Effects of single-node cutting method on the propagation and storage root growth of sweetpotato seedlings

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Abstract: The efficient propagation of virus-free sweetpotato seedlings is a critical challenge for maintaining stable production. This study evaluated two propagation methods—single-node cutting (SNC) and tuberous root propagation (TRP)—in two cultivars (Beniharuka and Himeayaka). Compared to TRP, SNC significantly improved seedling propagation efficiency, producing over 12 times more transplants in 70 days. SNC seedlings also showed enhanced photosynthetic performance before transplanting. After transplanting to the field, SNC seedlings achieved significantly higher storage root yield (30%-50% increase) without compromising root quality, including starch and sugar content. These findings demonstrate that the SNC method is a highly efficient and practical approach for sweetpotato seedling production. The adoption of this method could contribute significantly to improving the sustainability and productivity of sweetpotato cultivation globally.

Keywords: sweetpotato, single-node cutting method, tuberous root propagation method, photosynthesis, transplant quality, yield and quality of storage roots

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1 Introduction

Sweetpotato (*Ipomoea batatas* (L.) Lam.) is a Convolvulaceae herbaceous perennial vine with long, tapered tubers and heart-shaped leaves. Sweetpotato is a popular tuber crop high in protein, carbohydrates, minerals (calcium, iron, and potassium), carotenoids, dietary fiber, and vitamins[1]. Sweetpotato can be used as a staple food, tuber vegetable, recreational food, and livestock feed, considered to be the most important cash crop[2]. In the year 2021, there were 7.41 million hm^2 of sweetpotato planted globally, contributing to a total production of 89 million t[3]. Africa and Asia are the major sweetpotato producing areas, accounting for 95% of worldwide production. China has the largest area of sweetpotato production in the world [3].

Sweetpotato production is mostly based on seedling transplanting. Tuberous root propagation (TRP) is often used in the production of sweetpotato vines. The vine is divided into sections containing four to five leaves and measuring 15-20 cm in length. These sections are used as seedlings once the vine has reached a certain length. TRP method is primarily carried out in small arches or plastic greenhouses, where the production of seedlings is mostly limited by temperature and light. As a result, TRP methods are not always sufficient to produce large-scale, uniform, disease-free planting material year-round to meet the demand of farmers for consistent and stable production of storage roots[4]. Additionally, because sweetpotato cuttings have no roots, they quickly wither after being transplanted in the field and require a few weeks to re-root, which is not optimal for the seedlings' early growth[5]. More research is being done on creative methods for cultivating sweetpotato seedlings as soilless culture technology is used to produce seedlings. Studies have shown that stem-node cuttings can be used to produce sweetpotato seedlings. The original material for stem-node cuttings can be single or multiple nodes. Liu et al. found that sweetpotato cuttings could develop roots and axillary buds in a short period of time after dividing the stems into different stem nodes, including single-node, double-node, and three-node top bud sections, resulting in sweetpotato seedlings growing quickly and early[6]. Guo et al. found that double-node sweetpotato cuttings transplanted with roots were more resistant to adversity, grew faster, set tubers earlier, produced more tubers and had higher yields than those without roots[7]. Therefore, single-node cuttings (SNC) method can significantly improve the efficiency of seedling production.

With the development of lighting technology, plant factories with artificial lighting have the advantages of high production efficiency, fewer pests and diseases, easy to standardize, and other traditional production methods are difficult to compare. Under the environment of plant factory, a suitable light formula can be formulated according to the growth and development needs of seedlings, and the physiological and metabolic process of seedlings can be precisely regulated, so as to achieve the disease-free, standardized, and rapid production of seedlings. Research on sweetpotato plug seedlings and virus-free seedlings breeding technology utilizing plant factories with artificial lighting has been proved to be promising[5,8]. In recent years, with the development of hydroponic technology, a series of studies have been conducted on sweetpotato hydroponic seedlings. Mortley et al. and Hill et al. developed a hydroponic technique for sweetpotato production[9,10]. Using stem-node cuttings to produce sweetpotato seedlings in a hydroponic system may be an efficient production strategy.

Sweetpotato viral disease is a global disease that causes significant harm to sweetpotato productivity and quality in

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agricultural locations across the world[11]. In temperate zone production, the crop is generally affected by a complex of potyviruses and possibly other unknown viruses that may lead to yield reduction of up to 20% to 40%[12]. Pathogens collect in storage roots and plant vines and are passed on to the next generation through asexual reproduction, where they accumulate and cause a drop in yield over time, leading to the decline of cultivars[13]. There is currently no specialized pesticide for the efficient control of sweetpotato viral disease, and there are no cultivars with significant virus disease resistance[14]. Using virus-free seedlings is a more efficient way to manage the virus diseases[15]. The primary strategy for managing the sweetpotato virus disease now involves cutting off the top 0.2-0.4 mm of stems and growing virus-free seedlings from stem tips[16]. Most studies use stem nodes or leaves as initial production materials. The experiment showed that virus-free sweetpotato seedlings have improved photosynthetic rates, increased accumulation of photosynthetic products, better microscopic transport systems, vigorous nutritional growth in the early reproductive stage, healthy adaptation, and a quick recovery time after transplanting[17]. As a result, using virus-free sweetpotato seedlings as the base material for seedling production successfully reduces the probability of seedlings being contaminated with worms and viral infections, and the seedling quality is superior to that of traditional methods.

Photosynthesis, the main driver of plant growth and production in terrestrial ecosystems, is the source of energy needed to sustain growth[18]. Plants with high photosynthesis develop quickly and are resistant to adversity[19]. Healthy photosynthesis of seedlings promotes not only biomass increase but also quick recovery after transplantation[20]. As a result, the photosynthesis of sweetpotato seedlings before and after transplantation is a significant factor for determining the quality of seedlings. Bhagsari suggested that canopy photosynthesis evaluation of sweetpotato germplasm may be more relevant when the storage root sink is at an advanced stage of development[21]. Furthermore, the quantity and quality of storage roots harvested after transplanting must be evaluated to determine whether the propagation method can be used in actual production. Studies on sweetpotato seedling propagation methods have so far focused on seedling growth characteristics without transplanting seedlings in the field, with less attention paid to seedling adaptability after transplanting and tuber yield and quality. A good seedling culture method should not only be efficient, but the seedlings should grow well and be adaptable for transplanting. Therefore, photosynthetic indicators and yield of sweetpotato seedlings before and after transplanting can be important indicators for evaluating seedling quality.

This study investigated the viability of the single-node seedling (SNC) method using plant factories and hydroponics. As the original seedling material, virus-free sweetpotato seedlings were used. By analyzing photosynthetic characteristics of seedlings before and after transplanting, and investigating the yield and quality of harvested storage roots after transplanting, this study evaluated whether the method could be used for sweetpotato seedling production. This is intended to give technical assistance for the effective production of sweetpotato seedlings in an industrial setting. Therefore, this study aimed to compare the propagation efficiency and storage root performance between SNC and TRP methods, and to evaluate their potential for practical sweetpotato seedling production.

2 Materials and methods

2.1 Plant materials and environmental conditions

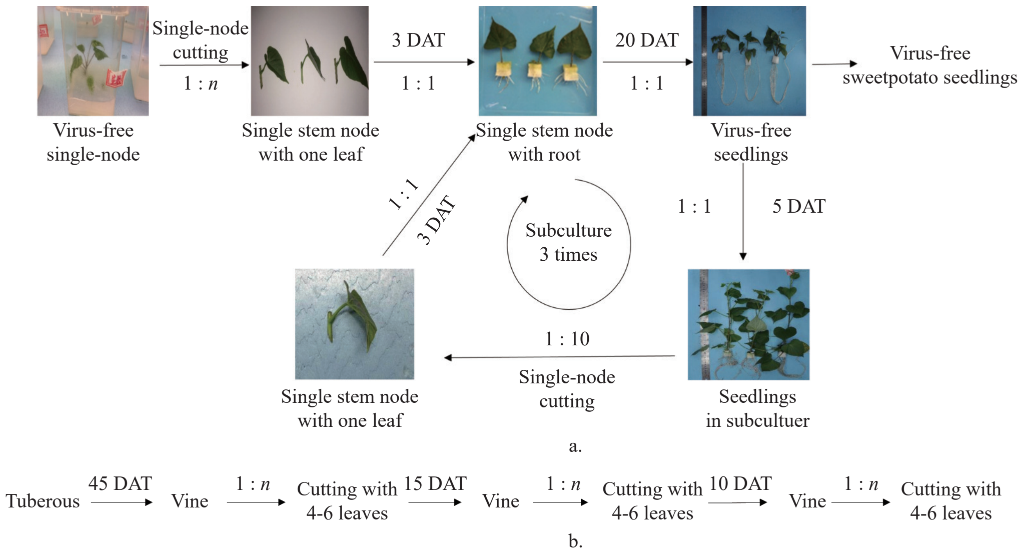
Healthy sweetpotato seedlings were selected and 0.2-0.4 cm stem tips were used as raw material for tissue culture to obtain virus-free seedlings. Single stem-node with one unfolded leaf and a length of about 1.0-2.0 cm below the leaf armpit were cut from virus-free seedlings and used as initial seedling material. Two cultivars, Beniharuka (J1) and Himeayaka (J2), were used for the experiment. The transplants propagation with one leaf node were conducted in an environmentally controlled plant factory with artificial lighting (China Agricultural University, Beijing). The environment in the growth chamber was controlled as follows: 12 h/12 h light/dark photoperiod with a photosynthetic photon flux density of 150 μmol/(m^2·s) (RBFr-LED, 300-800 nm, R:B=5.6 and R:FR=10.3, Beijing Lighting Valley Technology Co., Ltd., China). The air temperature in the light and dark periods was 24°C±1°C and 19°C±1°C, respectively. The relative humidity was 70%±10%. The CO_2 concentration was 800±50 μmol/mol in the light period and 400±50 μmol/mol in the darkness.

Seed tubers of the sweetpotato cultivars Beniharuka (J1) and Himeayaka (J2), weighing between 150 and 250 g, were selected for the experiment. The seed tubers were placed in a foamed plastic box (430 mm×260 mm×1700 mm). Cuttings of 15-20 cm length with four to six leaves were harvested from these sprouts. Each tuber was allowed to regenerate after cutting. After small amounts of a mixture of peat and perlite (volume ratio 4:1) were poured into the box, the seed tubers were placed on the substrates in the box, and the substrates were poured on again to fill the box. The tubers were incubated under natural sunlight in a greenhouse (China Agricultural University, Beijing). The greenhouse had the following environmental conditions: air temperature of around 10°C-25°C, relative humidity of 20%±50%, and CO_2 concentration of about 450-550 μmol/mol.

2.2 Experimental design

Fifteen single-node transplants with one leaf of two cultivars were cut as initial plantlets, then set into a sponge cube (23 mm×23 mm×23 mm) filled with deionized water to grow roots. After three days, these single-node cuttings rooted, which then were transplanted into a cultivation tray plug (1200 mm×900 mm×70 mm) in a growth chamber with artificial lights (Figure 1a). The initial culture area was controlled at 0.1 m^2 in which propagules were planted in the center (260 mm×420 mm). During the experiment, hydroponic cultivation system was used with Japanese horticultural experimental nutrient solution irrigation (pH: 6.0-6.5, EC: 2.2-2.5 mS/cm)[22]. Twenty-five days after transplanting (25 DAT), seven to 10 leaves of each propagule were grown, then using the same single-node cutting method with the first plantlets left one leaf and one node (Figure 1a). All these cutting propagules were planted to a larger culture area with 1 m^2 in the same growth chamber. After another 25 days (50 DAT), all propagules were cut into single leaf nodes. All the above single nodes were transplanted to a 10 m^2 tray in the same growth chamber. After another 20 days (70 DAT), the third single-node cuttings grew into transplants with four to six leaves suitable for sweetpotato production.

Forty-five days after transplanting, vine cuttings were propagated from seed tubers. Each cutting had four to six leaves at a depth of 15-25 cm (Figure 1b). Each seed tuber left a node for subsequent regeneration. After 15 days (60 DAT), the second cutting was harvested. Each cutting also had four to six leaves at a depth of 15-25 cm. After another 10 days (70 DAT), the third cutting was harvested.



Note: a and b represent two approaches: the Single-Node Cutting (SNC) method and the Tuberous Root Propagation (TRP) method.

Figure 1 Flowchart of propagation process for sweetpotato seedlings

All these cuttings, regenerated from single-node cuttings and seed tubers, were transplanted to the experimental field (40.2°N, 116.4°E; Beijing, China) on May 22nd 2018 and harvested on October 17th. The soil of the experimental field was sandy loam with a pH of 7.98 and an electronic conductivity of 324 μs/cm, which contained organic matter 17%, available phosphorus 17.5 mg/kg, and available potassium 129.8 mg/kg. The experimental area was 38.4 m^2 (4.0 m×9.6 m). The ridge distance was 1.0 m, and the hill distance was 0.4 m (planting density 16 000 hills/hm^2). Two different planting methods were used: SNC and TRP. For SNC, the seedlings had four to six leaves. For TRP, the cuttings were planted at a depth of 15-25 cm. Each group was planted in duplicate. Subareas of 2.0 m×1.6 m (two ridges of eight plants) in each plot were harvested to compare yield characteristics.

2.3 Measurements

2.3.1 Measurements during transplants propagation

Plant height, stem diameter, fresh and dry weight of shoots and roots, and number of leaves of SNC seedlings were measured on 18 and 25 DAT, using eight biological replicates per treatment. Dry matter content and healthy seedling index were calculated. The yield of SNC and TRP seedlings was counted separately for three generations, i.e., the SNC method was measured at 25, 50, and 70 DAT, while the TRP method was sampled at 45, 60, and 70 DAT. Eight plants were randomly selected from each treatment. Dry matter content and healthy seedling index were calculated by the following equations:

$$\text{Dry matter content} = \frac{\text{shoot dry weight}}{\text{shoot fresh weight}} \times 100\% \qquad (1)$$

$$\text{Healthy seedling index} = \left(\frac{\text{stem diameter}}{\text{plant height}} + \frac{\text{root dry weight}}{\text{shoot dry weight}}\right) \times \text{total dry weight} \qquad (2)$$

The chlorophyll content of SNC and TRP sweetpotato seedlings was investigated at 70 DAT. Arnon's method[23]was used to calculate the leaf chlorophyll and carotenoid contents. In brief, 0.1 g slices of sweetpotato transplants' leaves were extracted in 10 mL 80% (v/v) acetone for over 48 h in the dark. The absorbance of extracting solution at 663 nm, 645 nm, and 470 nm were measured by a spectrophotometer (UV-3150, Shimadzu Co., Japan).

The photosynthetic parameters of SNC and TRP sweetpotato seedlings were measured at 70 DAT. A portable photosynthesis system (LI-6400XT, LI-COR Inc., Lincoln, USA) was used to measure the photosynthetic parameters including net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Cond), and intercellular CO_2 concentration (Ci). In the leaf chamber, light intensity, leaf chamber temperature, air velocity, and CO_2 concentration were controlled at 150 μmol/(m^2·s), 24 °C, 500 μmol/s, and 400 μmol/mol, respectively.

The chlorophyll fluorescence parameters of SNC and TRP sweetpotato seedlings were measured at 70 DAT. These included the potential maximum photochemical efficiency of PSII (Fv/Fm), the performance index based on absorption of light energy (PIabs), absorption per reaction center (ABS/RC), and dissipated energy per reaction center (DIO/RC). PIabs is a comprehensive indicator that reflects the overall performance of photosystem II (PSII) in terms of light absorption, energy transfer, and photochemical efficiency, providing insights into the health of the photosynthetic system. Higher PIabs values indicate better performance and efficiency of the photosynthetic apparatus. ABS/RC represents the amount of light absorbed by the reaction centers per unit of reaction centers, indicating the efficiency of light absorption. DIO/RC measures the energy dissipated as heat per reaction center, reflecting the proportion of absorbed light energy that is lost as heat rather than used for photosynthesis, which serves as an indicator of energy utilization efficiency and plant stress levels. These parameters were calculated using a multi-function plant efficiency analyzer (M-PEA, Hansatech Instruments Ltd., UK). Before measurement, the sample leaves were dark-adapted for 30 min.

2.3.2 Measurements during cultivation

At 73 DAT, eight plants were randomly selected from each treatment. Photosynthetic characteristics were measured on the fourth fully expanded leaf from the apex with a portable photosynthesis system (LI-6400XT, LI-COR Inc., Lincoln, USA). Light intensity, leaf chamber temperature, air velocity, and CO_2 concentration were controlled at 800 μmol/(m^2·s), 35°C, 500 μmol/s, and 400 μmol/mol, respectively.

At 157 DAT, eight plants were randomly sampled of each treatment for assessing yields. At harvest, storage roots weight per plant and total weight of storage roots were recorded. However, some of the storage roots harvested were too small to be sold. Excluding these unsaleable storage roots, commodity storage roots weight per plant and total weight of commercial storage roots were counted. Yield and rate of commodity were calculated. The reducing sugar content was determined by 3,5-Dinitrosalicylic acid method, and the starch content was determined by anthrone sulfuric acid method[24]. Total yield (t/hm^2) was calculated by extrapolating the measured yield from subplots of 2.0 m×1.6 m, based on a planting density of 16 000 hills/hm^2. Yield and rate of commodity were calculated by the following equations:

$$\text{Yield} = \text{Total weight of storage roots}/S \qquad (3)$$

$$\text{Rate of commodity} = \frac{\text{Total weight of storage roots}}{\text{Total weight of commercial storage roots}} \times 100\% \qquad (4)$$

where, S is the area of field under sweet potato cultivation, hm^2.

2.4 Statistical analysis

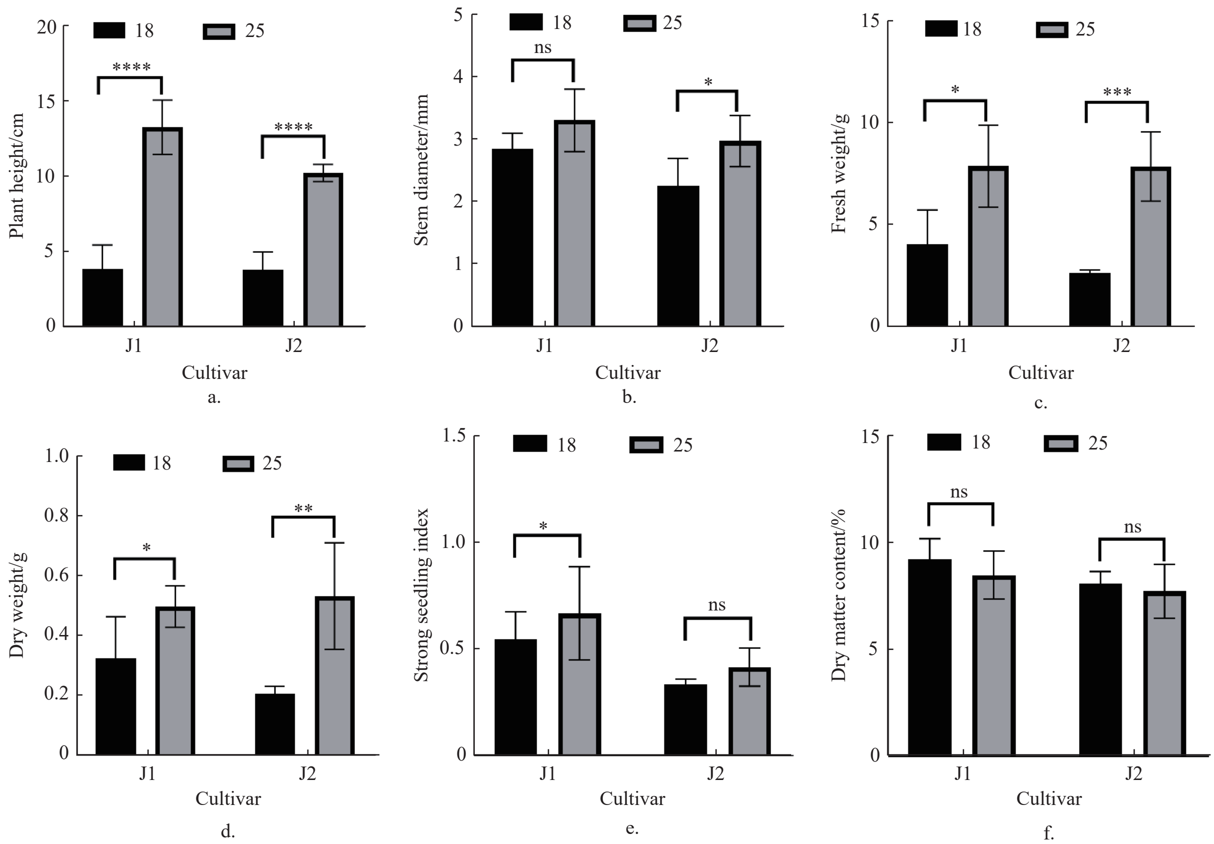
An analysis of variance (ANOVA) was conducted to examine the effects of propagation method on the growth, yields, and quality using IBM SPSS Statistics 23 (IBM, Inc., Chicago, IL, USA). Fisher's Least Significant Difference (LSD) method was used to make post-hoc multiple comparisons at a=0.05 level (n=8).

3 Results and discussion

3.1 Growth characteristics of SNC sweetpotato seedlings

SNC seedlings of both cultivars grew up to 3.8 cm in height at 18 DAT, and both topped more than 10 cm at 25 DAT, a threefold increase (Figure 2a). At 25 DAT, the stem diameter of seedlings of both cultivars reached 3 mm (Figure 2b). At 25 DAT, the fresh weight of seedlings of the two cultivars was 7.95 g and 7.93 g, which were two and three times the fresh weight of seedlings at 18 DAT, respectively (Figure 2c). The dry weight of seedlings of the two cultivars at 25 DAT was 0.50 g and 0.54 g, which were 1.5 times and 2.5 times the dry weight of seedlings at 18 DAT, respectively (Figure 2d). At 18 and 25 DAT, the dry matter content of seedlings from both cultivars was around 8%, and the healthy seedling index was around 0.5 (Figure 2e-2f). The leaves of SNC seedlings of both cultivars developed quadratic function with time, with the first true leaves developing at five DAT and continuing to grow up to 10 DAT (Figure 3).

The quantity of new leaves on seedlings has a direct impact on seedling production efficiency, as well as seedling growth and development[22]. The rate of leaf development determines the yield of seedlings, and quantitative monitoring of leaf number is an effective means of assessing reproductive efficiency, defined here as the ability of seedlings to produce new leaves or propagules over time, directly reflecting propagation potential[25]. Sweetpotato seedlings grown by single-node cutting method can significantly improve seedling production efficiency. Liu et al. cultivated sweetpotato cuttings in plug trays after cutting the stems into single-node, double-node, and three-node top bud portions and discovered that sweetpotato cuttings could generate roots and axillary buds fast, resulting in seedlings that grew swiftly and early[6]. Saiful Islam et al. found that single-node leafy cuttings of sweetpotato plants can be used to produce low-cost, pathogen-free sweetpotato plug transplants[26]. These studies' conclusions are compatible with the findings of this study. Finally, the single-node cutting approach has the potential to considerably enhance the efficiency of sweetpotato seedling production.



Note: J1 represents the cultivar cv. Beniharuka, while J2 represents the cv. Himeayaka. According to LSD's multiple comparison (n=8), NS represents not significant. *, **, and *** represent significant difference at the 5%, 10%, and 1% levels, respectively. Values are means (SD) from eight biological replicates. The figures below are the same.

Figure 2 Growth characteristics of SNC sweetpotato seedlings at 18 and 25 days after transplanting

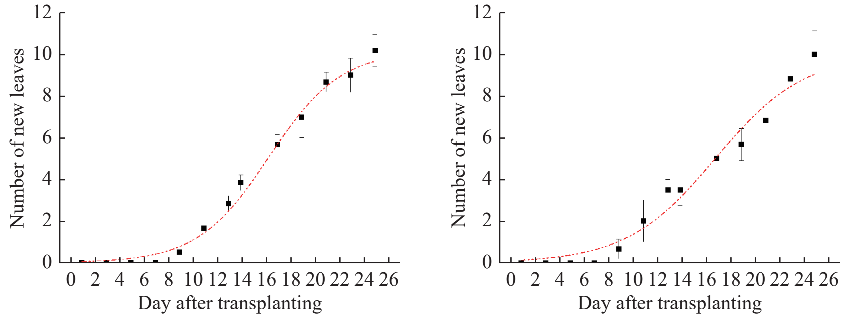


Figure 3 Time courses of increasing new leaf number in hydroponic sweetpotato seedlings produced by single-node cutting (SNC)

3.2 Photosynthetic and chlorophyll fluorescence characteristics of SNC and TRP sweetpotato seedlings

Sweetpotato seedlings from both methods had high total chlorophyll and carotenoid contents (Table 1). SNC seedlings of the J1 cultivar had higher total chlorophyll and carotenoid contents than TRP seedlings, but the difference between the two was not significant. SNC seedlings of the J2 cultivar had 9% higher total chlorophyll than TRP seedlings, while carotenoid contents were equal and neither was significantly different. There was no significant difference in the net photosynthetic rate of seedlings between the two methods. The net photosynthetic rate of SNC seedlings of the J1 cultivar was slightly higher than that of TRP seedlings, whereas the treatments of the J2 cultivar were almost the same between SNC and TRP. The stomatal conductance of SNC seedlings was significantly higher than that of TRP. The stomatal conductance of SNC seedlings of the J1 cultivar was 4.4 times that of TRP seedlings, while SNC seedlings of the J2 cultivar was 4.5 times that of TRP. SNC seedlings had significantly higher intercellular CO_2 concentrations than TRP treatments. SNC seedlings of the J1 cultivar had a 15% higher intercellular CO_2 concentration than TRP seedlings and a 17% higher concentration in the J2 cultivar. The transpiration rate was also significantly higher in SNC seedlings than in TRP treatments, which corresponded to the stomatal conductance trend. The transpiration rate of the J1 cultivar SNC seedlings was three times higher than that of TRP seedlings, while the J2 cultivar SNC treatment was four times higher than that of TRP. In conclusion, SNC seedlings may perform photosynthesis regularly based on the results of the chlorophyll concentration and photosynthetic measures of the leaves.

Table 1 Effect of seedling method on photosynthetic characteristics of sweetpotato seedlings

Cultivar	Treatment	Total chlorophyll content/mg·g^{-1}	Carotenoid content/mg·g^{-1}	Pn/μmol·m^{-2}·s^{-1}	Cond/mmol·m^{-2}·s^{-1}	Ci/μmol·mol^{-1}	Tr/mmol·m^{-2}·s^{-1}
J1	SNC	1.47±0.21	1.35±0.21	6.8±1.0	290±43	354±10	6.13±0.75
	TRP	1.45±0.05	1.24±0.17	6.2±1.0	66±11	301±34	1.98±0.25
J2	SNC	1.38±0.40	1.12±0.66	6.7±0.4	270±46	356±40	5.75±0.58
	TRP	1.26±0.03	1.12±0.09	6.8±0.2	59±10	296±26	1.45±0.75
ANOVA							
Seedling method-J1		NS	NS	NS	*	*	*
Seedling method-J2		NS	NS	NS	*	*	*

Note: SNC represents single-node cutting method and TRP represent tuberous root propagation method. J1 represents the cultivar cv. Beniharuka, while J2 represents the cv. Himeayaka. According to LSD's multiple comparison (n=8), NS and * represent no significant or significant difference at the 5% level, respectively. Values are means (SD) from eight biological replicates. The tables below are the same.

Photosynthesis is the process through which light energy is converted into chemical energy. Photosynthesis is essential for plant development and yield[27,28]. Bhagsari investigated the relationship between photosynthesis and sweetpotato yield and found that canopy photosynthesis was significantly and positively correlated with dry matter yield of storage roots[21]. Increasing photosynthesis in seedlings enhances its production of photosynthate which helps in their recovery after transplanting. SNC seedlings were shown to have attractive photosynthetic capacity in this experiment, and their photosynthetic characteristics were not statistically different from those of TRP sweet potato seedlings, even though they were higher. Stomatal conductance and transpiration rate are major photosynthesis rate factors. The results indicated that SNC seedlings had considerably greater stomatal conductance and transpiration rate than TRP seedlings. The reason could be that TPR seedlings, a section of vines with four to six leaves, had no root system and could not absorb water for transpiration through the root system, resulting in partial stomatal closure. Bandara et al. measured sweetpotato midday leaf water potential and soil water content and discovered a significant linear correlation among the two. By taking water from the substrate, the root system can change the water potential gradient in the plant[29]. Sung et al. found that leaf diffusive resistance increases as leaf water potential decreases, leading to stomatal closure[30]. Unlike SNC seedlings, TRP seedlings do not have a root system to absorb water from the substrate, which is not conducive to the formation of a water potential gradient in sweetpotato leaves, resulting in partial stomatal closure. Stomata are important in regulating the transpiration of higher plants. Stomatal closure leads to a decrease in transpiration rate. Decreases in stomatal conductance and transpiration rate lead to decreases in photosynthetic rate. Furthermore, photosynthesis is particularly sensitive to plant water status, and some research found that reducing the quantity of water utilized for irrigation produced carbon-limited outcomes in potato production[31]. Limited water is connected with decreased photosynthesis caused by both stomatal and non-stomatal restriction. Van Heerden and Laurie discovered that seedlings in drought suffer from photosynthetic inhibition due to stomatal closure[32]. Water status is critical for sweetpotato leaves to photosynthesis, and SNC seedlings are grown for roots, which promotes photosynthesis.

Fv/Fm values for SNC and TRP seedlings were around 0.8, with no significant differences between them (Table 2). The trend was consistent across both cultivars. The ABS/RC values of SNC seedlings were higher than those of the TRP treatment, but the differences were not significantly different, and the results were similar between the J1 and J2 cultivars. ABS/RC values grew by 48% for the J1 cultivar and 26% for the J2 cultivar of SNC seedlings. The DI_0/RC values of SNC seedlings were higher than those of the TRP treatment, but there was no statistically significant difference between the two, and the pattern was the same for the J1 and J2 cultivars. For the J1 cultivar, the DI_0/RC values of SNC seedlings grew by 28%, but for the J2 cultivar, they increased by 36%. Although the PIabs values of SNC seedlings of both J1 and J2 cultivars were lower than those of TRP seedlings, the differences between them were not significant. Overall, by comparing the PSII chlorophyll fluorescence parameters of the two seedling methods, it was confirmed that SNC seedlings have equal photosynthetic capacity as TRP seedlings.

Table 2 Effect of seedling method on chlorophyll fluorescence characteristics of sweetpotato seedlings

Cultivar	Treatment	Fv/Fm	ABS/RC	DI_0/RC	PIabs
J1	SNC	0.80±0.03	3.07±0.48	0.43±0.12	1.59±0.09
	TRP	0.81±0.02	1.57±0.15	0.31±0.03	1.71±0.22
J2	SNC	0.80±0.04	2.50±0.24	0.55±0.13	1.38±0.28
	TRP	0.81±0.01	1.84±0.16	0.35±0.03	2.17±0.21
ANOVA					
Seedling method-J1		NS	NS	NS	NS
Seedling method-J2		NS	NS	*	NS

Chlorophyll fluorescence parameters are often used to characterize the photosynthetic capacity of plant leaves, reflecting not only the primary reaction processes of photosynthesis such as light energy absorption, excitation energy transfer, and photochemical reaction, but also related to processes such as electron-transfer reaction, ATP synthesis, and CO_2 fixation[33]. Chlorophyll fluorescence can reflect almost all changes in photosynthetic activities. Fv/Fm is the maximum maximal quantum yield of PSII, ABS/RC is the light energy absorbed per reaction center, DIo/RC is the energy dissipated per reaction center, and PIabs is the performance index based on absorbed light energy[34]. The Fv/Fm values of SNC seedlings of both cultivars were able to reach 0.8, which was not different from that of TRP seedlings, indicating that SNC seedlings have better photosynthetic potential. In addition, the ABS/RC and DIo/RC values, which characterize photosynthetic energy transfer, did not differ significantly between the two nursery methods. The PIabs values could also indicate that SNC seedlings have a healthy photosynthetic capacity. Although seedlings were propagated in different environments (climate chamber for SNC, greenhouse for TRP), all were transplanted into the same field conditions for yield evaluation. This minimized environmental bias and allowed fair comparison between propagation methods.

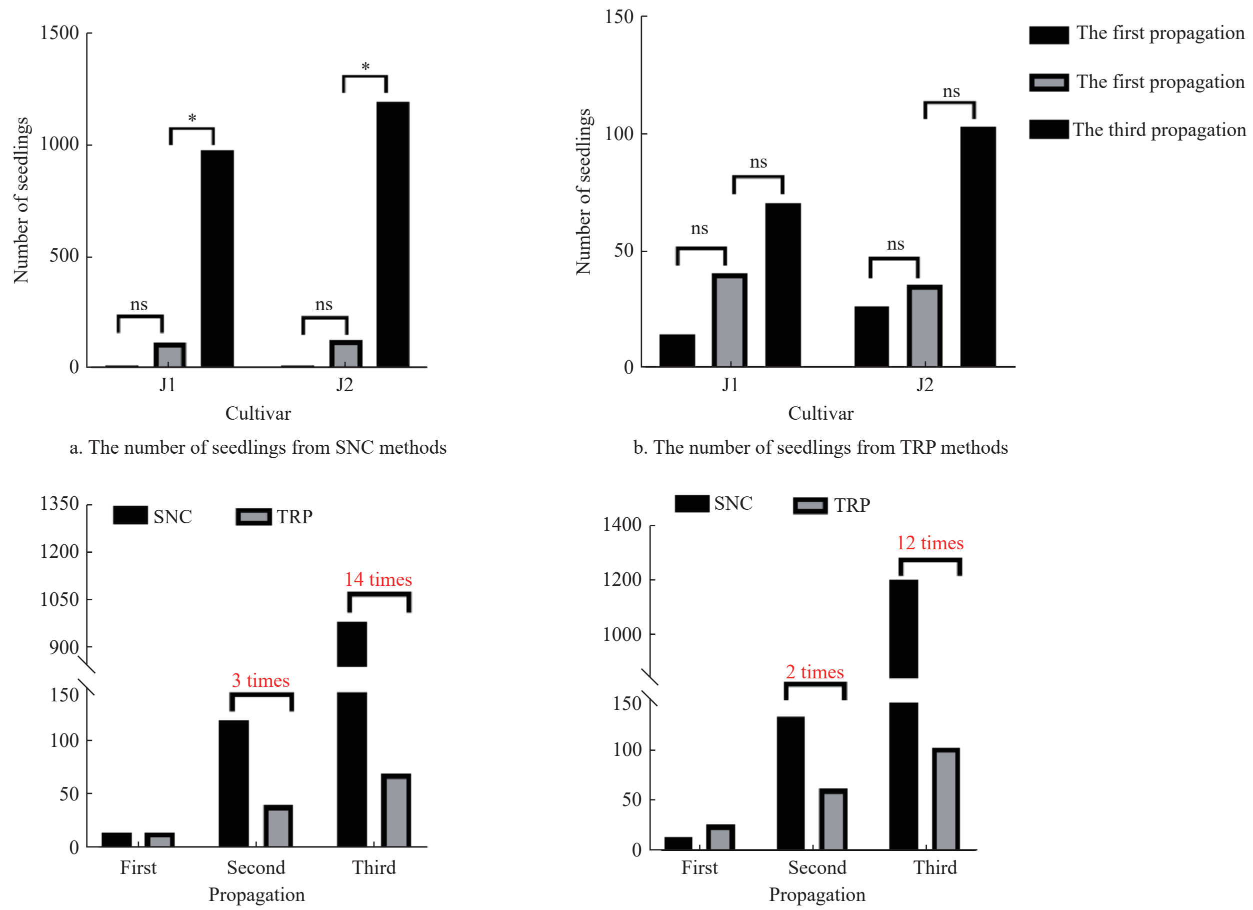
3.3 Yield of SNC and TRP sweetpotato seedlings

In the third propagation, the number of seedlings of both cultivars J1 and J2 was significantly increased by the SNC method for sweetpotato seedling production (Figure 4a). In the third propagation, the number of seedlings of both cultivars J1 and J2 was not significantly increased by the TRP method for sweetpotato seedling production (Figure 4b). After three generations (70 d) of cultivation in the same 0.1 m^2 initial culture area, the SNC method of the J1 cultivar produced 992 seedlings, 14 times more than the TRP treatment, and the J2 cultivar produced 1215 seedlings, 12 times more than the control (Figure 4c-4d). Breeding one generation with one stem node as the initial seedling material, about 10 new seedlings can be obtained using the SNC method (Figure 1a). In the first generation, there was little difference in the number of seedlings between the two ways; however, in the second generation, SNC seedlings were two to three times greater than TRP, and in the third generation, SNC seedlings were 10 times more numerous than TRP. The SNC seedlings are virus-free and can be used for long-term subculture. In this study, three generations of SNC seedlings were grown and SNC seedling production was more than ten times greater than TRP seedling production over the same period. The SNC method is much more efficient than the TRP method. Both seedling methods used vines with stem nodes and leaves as initial material. The TRP method used 15-20 cm sections of vine as initial material, while the SNC method used single stem nodes as initial material. One SNC seedling can produce 10 nodes after 25 d (Figure 1a). Tuberous roots can produce four to six leaves 45 d after transplanting (Figure 1b). This is the main reason why the SNC method is much more efficient than TRP. Moreover, continuously cutting the stem nodes could promote cell division and differentiation. Ma et al. discovered that node location (age) has significant effects on the developmental stage and amount of root primordia inside the stem, and that upper nodes (post-formation) are more capable of forming root primordia than lower nodes (pre-formation)[35]. TRP seedlings taken from the lower part (pre-formation) of vines were less able to grow roots after transplanting compared to SNC seedlings. The SNC method could significantly improve seedling propagation efficiency and quality, which is advantageous for industrial production of seedlings.

3.4 Photosynthetic characteristics of SNC and TRP sweetpotato seedlings after transplanting in the field

After transplanting in the field, the net photosynthetic rate of SNC seedlings of the JI cultivar was significantly higher than that of TRP seedlings, while the difference between the two seedlings of the J2 cultivar was not significant (Table 3). SNC seedlings of the J1 cultivar improved by 16% compared to TRP seedlings, whereas the J2 cultivar had little difference between the two. There were no significant differences in stomatal conductance between the two treatments for both J1 and J2 cultivars. The SNC treatment was higher than the TRP treatment for the J2 cultivar, whereas the opposite trend was observed for the J1 cultivar. The intercellular CO_2 concentrations in SNC seedlings were not significantly different from those in TRP treatments. The J1 cultivar SNC treatment had decreased intercellular CO_2 concentrations compared to TRP, but the J2 cultivar had the opposite effect. Although the transpiration rate of SNC seedlings was lower than that of TRP seedlings, the difference between them was not significant and the two cultivars performed similarly. In a word, SNC seedlings performed at the same level of photosynthetic ability as TRP seedlings in terms of the net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate.

Photosynthesis is a major determinant of sweetpotato yield. Bhagsari found a significant positive correlation between canopy photosynthesis of seedlings and dry matter production of storage roots[21]. Zong et al. found a significant positive correlation between net photosynthetic rate and tuber yield in each growth period of sweetpotato. They proposed that net photosynthetic rate, which could be assessed at any stage of growth, may be a crucial indicator in choosing sweetpotato cultivars[36]. Photosynthetic indicators were



Note: SNC represents single-node cutting method and TRP represents tuberous root propagation method. *X* times means that the number of seedlings from SNC treatments is *X* times higher than from TRP treatments.

Figure 4 Effect of two methods on number of sweetpotato seedlings

Table 3 Effect of seedling method on photosynthetic characteristics of sweetpotato seedlings after transplanting in the field

Cultivar	Treatment	Pn/ μmol·m^{-2}·s^{-1}	Cond/ mmol·m^{-2}·s^{-1}	Ci/ μmol·mol^{-1}	Tr/ mmol·m^{-2}·s^{-1}
J1	SNC	22.9±3.1	546±94	321±19	4.51±0.50
	TRP	19.2±1.9	701±46	331±2	4.81±0.48
J2	SNC	23.2±1.3	739±30	315±18	3.06±0.29
	TRP	23.9±2.2	727±27	302±19	3.09±0.80
ANOVA					
Seedling method-J1		*	NS	NS	NS
Seedling method-J2		NS	NS	NS	NS

utilized as the primary indicators in this study to evaluate seedlings, and net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate were measured before and after transplanting seedlings. The results indicated that stomatal conductance and transpiration rate of SNC seedlings were significantly higher than those of TRP seedlings in both cultivars before transplanting. After transplanting, the net photosynthetic rate of SNC seedlings of J1 cultivar was significantly higher than that of TRP seedlings, and the difference between the two treatments of J2 cultivar was not significant. Consequently, photosynthetic characteristics of seedlings after transplanting determine the yield of sweetpotato storage roots, and seedling growth after transplantation must be monitored. In addition, initial material for SNC seedlings was obtained from virus-free seedlings. Photosynthetic parameters such as chlorophyll content, stomatal conductance, net photosynthetic rate and transpiration rate, photosynthetic key enzymes ribulose-1,5-bisphosphate carboxylase and phosphoenolpyruvate carboxylase activities and gene (pepc, rbcS and rbcL) expression levels were higher in virus-free seedlings than in normal seedlings[37]. Wang et al. discovered that by raising chlorophyll content, photosynthetic key enzyme activities, and leaf gene expression levels, virus-free seedlings may increase photosynthetic efficiency and boost photosynthesis. By increasing the activity of antioxidant enzymes and decreasing the degree of membrane lipid peroxidation, the stress resistance of chewing cane was improved, resulting in improved yield and quality[38]. In conclusion, the measurement of photosynthetic indices of seedlings before and after transplanting confirmed that SNC seedlings have healthy photosynthetic capacity.

3.5 Yield of storage roots of SNC and TRP sweetpotato seedlings after transplanting in the field

The results showed that the storage roots yield of SNC seedlings was higher (Table 4). SNC seedlings of J1 cultivar had significantly higher total weight of storage roots per plant, total weight of commercial storage roots per plant, and total yield than TRP seedlings, increasing by 48.9%, 61.0%, and 49.3%, respectively. The J2 cultivar's seedlings showed similar patterns to the J1 cultivar, with total weight of storage roots per plant, total weight of commercial storage roots per plant, and total yield increasing by 30.4%, 34.1%, and 32.1%, respectively. Rate of commodity of the storage roots obtained by the two ways did not differ, and the two cultivars acted consistently. In conclusion, SNC seedlings can be used for production after transplanting to the field with short recovery time and high storage root yield.

Table 4 Effect of seedling method on yield of storage roots of sweetpotato seedlings after transplanting in the field

Cultivar	Treatment	Storage roots weight per plant/kg	Commodity storage roots weight per plant/kg	Yield/ t·hm^{-2}	Rate of commodity/ %
J1	SNC	0.67±0.01	0.66±0.01	40.0±5.85	99.4±1.27
	TRP	0.45±0.11	0.41±0.10	26.8±6.79	97.4±1.85
J2	SNC	0.60±0.12	0.59±0.12	36.2±6.99	97.1±2.37
	TRP	0.46±0.10	0.44±0.10	27.4±5.94	96.4±3.63
ANOVA					
Seedling method-J1		*	*	*	NS
Seedling method-J2		*	*	*	NS

Note: Yield values were extrapolated based on the planting density of 16 000 hills/hm^2.

To investigate whether SNC seedlings can replace TRP seedlings for commercial production, this study surveyed the storage roots yield of SNC seedlings. The results showed that the storage roots yield of SNC seedlings was higher. The reason for this result could be that virus-free seedlings have higher photosynthetic capacity and more metabolite accumulation. Moreover, SNC seedlings contain roots when transplanted, which can absorb water and nutrients from the soil faster and grow faster. Ying discovered that virus-free seedlings had significantly higher chlorophyll content, leaf area, number of leaves, vine base diameter, and number of lateral vines than traditional seedlings, implying that virus-free seedlings should be obtained through tissue culture to improve tuber yield[17]. Sweetpotato seedlings transplanted in the early stage of recovery had weak growth, low resistance, and were easily infected with the virus, but virus-free seedlings placed in the early stage of recovery had healthy growth, enhanced resistance, and the rate of disease susceptibility fell dramatically. Chen et al. found that virus-free sweetpotato seedlings had increased resistance to stress, earlier and faster tuber enlargement, and higher yield, generally increasing yields by more than 50%[39]. The above-ground and below-ground growth of sweetpotato seedlings were coordinated, and the high rate of photosynthetic product transport to the tuber was the key to their ability to produce abundant yields. Furthermore, as SNC seedlings have roots and do not need to grow new roots after transplanting, they can absorb water and nutrients from the soil in time to avoid wilting and promote growth, providing a good material basis for tuber formation and growth. In addition, when compared to TRP seedlings, total weight of storage roots per plant, total weight of commercial storage roots per plant, and total yield of SNC seedlings were enhanced after transplanting and could be used for production.

3.6 Quality of storage roots of sweetpotato seedlings after transplanting in the field

For cultivars J1 and J2, dry matter content and polyphenol oxidase activity were not statistically different between the two treatments (Figures 5a-5b). Compared to the TRP treatment, the starch content of storage roots of SNC seedlings was higher, 13% and 8% higher for cultivars J1 and J2, respectively (Figure 5c). SNC seedlings of J1 cultivar had significantly higher storage roots reducing sugar content than the TRP treatment. This may be attributed to enhanced photosynthetic performance and sugar metabolism in SNC seedlings, which were derived from virus-free materials. The difference in reducing sugar content of storage roots between the two treatments of cultivar J2 was significant (Figure 5d). The reducing sugar content after baking in SNC seedlings of cultivar J1 was higher than in the TRP treatment (Figure 5e). There was no significant variation in the reducing sugar content of sweetpotato storage roots after steaming, with a continuous trend in the two cultivars (Figure 5f). By investigating the soluble sugar and starch content of sweetpotato storage roots, this study found that the quality of sweetpotato storage roots from SNC seedlings was better than that of TRP treatments after transplanting them to the field. This indicates that SNC seedlings can be used for production.

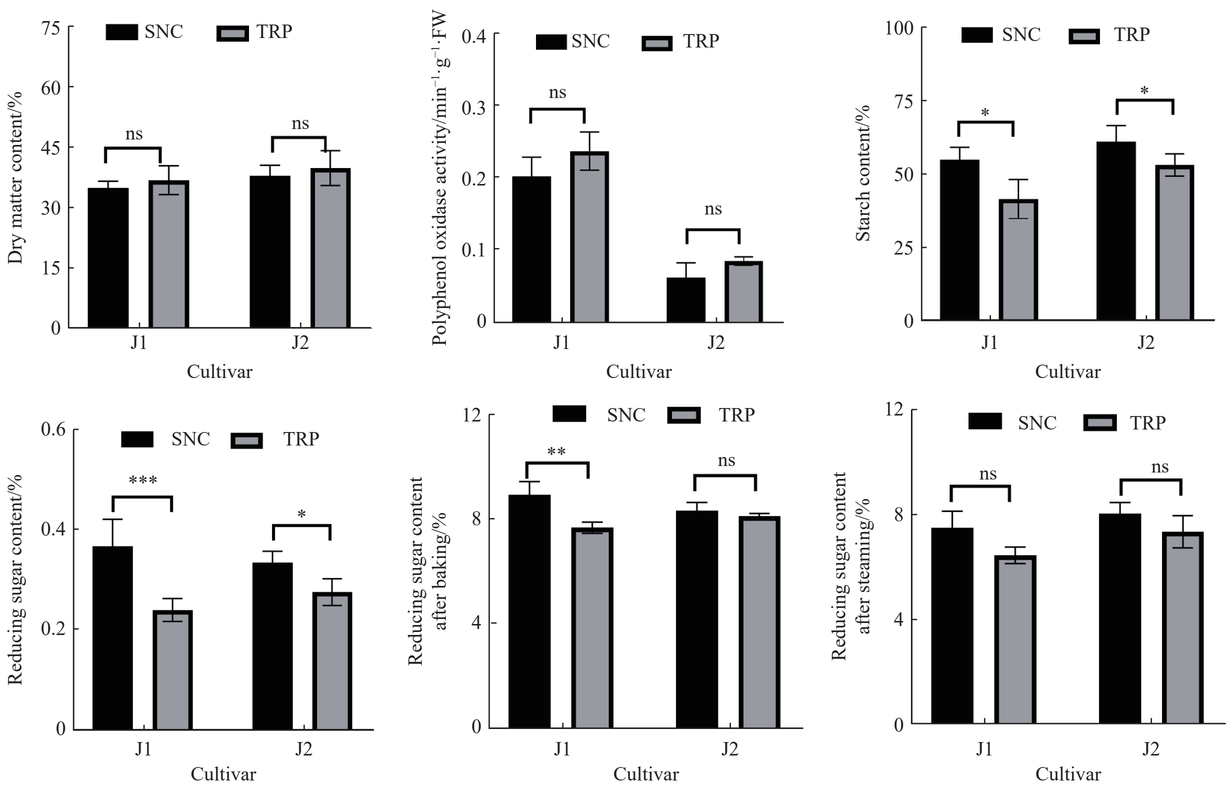


Figure 5 Effect of two methods on quality of storage roots of sweetpotato seedlings after transplanting in the field

In addition to the yield of storage roots, this study also investigated the dry matter content, soluble sugar and starch content of sweetpotato storage roots, hoping to evaluate the quality of SNC seedlings in a comprehensive manner. SNC seedlings had greater storage roots dry matter rate, starch content, and reducing sugar content after steaming than TRP seedlings, although the differences were not significant. Wang et al. discovered that sugarcane virus-free seedlings had considerably greater biomass and sucrose content than normal seedlings. The stress resistance, yield, and quality of the virus-free seedlings improved as they grew[40,41]. Both this study and the results of this paper indicate that virus-free seedlings have higher biomass accumulation and more active sugar metabolism. There is no difference in polyphenol oxidase activity between SNC and TRP sweetpotato storage roots. Polyphenol oxidase (PPO), an enzyme that oxidizes polyphenols, is widely found in plants such as apples and potatoes, making them gradually turn brown to black when exposed to air during consumption or processing, which seriously affects appearance and quality[42]. Compared to TRP sweetpotato storage roots, SNC storage roots can also be exposed to air for long periods without oxidation and there is no difference in eating taste. In conclusion, storage roots obtained from SNC seedlings were at the same level or performed better than the TRP treatment in terms of starch content, sugar content after cooking, and storage time.

4 Conclusions

The results indicated that single-node cutting method (SNC) could significantly improve the efficiency of sweetpotato seedling production, which was about 12 times higher than that of the conventional method. The photosynthesis level of sweetpotato seedlings produced by this method was at the same level or higher than that of seedlings obtained by the conventional method, with the same pattern before and after transplanting. When the seedlings were used for sweetpotato production, their storage root yield increased and their quality did not deteriorate. Thus, the single-node cutting method can be used for sweetpotato seedling production. Although the SNC method demonstrated excellent propagation efficiency and field performance in two cultivars under controlled and field conditions in Beijing, further research involving more cultivars and diverse geographic locations is necessary to confirm its general applicability.

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