultivar | K₂O Rate (kg ha⁻¹) | Pn (µmol CO₂ m⁻² s⁻¹) | gs (mol H₂O m⁻² s⁻¹) | Ci (µmol CO₂ mol⁻¹) | Non-Ls | Ls |
|----------|---------------------|------------------------|----------------------|---------------------|--------|----|
|          | 35 DAT  | 55 DAT  | 35 DAT  | 55 DAT  | 35 DAT  | 55 DAT  | 35 DAT  | 55 DAT  | 35 DAT  | 55 DAT  |
| Xushu 32 | 0       | 30.85 ab | 28.3 b  | 0.63 d | 0.72 b  | 321.9 bc | 339.9 a  | 514.6 a  | 471.9 ab | 0.16 a  | 0.12 bc |
| 75       | 31.14 ab | 29.16 ab | 0.69 cd | 0.77 ab | 325.2 b  | 330.5 bc | 473.2 ab | 431.8 bc | 0.13 b  | 0.11 abc |
| 150      | 31.59 a  | 29.12 ab | 0.73 bc | 0.89 a  | 328.4 a  | 327.9 c  | 449.6 bc | 366.9 c  | 0.14 b  | 0.11 c  |
| 225      | 30.98 ab | 30.29 a  | 0.82 a  | 0.79 ab | 332.3 b  | 332.3 bc | 397.6 bc | 435.5 ab | 0.16 a  | 0.11 abc |
| 300      | 30.34 b  | 29.85 a  | 0.78 ab | 0.69 b  | 322.3 b  | 335.2 ab | 414.3 c  | 492.6 ab | 0.14 ab  | 0.11 ab  |
| 375      | 28.11 c  | 29.95 a  | 0.73 bc | 0.63 b  | 320.7 c  | 332.7 bc | 442.8 bc | 533.5 ab | 0.15 a  | 0.11 a  |
| CV across K₂O rate (%) | 3.71 | 2.25 | 8.25 | 10.91 | 0.78 | 1.13 | 8.52 | 11.51 | 7.65 | 4.62 |
| Ningzishu 1 | 0       | 30.97 b | 27.94 a  | 0.69 bc | 0.49 b  | 319.6 c  | 338.7 b  | 472.8 ab | 699.7 a  | 0.17 a  | 0.12 ab |
| 75       | 31.09 b | 31.24 a  | 0.8 a   | 0.79 a  | 326.3 b  | 330 b    | 407.6 b  | 422 b    | 0.15 b  | 0.11 bc  |
| 150      | 33.96 a  | 30.79 ab | 0.83 a  | 0.8 a   | 332.1 a  | 327.5 b  | 402.5 b  | 424.4 b  | 0.14 b  | 0.11 bc  |
| 225      | 31.46 b  | 30.14 ab | 0.77 ab | 0.75 a  | 325 b    | 326.2 b  | 422 b    | 448.1 b  | 0.15 ab  | 0.11 c  |
| 300      | 29.23 c  | 29.5 b   | 0.65 c  | 0.69 a  | 321.5 b  | 340.3 a  | 509 a    | 502.7 b  | 0.14 b  | 0.11 c  |
| 375      | 29.38 c  | 30.14 ab | 0.63 c  | 0.48 b  | 318.1 c  | 330.8 b  | 505.2 a  | 703.7 a  | 0.13 a  | 0.11 a  |
| CV across K₂O rate (%) | 5.05 | 3.52 | 10.32 | 19.82 | 1.41 | 1.26 | 9.79 | 22.13 | 9.00 | 9.28 |
| Analysis of variance | * * | ns | ** | ns | ns | ns | ns | ns | ns | * ns |

With the increase of the K₂O rate, Ls in sweet potato leaves decreased or decreased first and then increased (Table 1), and the variation range of Non-Ls was significantly higher than that of Ls. Compared to the no-K treatment, the Ls and Non-Ls values in Xushu 32 leaves after the 75–375 kg K₂O ha⁻¹ treatments were decreased by (−19)–(−2%) and (−23)–(−22%), respectively, and those in Ningzishu 1 were reduced by (−4)–(−21%) and (−31)–(−38%), respectively. Therefore, with the increase of the K₂O rate, leaf Pn in sweet potato was more affected by non-stomatal limitation factors.
The difference between the $L_s$ value and Non-$L_s$ value in sweet potato leaves was only found between cultivars and among K$_2$O rates ($p < 0.05$, Table 1), and the CV of Ningzishu 1 among the six K$_2$O rates was higher than that of Xushu 32. However, the interaction effect between cultivars and K$_2$O rates was not stable due to the influence of years.

### 2.2. Dynamic Accumulation and Simulation of Sweet Potato Biomass

In sweet potato, plant biomass increased following a logistic model by DAT, and significant differences were observed among the six K$_2$O rates (Figure 1a). With the increase of the K$_2$O rate, the maximum growth rate appears later ($T_m$), and the period of rapid plant biomass increase ($T$) was first prolonged and then slightly shortened, but it was still longer than that of no-K treatment, resulting in a high biomass per plant (Table 2).

Compared to no-K treatment, the plant biomass after the 75–375 kg K$_2$O ha$^{-1}$ treatments for Xushu 32 and Ningzishu 1 increased by 3–30% and 15–47%, respectively. There were significant differences between cultivars and among K$_2$O rates, but their interactions were not observed. In terms of the CV among the six K$_2$O rates, the CV of $V_T$ was higher than those of $T_m$ and $T$.

![Figure 1. Sweet potato biomass accumulation of plant and storage root under different K$_2$O rates in 2017 and 2018. (a) Plant biomass accumulation; (b) storage root biomass accumulation. Each data point is the mean ± SE of at least three replications. * and ** mean significance among the different K$_2$O rates at the $p < 0.05$ and 0.01 probability levels, respectively.](image)

Little biomass accumulation in SPSR was observed before 60 DAT; nevertheless, the biomass accumulation in SPSR increased rapidly after 60 DAT, i.e., the SPSR expansion period. There were significant differences among the six K$_2$O rates, the biomass after the 75–375 kg K$_2$O ha$^{-1}$ treatments in SPSR accumulated with a quicker speed than that of no-K treatment (Table 3), and consequently, the SPSR biomass at harvest was increased with the K$_2$O rate increasing, with peak values for the 225–300 kg K$_2$O ha$^{-1}$ treatments (Figure 1b). Additionally, the CV of $V_T$ was higher than those of $T_m$ and $T$, except that of $T$ in Xushu 32.
Table 2. Effects of K$_2$O rates on the eigenvalues of sweet potato biomass accumulation in 2017 and 2018.

| K$_2$O Rate (kg ha$^{-1}$) | Equations | 2017 |  |  |  | Equations | 2018 |  |  |  |
|---------------------------|-----------|------|---|---|---|-----------|------|---|---|---|
|                           | r | $T_m$ (d) | $T_c$ (d) | $V_T$ (g d$^{-1}$) | r | $T_m$ (d) | $T_c$ (d) | $V_T$ (g d$^{-1}$) |
|                            | Xushu 32 |
| 0                         | 0.975 ** | 78.85 | 46.21 | 1.96 | 0.975 ** | 71.28 | 47.89 | 2.00 |
| 75                        | 0.983 ** | 80.41 | 49.69 | 1.93 | 0.979 ** | 73.84 | 49.69 | 2.18 |
| 150                       | 0.997 ** | 84.87 | 49.69 | 1.93 | 0.997 ** | 75.20 | 49.69 | 2.48 |
| 225                       | 0.995 ** | 84.76 | 49.69 | 1.98 | 0.993 ** | 72.57 | 48.77 | 2.51 |
| 300                       | 0.996 ** | 82.73 | 47.89 | 2.70 | 0.999 ** | 75.27 | 49.69 | 2.69 |
| 375                       | 0.980 ** | 77.12 | 44.64 | 2.58 | 0.999 ** | 71.46 | 47.03 | 2.72 |
|                            | Ningzishu 1 |
| 0                         | 0.972 ** | 71.69 | 44.64 | 1.63 | 0.987 ** | 71.84 | 47.89 | 1.68 |
| 75                        | 0.950 ** | 74.25 | 47.03 | 1.79 | 0.996 ** | 78.51 | 49.69 | 2.12 |
| 150                       | 0.981 ** | 78.68 | 47.89 | 1.95 | 0.990 ** | 75.23 | 49.69 | 2.30 |
| 225                       | 0.932 ** | 74.41 | 47.03 | 2.01 | 0.978 ** | 78.10 | 56.04 | 2.22 |
| 300                       | 0.951 ** | 77.60 | 51.64 | 1.99 | 0.999 ** | 72.44 | 48.77 | 2.36 |
| 375                       | 0.943 ** | 70.05 | 48.77 | 2.07 | 0.999 ** | 71.16 | 48.77 | 2.37 |

* $T_m$ is the DAT when biomass accumulation reached the maximum rate of increase; $T_c$ is the period of fast biomass accumulation phase; $V_T$ is the average biomass accumulation rate during the fast biomass accumulation duration. ** means significant at the $p < 0.01$ probability levels.

Table 3. Effects of K$_2$O rates on the eigenvalues of tuberous biomass accumulation in 2017 and 2018.

| K$_2$O Rate (kg ha$^{-1}$) | Equations | 2017 |  |  |  | Equations | 2018 |  |  |  |
|---------------------------|-----------|------|---|---|---|-----------|------|---|---|---|
|                           | r | $T_m$ (d) | $T_c$ (d) | $V_T$ (g d$^{-1}$) | r | $T_m$ (d) | $T_c$ (d) | $V_T$ (g d$^{-1}$) |
|                            | Xushu 32 |
| 0                         | 0.985 ** | 78.12 | 30.27 | 12.51 | 0.988 ** | 82.77 | 26.08 | 10.74 |
| 75                        | 0.981 ** | 79.55 | 30.98 | 14.51 | 0.989 ** | 83.82 | 28.94 | 10.84 |
| 150                       | 0.996 ** | 79.16 | 32.92 | 14.19 | 0.991 ** | 83.75 | 30.98 | 11.12 |
| 225                       | 0.988 ** | 80.77 | 33.34 | 15.03 | 0.991 ** | 84.60 | 33.34 | 11.05 |
| 300                       | 0.998 ** | 83.77 | 36.08 | 14.40 | 0.997 ** | 83.30 | 33.77 | 11.82 |
| 375                       | 0.996 ** | 77.93 | 33.34 | 14.08 | 0.999 ** | 79.56 | 32.92 | 11.89 |
|                            | Ningzishu 1 |
| 0                         | 0.974 ** | 84.14 | 27.72 | 10.80 | 0.976 ** | 80.62 | 25.82 | 7.33 |
| 75                        | 0.998 ** | 88.38 | 30.98 | 12.23 | 0.993 ** | 81.04 | 28.63 | 8.38 |
| 150                       | 0.982 ** | 90.06 | 32.12 | 13.69 | 0.997 ** | 81.38 | 32.92 | 8.69 |
| 225                       | 0.996 ** | 87.97 | 32.51 | 14.50 | 0.999 ** | 79.56 | 34.65 | 9.03 |
| 300                       | 0.997 ** | 88.07 | 35.12 | 13.24 | 0.998 ** | 74.93 | 28.02 | 10.53 |
| 375                       | 0.996 ** | 85.58 | 30.98 | 14.81 | 0.999 ** | 75.11 | 28.02 | 10.33 |

* $T_m$ is the DAT when biomass accumulation reached the maximum rate of increase; $T_c$ is the period of fast biomass accumulation phase; $V_T$ is the average biomass accumulation rate during the fast biomass accumulation duration. ** means significant at the $p < 0.01$ probability levels.
The dynamic accumulation of plant biomass in sweet potato per DAT was simulated using the Equations (1)–(3), and differences were found in the accumulation of SPSR biomass for the six K2O treatments (Table 2). The no-K treatment or 375 kg K2O ha\(^{-1}\) treatment lasted for the shortest \(T\) and the lowest \(V_T\). The 150 kg K2O ha\(^{-1}\) treatment caused a 20–37% higher \(V_T\) and a 1.8–3.5 d longer \(T\) compared to no-K treatment, and the 300 kg K2O ha\(^{-1}\) treatment caused a 22–40% increased \(V_T\) and a 0.9–7.0 d longer \(T\) compared to no-K treatment.

Differences were also observed among the six K2O treatments with regards to SPSR biomass accumulation (Table 3). When all treatments were averaged, the accumulation duration of SPSR biomass was 17.5 d shorter, but faster than that of plant biomass. The 75–375 kg K2O ha\(^{-1}\) treatments caused a 15–21% higher \(V_T\) and a 3.8–5.3 d longer \(T\) compared to no-K treatment.

### 2.3. Distribution of Sweet Potato Biomass

The accumulation and distribution of sweet potato biomass were remarkably affected by years, cultivars, and K2O rates (Table 4). K application enhanced biomass production and the partition ratio to SPSR. Compared with no-K treatment, the averaged biomass after the 75–300 kg K2O ha\(^{-1}\) treatments were increased by 7–26% for Xushu 32 and 21–44% for Ningzishu 1, respectively, and the averaged partition ratio to SPSR after the 75–300 kg K2O ha\(^{-1}\) treatments were increased by 5–12% for Xushu 32 and 7–17% for Ningzishu 1.

### 2.4. Yield Components

In this study, the storage root number appeared to be similar between cultivars, while the fresh weight per storage root, as well as storage root yield, were significantly affected by years, cultivars and K2O rates (Table 4). Compared to the no-K treated sweet potato, K led to an increased yield. Averaged across DAT for each K2O treatment over two years, compared to the no-K treatment, the storage root yield after the 75–300 kg K2O ha\(^{-1}\) treatments were increased by 13–29% for Xushu 32 and 17–48% for Ningzishu 1, respectively, whereas the storage root yield after the 375 kg K2O ha\(^{-1}\) treatment was only increased by 26% for Xushu 32 and 45% for Ningzishu 1, respectively. When all data from K2O rates were pooled, the storage root yield had a significant positive relationship with \(Pn\) (Figure 2). In the following study, three K2O rates (0, 150 and 300 kg K2O ha\(^{-1}\)) were selected to analyze the changes of the conversion of sucrose-to-starch in the SPSR.

![Figure 2](image-url)
| K2O Rate (kg K2O ha⁻¹) | Storage Root Number (No. Plant⁻¹) | Fresh Weight Per Storage Root (g) | Storage Root Yield (Mg ha⁻¹) | Plant Biomass (g Per Plant) | The Biomass Rate of Storage Root/Whole Plant |
|------------------------|----------------------------------|----------------------------------|-------------------------------|---------------------------|---------------------------------------------|
|                        |                                  |                                  |                               |                           |                                             |
|                        | 2017  | 2018  | 2017  | 2018  | 2017  | 2018  | 2017  | 2018  | 2017  | 2018  |                                             |
| Xushu 32               |                                  |                                  |                               |                           |                                             |
| 0                      | 3.67 a | 3.33 a | 163.9 e | 143.1 abc | 32.79 c | 22.18 c | 155.66 c | 155.3 c | 0.78 a | 0.80 c |                                             |
| 75                     | 4.33 a | 4.33 a | 181.7 d | 127.4 c  | 36.61 b | 25.67 bc | 160.87 bc | 170.3 bc | 0.82 a | 0.83 abc |                                             |
| 150                    | 4.33 a | 4.33 a | 190.1 c | 140.5 bc | 38.3 ab | 28.30 ab | 187.53 a | 187.4 ab | 0.87 a | 0.85 ab  |                                             |
| 225                    | 4.33 a | 4.00 a | 200.5 b | 160.2 ab | 40.4 a  | 29.79 ab | 181.88 ab | 178.3 abc | 0.87 a | 0.87 a  |                                             |
| 300                    | 4.33 a | 4.33 a | 206.8 a | 154.1 ab | 40.28 a | 31.05 a | 193.79 a | 196.9 ab | 0.88 a | 0.87 a  |                                             |
| 375                    | 4.00 a | 4.00 a | 207.8 a | 165.2 a  | 38.65 ab | 30.72 ab | 184.28 ab | 202.1 a  | 0.88 a | 0.82 bc  |                                             |
| Ningzishu 1            |                                  |                                  |                               |                           |                                             |
| 0                      | 4.00 a | 2.33 b | 141.9 c | 148.5 a  | 26.40 b | 16.11 b | 118.71 d | 120.3 c  | 0.77 b | 0.71 c  |                                             |
| 75                     | 4.33 a | 3.33 ab | 162.2 b | 129.7 c  | 29.58 b | 20.10 ab | 136.27 cd | 154.1 b  | 0.84 a | 0.74 bc  |                                             |
| 150                    | 4.33 a | 3.67 a | 189.0 a | 132.6 bc | 34.99 a | 22.82 a | 143.78 bc | 165.0 ab | 0.85 a | 0.81 ab  |                                             |
| 225                    | 5.00 a | 4.00 a | 183.5 a | 126.2 c  | 38.02 a | 24.87 a | 158.34 abc | 176.2 a  | 0.86 a | 0.83 ab  |                                             |
| 300                    | 3.67 a | 3.67 a | 195.3 a | 142.4 ab | 37.95 a | 24.27 a | 165.03 ab | 175.9 a  | 0.87 a | 0.85 ab  |                                             |
| 375                    | 4.00 a | 3.67 a | 192.3 a | 142.5 a  | 37.31 a | 24.29 a | 167.83 a | 177.0 a  | 0.87 a | 0.85 a  |                                             |

Significance of factors

| Year                  | 6.594 * | 360.157 ** | 402.395 ** | 9.863 ** | 5.821 * |
|-----------------------|---------|------------|------------|----------|---------|
| Cultivar (C)          | ns      | 34.289 **  | 78.879 **  | 78.563 ** | 6.179 * |
| K2O rate (K)          | 2.637 * | 19.075 **  | 29.349 **  | 27.047 ** | 10.001 **|
| Y × C                 | ns      | ns         | ns         | ns       | ns      |
| Y × K                 | ns      | 10.401 **  | ns         | ns       | ns      |
| C × K                 | ns      | ns         | ns         | ns       | ns      |
| Y × C × K             | 2.665 * | ns         | ns         | ns       | ns      |

* Values followed by different letters within a column are significantly different at p < 0.05. * and ** are significant at p < 0.05 and p < 0.01, respectively. ns means no significance.
2.5. Changes of Sucrose and Starch Content in SPSR

Sucrose content in SPSR first declined and then increased with DAT for the three K$_2$O rates (Figure 3a), consistent with a previous study [26]. Sucrose content in SPSR decreased with an increasing K$_2$O rate and the difference of the sucrose content among the three K$_2$O rates increased with DAT. In addition, the decrease range (Δ%) of sucrose in SPSR for Ningzhishu 1 (9–15%) was remarkably higher than that of Xushu 32 (17–34%).

![Figure 3](image_url)

**Figure 3.** Changes of carbohydrate contents in SPSR under different K$_2$O rates in 2017 and 2018. (a) Sucrose content; (b) amylose content; (c) amylopectin content; (d) starch accumulation. * is significant among the three K$_2$O rates at p < 0.05.

The contents of amylose and amylopectin in SPSR first increased and then declined or increased further with DAT for the three K$_2$O rates (Figure 3b,c). K application reduced amylose and amylopectin content in SPSR. Compared to no-K treatment, K application decreased the average amylose and amylopectin content across DAT by 9–13% and 6–11% for Xushu 32, respectively, and by 10–23% and 13–19% for Ningzhishu 1, respectively.

The trend of total starch accumulation with DAT was similar to that of storage root biomass (Figures 3d and 2b). Compared to the no-K treatment, K application enhanced starch accumulation across DAT by 11–14% and 11–21% for Xushu 32 and Ningzhishu 1, respectively.
2.6. Enzyme Activities Involved in the Sucrose–Starch Metabolism in SPSR

The activities of AI, AKI and SuSy in SPSR remained increasing with DAT for the three K₂O rates (Figure 4a–c). As the K₂O rates increased, AI and AKI activities were significantly reduced, while SuSy and SPS activities were significantly enhanced (Figure 4).

![Figure 4. Effect of the K₂O rates on sucrose-metabolizing enzyme activities in SPSR. (a) AI activity; (b) AKI activity; (c) SuSy activity; (d) SPS activity. * is significant among the three K₂O rates at p < 0.05.](image)

The pattern of AGPase, SSS and SBE were similar, increasing from 30 DAT, reaching their peak values at 55–90 DAT in 2017 and 90–116 DAT in 2018 and then declining quickly at 120 DAT (Figure 5a–c). Compared to the no-K treatment, K application enhanced the activities of AGPase, SSS and SBE significantly by 19–110%, 13–144% and 9–26%, respectively.

Furthermore, except for SuSy, AGPase and SSS in SPSR for Xushu 32, the peak values of AI and AKI in SPSR for both cultivars were negatively correlated with leaf K content (p < 0.05, Figure 6a,b), whereas peak values of SuSy, SPS, AGPase, SSS and SBE were significantly positively correlated with leaf K content (Figure 6c,d and Figure 7a–f).
Figure 5. Effect of K$_2$O rates on starch-metabolizing enzyme activities in SPSR. (a) AGPase activity; (b) SSS activity; (c) SBE activity. * is significant among the three K$_2$O rates at $p < 0.05$.

Figure 6. Relationships between the peak values of sucrose-metabolizing enzyme activities in SPSR and leaf K content in 2017 and 2018. (a) acid invertase (AI) activity; (b) alkaline invertase (AKI) activity; (c) SuSy activity; (d) SPS activity. The solid and dotted lines represent Xushu 32 and Ningzishu 1, respectively. * and ** are significant among the three K$_2$O rates at $p < 0.05$ and $p < 0.01$, respectively.
Figure 7. Relationships between the peak values of starch-metabolizing enzyme activities in SPSR and leaf K content in 2017 and 2018. (a) AGPase activity in 2017; (b) AGPase activity in 2018; (c) SSS in 2017; (d) SSS in 2018; (e) SBE in 2017; (f) SBE in 2018. The solid and dotted lines represent Xushu 32 and Ningzishu 1, respectively. * and ** are significant among the three K2O rates at \( p < 0.05 \) and \( p < 0.01 \), respectively.

2.7. Genes Transcription Involved in the Sucrose–Starch Metabolism in SPSR

Significant differences were observed in the transcription of \( Sps, Susy, AGP, SSS \) and \( SBE \) in SPSR among three K\(_2\)O rates (Figure 8). The transcriptional levels of those genes at 90 DAT were ranked as 300 > 150 > 0 kg K\(_2\)O ha\(^{-1}\) among the three K\(_2\)O rates. Moreover, the transcriptional level showed a descending order of \( AGP > SSS67 > SSS34 > Susy > SBEI-1a > SBEII > Sps \).

Figure 8. Expression of carbohydrate-metabolizing genes in SPSR under different K\(_2\)O rates in 2018. Blue and red represent decreased and increased expression levels, respectively. Values followed by different letters among the three K\(_2\)O rates are significantly different at \( p < 0.05 \).

2.8. Relationship Between Enzyme Activities Related to Sucrose and Starch with Starch Accumulation and Storage Root Yield

A correlation analysis of key enzyme activities, involved in sucrose-to-starch conversion, with storage root yield and total starch accumulation showed that SPS, \( SuSy \), AGPase, SSS and SBE in SPSR was significantly positively correlated with the total starch
accumulation; AGPase and SSS in SPSR was significantly positively correlated with the yield of storage root (Table 5).

Table 5. Correlation coefficients among sweet potato yield, starch accumulation and sucrose–starch metabolizing enzymes for three different K$_2$O rates. $n = 12$, $R_{0.05} = 0.5760$, $R_{0.01} = 0.7079$.

| Correlation with          | SPS | SuSy | AGPase | SSS | SBE |
|--------------------------|-----|------|--------|-----|-----|
| Starch accumulation      | 0.5833 * | 0.7208 ** | 0.5881 * | 0.5779 * | 0.7665 ** |
| Storage root yield       | ns  | ns   | 0.7992 ** | 0.7780 ** | ns  |

* and ** are significant at $p < 0.05$ and $p < 0.01$, respectively. ns means no significance.

3. Discussion

Previous studies have shown that K can improve crop stress resistance by optimizing gas exchange, stomatal conductance, protein synthesis, enzyme activation and photosynthetic transport [27,28]. Statistically, at present, about 60% of the total cultivated land area is short of K in China, among which land with a serious shortage of K (available K < 70 mg kg$^{-1}$) occupies 22.6% of the total cultivated land area [29]. The yield and quality decline of SPSR, caused by soil K deficiency, will be increasingly aggravated, which has become one of the important reasons to restrict the production in SPSR. It has been reported that K application of 240–300 kg K$_2$O ha$^{-1}$ at basal was given high storage root yields [19], in accordance with the yield of SPSR observed in this study. It was found that the K application of 225–300 kg K$_2$O ha$^{-1}$ could enhance the storage root yield by 28–30% and 46–48% for Xushu 32 and Ningzishu 1, respectively, compared to the no-K treatment (Table 5).

Therefore, this study further analyzed the physiological mechanisms of K application to improve sweet potato yield. A growth analysis showed that K application significantly affects sweet potato growth, source–sink relation and yield formation.

3.1. Plant Growth, Biomass Accumulation and Distribution in SPSR was Improved by Appropriate K Application

As is known, K application could be favorable to plant growth and yield formation, especially in terms of nutrients [21,30]. K application improved sweet potato plant agronomic traits overall [31], showing a longer fast accumulation phase duration ($T$) of plant and SPSR biomass accumulation with a higher growing speed ($V_{T}$, Tables 2 and 3) for both cultivars. Similar results have been observed in cotton (Gossypium hirsutum L.) [32]. In terms of biomass accumulation, higher biomass accumulation in SPSR were observed in the K-supply treatments for Xushu 32 and Ningzishu 1 (Table 4), with more than 75% of the biomass accumulation observed in SPSR. In this study, the effect of K application on the yield of SPSR were mainly attributed of the impact on biomass production.

The production of biomass depends on leaf photosynthetic characteristics and sucrose-to-starch conversion in SPSR [26,30,33]. In this study, K application enhanced sweet potato photosynthetic capacity at 35 DAT and 55 DAT, accompanied with an increased storage root weight (Table 4). With the increase of the K$_2$O rate, leaf $Pn$, $gs$ and $Ci$ all increased first and then decreased, and the peak value was between 150–225 kg K$_2$O ha$^{-1}$. Previous studies showed that with a treatment of mild K deficiency, the decrease of $Pn$ was mainly due to low $gs$ [34]. In this study, low $gs$ and $Pn$ with regards to decreased $Ci$ were found in the no-K treatment for both cultivars at 35 DAT, suggesting that stomatal limitation was the dominant factor leading to the decrease of $Pn$. Subsequently, because of the lower $gs$ and $Pn$ accompanied with increased $Ci$, it seemed that non-stomatal factors played more critical roles in reducing $Pn$ in the no-K treatment. These results was consistent with reports on cotton [32] and hickory (Carya cathayensis Sarg.) [35]. If the reduction of $Pn$ was mainly caused by an increase of $Ls$, then $Ci$ must decrease; if the reduction of $Pn$ was dominated by an increase in non-stomatal limitations, $Ci$ would be increased. In this study, the reduced $Pn$ was apparent along with an increase in $Ls$ in the no-K treatment (Table 1), but $Ci$ increased at 55 DAT, indicating that the reduction of $Pn$ was largely due to non-stomatal factors at
55 DAT for Xushu 32 and Ningzishu 1. Therefore, the conclusion obtained from the $L_s$ value was in accordance with the above interpretation and analysis in Table 1.

### 3.2. Appropriate K Application Is Beneficial to Sucrose-to-Starch Conversion in SPSR

Starch is the major form of fixed carbon in SPSR, about 50–80% of SPSR’s dry biomass [4,5]. Rational fertilization is of great importance to obtain a high starch yield. In this study, K application enhanced starch accumulation in SPSR compared to the no-K treatment (Figure 3d). However, the content of starch in SPSR, as the sum of amylose and amylopectin (Figure 3b,c), was decreased by K application, probably because of the dilution effect of protein [36]. Starch synthesis in plastids is closely related to the hydrolysis of sucrose in cytoplasm [17], which involves many enzymes and their coding genes that convert sucrose to starch during SPSR development.

The local content of sucrose in SPSR varies with the enzymes involved in the synthesis and breakdown of sucrose, which further determines the sucrose unloading from phloem [37]. Additionally, the decomposition products of sucrose, glucose and fructose could provide essential substrates to biosynthesis starch [38]. The main enzymes are SPS for sucrose synthesis, and AI, AKI and SuSy (cleavage) for sucrose decomposition [39]. A reduced sucrose content in SPSR was found with the increase of K$_2$O rates (Figure 3a), possibly causing more sucrose to break down to produce more substrates to synthesize starch. It was reported that in tuber crops, the main component of sucrose decomposition are invertase in the early stage of root formation and SuSy (cleavage) in the late stage [15]. Compared to the SPSR of the no-K treatment, the K-treated SPSR had higher SuSy (cleavage) and lower AI; moreover, AKI decreased, indicating that K application increased sucrose decomposition by increasing the transcriptional level and enzymatic activity of SuSy. Surprisingly, K application not only brought in higher SuSy activity for cleavage but also higher SPS activity in SPSR (Figure 4c,d). In comparison, K application also improved the transcriptional level of $Susy$ relative to that of $Sps$ in the K-treated SPSR (Figure 8), and the SuSy activity was much higher than the SPS activity. Moreover, SuSy (cleavage) activity was significantly positively correlated with starch accumulation and storage root yield (Table 5). The results were confirmed by the higher ABA in SPSR at the stage of storage root expansion [40], which could inhibit SuSy and enhance the synthesis of starch [41]. Therefore, SuSy activity for cleaving in the K-treated SPSR considerably exceeded the SPS activity for synthesis, thus promoting the decomposition of sucrose and providing sufficient substrates for the synthesis of starch.

AGPase, SSS and SBE play critical roles in starch synthesis [17]. AGPase, a speed-limiting enzyme in starch synthesis, catalyzes ADP-glucose synthesis, used for the synthesis of starch polymers [16]. In tuber root, higher sucrose from phloem unloading could up-regulate the transcriptional level of AGP [42]. K application could produce more carbon from leaf to SPSR during SPSR development (Figure 2), thus up-regulating AGP (Figure 8) and improving the AGPase activity in the K$_2$O-treated SPSR (Figure 7a). SSS and SBE play critical roles in the synthesis of amylose and amylopectin [16,17]. Appropriately increasing the fertilizer to soil could improve the enzyme activities and transcriptional levels of SSS and SBE, thus leading to a high starch yield in tuber root and grain [43,44], consistent with our study, showing that the activities of SSS and SBE and transcriptional levels of $SSS34$, $SSS67$, $SBEI$-1a and $SBEII$ in SPSR were increased by K application. In this study, starch accumulation, as well as the yield in SPSR, was significantly positively correlated with AGPase and SSS (Table 5), in agreement with previous studies [19,26]. In this study, the most noteworthy is that the ↑△AGPase and ↑△SSS were more than ↑△SBE. Additionally, the transcriptional level of AGP was higher than those of $Susy$, $SSS34$ and $SSS67$ in SPSR (Figure 8). The results indicated that K application promoted the synthesis of starch and storage root yield through improving the activities and transcriptional levels of AGPase and SSS, and AGPase is the speed-limiting enzyme in SPSR for the synthesis of starch in the K-treated SPSR. Based on the results and discussion above, we propose a schematic
overview of K application on sweet potato growth and the conversion of sucrose to starch in SPSR (Figure 9).

Figure 9. Increases in metabolite concentrations and enzyme activities in the K-treated sweet potato are shown in red, and decreases are shown in blue. \( T \) is the period of fast biomass accumulation phase; \( V_T \) is the average biomass accumulation rate during the fast biomass accumulation duration.

The two cultivars, Xushu 32 and Ningzishu 1, exhibited similar patterns in carbon assimilation following K application. However, Ningzishu 1 showed a larger change for sucrose–starch metabolism in the K-treated sweet potato compared to Xushu 32. Based on the analysis of this study, in the process of sucrose–starch metabolism, SuSy plays a critical role in sucrose decomposition, and AGPase and SSS are the important roles in starch synthesis. In this study, for the two cultivars, there were different sensitivities to K in SuSy, AGPase and SSS activities in SPSR. The average SuSy increased by 6–12% in the K-treated SPSR (150 and/or 300 kg K\(_2\)O ha\(^{-1}\)) compared to the no-K treated SPSR for Xushu 32, and by 7–18% for Ningzishu 1. The average AGPase and SSS increased by 19–46% and 13–38% for Xushu 32, respectively, and by 46–110% and 52–144% for Ningzishu 1, respectively. In addition, for Ningzishu 1, there were linear relationships between these peak values of SuSy, AGPase and SSS and leaf K content, whereas no correlation was found between these peak values for Xushu 32 and leaf K content (Figure 7), suggesting that Ningzishu 1 was much more influenced by K than Xushu 32. Therefore, K application enhanced the conversion of sucrose to starch more in Ningzishu 1 compared with Xushu 32.

4. Materials and Methods

4.1. Experimental Design

In the field experiments, two sweet potato cultivars, Ningzishu 1 (low-K sensitive) and Xushu 32 (low-K tolerant) [24,25], were grown in 2017 and 2018 at the Xuzhou Academy of Agricultural Sciences, Xuzhou, Jiangsu, China (117°29′ E, 34°29′ N). The soil at the experimental site was loamy and yellow fluve-aquic (pH 7.8). In 20-cm deep soil, in 2017, the content of organic matter was 16.8 g kg\(^{-1}\), the content of hydrolysable N was 91 mg kg\(^{-1}\) and the content of available K and P were 71 and 23 mg kg\(^{-1}\), respectively; in 2018, the content of organic matter was 16.2 g kg\(^{-1}\), the content of hydrolysable N was 94 mg kg\(^{-1}\) and the content of available K and P were 85 and 20 mg kg\(^{-1}\), respectively.

The experiment was designed with three completely random replications. A total of 150 kg ha\(^{-1}\) N (urea) and 75 kg ha\(^{-1}\) P\(_2\)O\(_5\) (calcium superphosphate) was applied as a uniform fertilizer application. The treatments consisted of six K\(_2\)O rates: 0, 75, 150, 225, 300 and 375 kg K\(_2\)O ha\(^{-1}\) using potassium sulphate. The control was treatment of 0 kg K\(_2\)O ha\(^{-1}\). All these fertilizer applications were applied as base fertilizer in each treatment.
The row spacing × plant spacing was 0.85 m × 0.25 m, and the final planting density was 46,500 plants ha⁻¹.

4.2. Sampling and Processing

Three continuous sweet potato plants were collected from the central row of each treatment. Sweet potato biomass was determined at different growth stages from 35 days after transplanting (DAT) to 120 DAT in 2017 and from 30 to 120 DAT in 2018. The collected plants were divided into stem + vine, leaf, root and storage root for each treatment. Subsequently, the samples were first oven-dried at 105 °C for half an hour and then at 65 °C until the weight was constant.

Simultaneously, the fresh storage roots (diameters > 0.5 cm) were collected and washed with dd H₂O. Each fresh SPSR was divided into two parts from the middle of SPSR, and cut into slices. One part was immediately frozen in liquid N₂ and saved in a refrigerator (−80 °C) to measure the sucrose–starch metabolizing enzyme activities and their gene transcriptions. The other was oven-dried for carbohydrate analysis.

At the mature stage, the SPSR per plant was collected by hand for each treatment. The number of storage root per plant, fresh weight per storage root and storage root yield, as yield components, were counted and recorded.

4.3. Leaf Photosynthesis Parameters

At 35 DAT and 55 DAT, Pn, stomatal conductance (gs) and intercellular CO₂ concentration (Ci) in functional leaves (the third leaf from the top) were measured by a Li-6400 (Li-COR Inc., NE, USA) on a sunny day at 9:00–11:00 am. Measurements were made using under 1000 µmol m⁻² s⁻¹ light intensity with 400 µmol mol⁻¹ CO₂ and (65 ± 5)% relative humidity.

4.4. Sucrose and Starch Analysis

Dried storage root tissues were ground by a ball mill instrument. A total of 50 mg of the ground sample was put into a tube of 2 mL and extracted twice using 80% ethanol (500 µL) at 80 °C. Thereafter, the extracting solution was diluted into 1.8 mL and mixed for the determination of sucrose according to previously described protocols [14].

The content of amylose and amylopectin were determined following previous methods with some modifications [16]. Briefly, a 50-mg storage root tissue was weighed into a 15 mL centrifuge tube, followed by adding 250 µL of 100% ethanol and 1.25 mL NaOH (1 M), and the centrifuge tube was cooled after boiling for 15 min, after which 11 mL dd H₂O was added and the sample was vigorously mixed for subsequent measurements. Then, 0.3 mL of extract was added into another centrifuge tube containing 50 µL acetic acid (1 M), 50 µL iodine solution (0.2 g I₂ and 2.0 g KI in 100 mL dd H₂O) and 2.1 mL of dd H₂O, mixed vigorously and let stand for 20 min. The OD values were recorded at 630 nm and 460 nm for amylose, and at 550 nm and 740 nm for amylopectin using a microplate reader.

4.5. Sucrose–Starch Metabolizing Enzymatic Extraction and Analysis

A total of 0.1 g of the frozen storage root sample was homogenized with 1.8 mL cool extraction buffer. Tris-HCl (pH 7.5, 100 mM), MgCl₂ (8 mM), EDTA (2 mM), glycerin (12.5%, v/v) and β-mercaptoethanol (50 mM) were contained in the extraction buffer. The supernatant after centrifugation at 10,000 × g at 4 °C was collected to analyze the activities of enzymes (SPS, SuSy, AI, AKI, AGPase, SSS, and SBE) in sucrose–starch metabolism.

SPS activity was measured using the UDP-Glucose method and SuSy activity in the direction of sucrose decomposition was determined using the UDP method, as previous studies described [45,46]. The activities of AI and AKI were analyzed using the 3,5-dinitro salicylic acid (DNS) method, by sucrose (1 M) in acetic acid–NaOH (200 mM, pH 5.0) for AI or sodium acetate–acetic acid (100 mM, pH 7.5) for AKI.

AGPase and SSS were assayed by measurement of the formation of NADPH using a microplate reader according to a previous study with some modifications [47,48]. The
reaction mixture I for AGPase contained Hepes-NaOH (100 mM, pH 7.4), ADPG (1.2 mM), sodium pyrophosphate (3 mM), MgCl$_2$ (5 mM) and DTT (4 mM). The reaction mixture II for AGPase contained NADP (10 mM), P-glucosamutase (0.4 U) and glucose 6-phosphate dehydrogenase (0.4 U). The reaction mixture I for SSS contained Hepes-NaOH (50 mM, pH 7.4), ADPG (1.6 mM), amylopectin (0.1 mg) and DTT (15 mM). The reaction mixture II for SSS contained Hepes-NaOH (50 mM, pH 7.4), phosphoenolpyruvic acid (4 mM), KCl (200 mM), MgCl$_2$ (10 mM) and pyruvate kinase (1.2 U). The reaction mixture III for SSS contained Hepes-NaOH (50 mM, pH 7.4), glucose (10 mM), MgCl$_2$ (20 mM), NADP (2 mM), hexokinase (1.4 U) and glucose 6-phosphate dehydrogenase (0.35 U). SBE (EC 2.4.1.18) was determined as described previously [47,48].

The units of enzyme activity were recorded as U min$^{-1}$ g$^{-1}$ FW for SBE, µmol min$^{-1}$ g$^{-1}$ FW for AGPase and SSS and mg g$^{-1}$ FW for others.

4.6. RNA Extraction and Gene Transcriptional Analysis in SPSR

Total RNA from SPSR were extracted according to the manufacturer’s instructions of RNA-prep pure plant kit (DP441-50T, Tiangen Biotech, Beijing, China). Two µg of Total RNA was reverse transcribed using a Prime Script RT reagent cDNA kit (TaKara, Dalian, China). The primers of related genes are shown in Supplementary Table S1. GAPDH was used as a reference gene, and the relative transcriptional levels of Sps, Susy, AGP, SSS34, SSS67, SBEI-1a and SBEII were calculated using the 2$^{-\Delta\Delta CT}$ method [49,50].

4.7. Weather Data and Statistical Analysis

Weather data in Xuzhou were obtained from the National Meteorological Information Center. The mean daily temperature and total sunshine hours during the sweet potato growth stage (from June to October) was lower in 2017 than in 2018 (Table 6). However, the total precipitation in August was higher in 2018 than in 2017.

Table 6. Meteorological data during the sweet potato growing season in 2017 and 2018.

| Meteorological Data | Year | June | July | August | September | October | Total |
|---------------------|------|------|------|--------|-----------|---------|-------|
| Mean daily temperature (°C) | 2017 | 25.8 | 29.9 | 27.7 | 23.1 | 15.4 | 24.4 |
| Total sunshine hours (h) | 2017 | 265.2 | 241.2 | 210.3 | 191.8 | 157.8 | 1066.3 |
| Total precipitation (mm) | 2017 | 92.1 | 279.9 | 105.1 | 225.3 | 212.2 | 238.0 | 1200.7 |
| | 2018 | 282.8 | 242.4 | 225.3 | 212.2 | 238.0 | 1200.7 |
| | 2018 | 92.1 | 279.9 | 105.1 | 210.3 | 238.0 | 1200.7 |
| | 2018 | 48.7 | 248.5 | 362.3 | 62.6 | 0.2 | 722.2 |

Origin and Microsoft Excel 2019 were used for data analysis. SPSS was used for the analysis of variance through using the difference between the average values identified by the Tukey test (HSD, $p < 0.05$) and for the regression analysis. The stomatal limitation value ($Ls = 1 - Ci/Ca$ (Ca is the external CO$_2$ concentration) is used to represent the stomatal limit value. The limitation of non-stomatal factors (Non-$Ls$) is usually expressed as a $Ci/gs$ ratio.

Crop biomass dynamic accumulation is extensively described by the following logistic model:

$$y = \frac{y_{max}}{1 + ae^{-b \times DAT}}$$  \hspace{1cm} (1)

where $y$ (g per plant) is sweet potato biomass, $DAT$ (d) is days after transplanting, $y_{max}$ (g) is the maximum biomass and $a$ and $b$ are parameters.

From Equation (1):

$$DAT_0 = \frac{a}{b}, \hspace{0.5cm} DAT_1 = -\frac{1}{b} ln \frac{2 + \sqrt{3}}{a}, \hspace{0.5cm} DAT_2 = -\frac{1}{b} ln \frac{2 - \sqrt{3}}{a}, \hspace{0.5cm} DAT_m = \frac{lna}{b}$$  \hspace{1cm} (2)
The accumulation of sweet potato biomass was at the maximum rate when $\text{DAT} = \text{DAT}_0$:

$$V_{max} = \frac{b \times y_{max}}{4}, \quad V_T = \frac{y_2 - y_1}{\text{DAT}_2 - \text{DAT}_1}$$

(3)

where $\text{DAT}_1$ and $\text{DAT}_2$ are the DAT when biomass rapid increase started and when the rapid increase terminated, respectively, $\text{DAT}_m$ is the DAT when the rate of biomass accumulation reached maximum, $T (d) = \text{DAT}_2 - \text{DAT}_1$ is the rapid biomass increase period, $V_{max}$ is the maximal rate of biomass accumulation and $V_T$ is the average rate of biomass accumulation.

5. Conclusions

1) Compared to the no-K treatment, sweet potato yield was remarkably improved by K application, although treatments at high levels of K, i.e., 375 kg K$_2$O ha$^{-1}$, significantly decreased storage root yield. K application enhanced biomass accumulation of plant and storage root yield by promoting $V_T$ and $T$.
2) K application enhanced the photosynthetic capacity of sweet potato at 35 DAT and 55 DAT, accompanied with an increased storage root weight. The reduction of $Pn$ induced by K deficiency was mainly caused by stomatal limitation at 35 DAT and by non-stomatal factors at 55 DAT.
3) K application promoted the starch accumulation mainly by improving the activities of SuSy, AGPase and SSS, and these genes’ transcriptions, and AGPase is the speed-limiting enzyme for the synthesis of starch in SPSR of the K-treated treatments.
4) Future research is required to determine the correlation of K$^+$ transport activity in root and K tolerance in sweet potato, in order to breed low-K-tolerant sweet potato cultivars in the future.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ijms22094826/s1. Table S1. Specific primers for gene amplification.

Author Contributions: Conceptualization, J.L., Z.T., and Z.L.; performed experiments, Y.G., Z.T., and H.X.; formal analysis, Y.G., H.X., M.S., and M.L.; writing—original draft preparation, J.L., and Y.G.; writing—review and editing, J.L. and Z.L.; supervision, S.P. and Z.L.; funding acquisition, Z.L., J.L., and Z.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the China Agriculture Research System (CARS-10-B3), the Natural Science Foundation of the Jiangsu Higher Education Institutions of China (19KJB210012), the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), the National Key Research and Development Project (2018YFD1000704, 2018YFD1000700), and the Postgraduate Research & Practice Innovation Program of Jiangsu Province (KYCX20_2317).

Data Availability Statement: The data used to support the findings of this study are included in the article.

Acknowledgments: Ningzishu 1 seedlings were obtained from the Institute of Food Crops/Provincial Key Laboratory of Agrobiology, Jiangsu Academy of Agricultural Sciences. The authors would like to thank Yizhi Xie for all of his assistance with the sweet potato seedlings.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References
1. Liu, Q. Improvement for agronomically important traits by gene engineering in sweetpotato. Breed. Sci. 2017, 67, 15–26. [CrossRef]
2. Villordon, A.Q.; Clark, C.A. Variation in virus symptom development and root architecture attributes at the onset of storage root initiation in ‘Beauregard’ sweetpotato plants grown with or without nitrogen. PLoS ONE 2014, 9, e107384.
3. Firon, N.; LaBonte, D.; Villordon, A.; McGregor, C.; Kfir, Y.; Pressman, E. Botany and physiology: Storage root formation and development. In The Sweetpotato; Loebenstein, G., Thottappilly, G., Eds.; Springer: Dordrecht, The Netherlands, 2009; pp. 13–26.
32. Hu, W.; Jiang, N.; Yang, J.; Meng, Y.; Wang, Y.; Chen, B.; Zhao, W.; Oosterhuis, D.M.; Zhou, Z. Potassium (K) supply affects K accumulation and photosynthetic physiology in two cotton (Gossypium hirsutum L.) cultivars with different K sensitivities. Field Crop. Res. 2016, 196, 51–63. [CrossRef]

33. Du, X.; Kong, L.; Xi, S.; Zhang, X. Split application improving sweetpotato yield by enhancing photosynthetic and sink capacity under reduced nitrogen condition. Field Crop. Res. 2019, 238, 56–63.

34. Bednarz, C.W.; Oosterhuis, D.M.; Evans, R.D.J.E.; Botany, E. Leaf photosynthesis and carbon isotope discrimination of cotton in response to potassium deficiency. Environ. Exp. Bot. 1998, 39, 131–139. [CrossRef]

35. Jin, S.H.; Huang, J.Q.; Li, X.Q.; Zheng, B.S.; Wu, J.S.; Wang, Z.J.; Liu, G.H.; Chen, M. Effects of potassium supply on limitations of photosynthesis by mesophyll diffusion conductance in Carya cathayensis. Tree Physiol. 2011, 31, 1142–1151. [CrossRef] [PubMed]

36. Hou, M.; Zhang, Y.; Liu, Y.; Wang, X.; Tang, W.; Yan, H.; Ma, D.; Li, Q. Effects of applying potassium at different stages on yield and quality of edible sweetpotato. Acta Agric. Boreali Sin. 2014, 29, 368–372. (In Chinese)

37. Yang, J.; Zhang, J.; Wang, Z.; Liu, K.; Wang, P. Post-anthesis development of inferior and superior spikelets in rice in relation to abscisic acid and ethylene. J. Exp. Bot. 2006, 57, 149–160. [CrossRef] [PubMed]

38. Mukerjea, R.; Robyt, J.F. Starch biosynthesis: Sucrose as a substrate for the synthesis of a highly branched component found in 12 varieties of starches. Carbohydr. Res. 2003, 338, 1811–1822. [CrossRef]

39. Bednarz, C.W.; Oosterhuis, D.M.; Evans, R.D.J.E.; Botany, E. Leaf photosynthesis and carbon isotope discrimination of cotton in response to potassium deficiency. Environ. Exp. Bot. 1998, 39, 131–139. [CrossRef]

40. Shi, C.; Wang, Z.; Zhao, B.; Guo, F.; Yu, S. Effect of potassium nutrition on some physiological characteristics and yield formation of sweet potato. Plant Nutri. Fert. Sci. 2002, 8, 81–85. (In Chinese)

41. Kermode, A.R.; Oishi, M.Y.; Bewley, J.D. Regulatory roles for desiccation and abscisic acid in seed development: A comparison of the evidence from whole seeds and isolated embryos. In Seed Moisture; Stanwood, P.C., McDonald, M.B., Eds.; Crop Science Society of America, Inc.: Madison, WI, USA, 1989; pp. 23–50.

42. Tiessen, A.; Hendriks, J.H.M.; Stitt, M.; Branscheid, A.; Gibon, Y.; Farré, E.M.; Geigenberger, P. Starch synthesis in potato tubers is regulated by post-translational redox modification of ADP-glucose pyrophosphorylase: A novel regulatory mechanism linking starch synthesis to the sucrose supply. Plant Cell 2002, 14, 2191–2213. [CrossRef]

43. Du, X.D.; Zhao, H.W.; Wang, J.G.; Liu, H.L.; Yang, L.; Xu, J.; Song, J.T. Changes in starch accumulation and activity of enzymes associated with starch synthesis under different nitrogen applications in japonica rice in cold region. Acta Agron. Sin. 2012, 38, 159–167. (In Chinese) [CrossRef]

44. Wang, J.M. Differential Activities and Transcriptional Levels of Starch Synthesis Related Enzymes Regulated by Potassium in High Starch Potatoes. Master’s Thesis, Northeast Agricultural University, Haerbin, China, 2016.

45. Shu, H.M.; Zhou, Z.G.; Xu, N.Y.; Wang, Y.H.; Zheng, M. Sucrose metabolism in cotton (Gossypium hirsutum L.) fibre under low temperature during fibre development. Eur. J. Agron. 2009, 31, 61–68. [CrossRef]

46. Liu, J.; Peng, J.; Xia, H.; Li, P.; Li, Z.; Sun, M.; Zheng, C.; Dong, H. High soil available phosphorus favors carbon metabolism in cotton leaves in pot trials. J. Plant Growth Regul. 2012, 31, 51–63. [CrossRef]

47. Nakamura, Y.; Umemoto, T.; Ogata, N.; Kuboki, Y.; Yano, M.; Sasaki, T. Starch debranching enzyme (R-enzyme or pullulanase) and quality of edible sweetpotato. Acta Agric. Boreali Sin. 2014, 29, 368–372. (In Chinese)

48. Liu, J.; Peng, J.; Xia, H.; Li, P.; Li, Z.; Sun, M.; Zheng, C.; Dong, H. High soil available phosphorus favors carbon metabolism in cotton leaves in pot trials. J. Plant Growth Regul. 2012, 31, 51–63. [CrossRef]

49. Park, S.C.; Kim, Y.H.; Ji, C.Y.; Park, S.; Jeong, J.C.; Lee, H.S.; Kwak, S.S. Stable internal reference genes for the normalization of Real-time PCR in different sweetpotato cultivars subjected to abiotic stress conditions. PLoS ONE 2012, 7, e51502. [CrossRef] [PubMed]

50. Xia, H.; Xu, T.; Zhang, J.; Shen, K.; Li, Z.; Liu, J. Drought-induced responses of nitrogen metabolism in Ipomoea batatas. Plants 2020, 9, 1341. [CrossRef]