

Comparison of Photosynthetic Characteristics and Reproductive Traits of Winter Squash (*Cucurbita maxima* L.) Between Cultivars

Yu Sung[†], Guei-Jia Liang[†]

Department of Horticulture, National Chung Hsing University, Taichung, Taiwan

Email address:

yusung@dragon.nchu.edu.tw (Yu Sung)

[†] Yu Sung and Guei-Jia Liang are co-first authors.

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Abstract: The production of winter squash used vertical trellising was popular in the greenhouse for easy to manage growth and fruiting. This study investigated the photosynthetic characteristics of six winter squash cultivars to explore the differences in crop production efficiency between cultivars by vertical trellising cultivation. Plant growth, photosynthetic characteristics and fruit quality of winter squash cultivars ‘Black lamp’, ‘Tastemaker’, ‘East elite’, ‘Julius’, ‘Sugar’ and ‘Lyric’ were surveyed. The photosynthetic rate (Pn) was measured on the 70th day after planting. We selected the three leaves above the fruit in which to compare the Pn between cultivars, and surveyed all leaves on ‘Black lamp’ and ‘East elite’ in order to make a comparison between nodes. The Pn values of ‘Black lamp’, ‘East elite’, ‘Lyric’ and ‘Tastemaker’ were significantly higher, at 14.02, 13.06, 12.33 and 12.19 $\mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$, respectively. The Pn of the leaves on 5–8th nodes decreased significantly, and the Pn of the 1st–5th nodes up to the fruit also decreased. With regards to reproductive traits, the first pistillate flowers of ‘East elite’ and ‘Black lamp’ bloomed on the 30th day after planting, while the other cultivars bloomed on the 35–37th day after planting. The six cultivars were classified according to the size of the fruit on the 48th day after pollination: ‘Lyric’ and ‘Tastemaker’ were classified into the largest group, at 1203 and 1106 cm^3 , respectively; the second group contained ‘East elite’ and ‘Julius’, at 870 and 687 cm^3 ; while ‘Sugar’ and ‘Black lamp’ were in the smallest group, at 353 and 217 cm^3 , respectively. The soluble sugar and total soluble solid (TSS) contents of ‘East elite’ were significantly elevated, their values being 630.6 g/kg and 13.3%, respectively. The starch content of ‘Lyric’ was the highest at 193.7 g/kg. “Black Light” was a fruit-number type, and the increase in fruit number could increase the yield/plant; “East elite” was a fruit-weight type, and the yield per plant was related to the weight of a single fruit. The influence of the unit leaf of “Black Lamp” on the fruit fresh weight was the lowest performance in the reproductive growth. In order to increase productivity of small fruit such as ‘Black lamp’ western pumpkin, the balance between the vegetative growth and the reproductive growth was necessary to be improved.

Keywords: Photosynthesis, Winter Squash, Cultivars, Reproductive Traits

1. Introduction

Vertical trellising of winter squash (*Cucurbita maxima* L.) in greenhouses generally provides fruit of better quality with a stable yield in Taiwan. According to Taiwan Agriculture and Food Agency (AFA) statistics regarding winter squash production, there was a farming area of 5,814 ha and an output of 92 million kg in 2016. The kabocha type of winter squash is the most famous table cultivar that is mainly bred and consumed in Japan and produced in New Zealand and Mexico.

Also, New Zealand has engaged in important research into cultivation and storage techniques [2, 6, 8].

Sugar, starch and total soluble solid (TSS) contents are factors that affect the quality of fruit of winter squash. Winter squash fruit with a TSS content of 10–15°Brix, a starch content of 60–90 g/kg DW, and a sugar content of 400–500 g/kg DW are of standard quality. If squash meet the criteria mentioned above, the fruit after cooking will be loose, but not soft, and will have a sweetness and texture like that of a sweet potato [1, 4]. These are the acceptable

conditions for the fresh market.

The leaves are a critical factor in supporting growth of squash fruit. The fruit of winter squash contains approximately 80% DW of starch, a kind of polysaccharide in which photosynthetic energy is stored efficiently in squash fruit [8, 14]. Leaves draw water and nutritional solution up for fruit initial expansion, and then produce sucrose for the fruit in order to support bearing seeds. The process of synthesizing sucrose from leaves is important for fruit quality and yield, and is affected by training methods, leaf age, fruit position and environment during cultivation [3, 9].

Cucurbita is a plant of high photosynthetic efficiency; however, little research on the photosynthesis of winter squash has been documented. The photosynthetic daily change in *C. moschata* and *C. maxima* seedlings was studied, and the results showed that the Pn became a single-peak inverted U-shaped curve according to daily change, and no photosynthetic midday depression was observed. Otherwise, winter squash ‘Spinning’ had the highest peak, the maximum photosynthesis rate reaching 25 $\mu\text{mol}/\text{m}^2$ at noon under 1600 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ relative photosynthetically active radiation (PAR) [7].

Many studies have explored the photosynthesis and fruit composition of winter squash, but few have discussed the relationships between vegetative factors and reproduction in different varieties. The aim of this study was to identify the basic vegetative and reproductive characteristics of different varieties. Furthermore, we investigated the production efficiency of different varieties and explored its relationship with photosynthesis.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Winter squash [*Cucurbita maxima* L. ‘Black lamp’, ‘Tastemaker’, ‘East elite’, ‘Julius’, ‘Lyric’ and ‘Sugar’] seeds were sown and grown in simple vegetable cages at National Chung Hsing University (Taichung, Taiwan). Cultivated as a hanging vine, the plant spacing was 90×60cm, and we kept only one fruit after the 15th node of the main vine. The side vines were removed, with the exception of the one on the fruiting node, on which three leaves were kept before topping. The main vine was topped when the plant tip reached the top of the steel cable. After 60 days of transplanting, we removed 4~6 aging leaves at the base of the plant to avoid pests and diseases.

Artificial pollination was carried out daily between 6 and 9 am during blooming. We removed malformed fruit and those that had stopped growing after 5–7 days of fruit-setting (the

fruit were of similar size to a fist). Fruit were harvested on around the 50th day after pollination, the timing being judged according to the fruit color (transformation from bright green to dark green), pedicels (lignification was up to 70%), and emergence of basal adventitious buds.

2.2. Plant Sampling

2.2.1. Measurements of Photosynthetic Properties

The photosynthetic properties of leaves were investigated using a portable photosynthesis analyzer (Li-6400XT, Li-COR inc., Lincoln, NE, USA) on the 70th day after transplant at 9:00–10:00 am. Parameters were set up as a 400 ppm CO₂ concentration, 500 $\mu\text{mol}/\text{s}$ gas flow rate, and natural light (PAR between 600 and 700 $\mu\text{mol}/\text{m}^2\cdot\text{s}$) to determine the net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular carbon dioxide concentration (Ci) and evapotranspiration rate (E). Three plants of each variety were analyzed; mature leaves were taken from the first node to the third node above the fruit, and two points were taken for each leaf. To examine the photosynthesis daily change, the Pn values of the leaves were measured from 6 am to 3 pm. Three measurements were taken for each variety, and four points per leaf were analyzed in order to calculate the daily variation of photosynthetic parameters of two varieties.

2.2.2. Carbon Fixed Amount Calculation

Daily amount of carbon fixation per plant: different periods of photosynthesis rate ($\text{mole}/\text{m}^2\cdot\text{s}$) time (s) × leaf area per plant (m^2) × 3.277 (g/mole).

2.2.3. Plant and Fruit Analysis

The growth and fruiting of the 6 varieties of winter squash were investigated. Each plant was analyzed in 4 replicates, with a total of 12 replicates per cultivar. The analysis was of a completely randomized design. In the early stage of growth, each variety was calibrated using 5 replicates per plant to assess plant fertility. The plants were used to determine the vine length, leaf number, node number, internodal length, leaf area, plant fresh weight, plant dry weight, fruit weight, and leaf area to fruit weight ratio (LAFR).

2.2.4. Fruit Quality Measurement

Collect the mature fruits of 6 varieties of winter squash were investigated. To determined the pulp weight, fruit height/width, fruit cavity height/width, pulp rate of volume (PRV).

The fruit volume (cm^3) and fruit cavity volume (cm^3) was calculated by the following equation:

$$\text{the fruit volume (cm}^3\text{)} = (\text{fruit height (cm)})/2 \times (\text{fruit width (cm)})/2 \times (\text{fruit width (cm)})/2 \times 3.14159 \times 3/4;$$

$$\text{the fruit cavity volume (cm}^3\text{)} = (\text{fruit cavity height (cm)})/2 \times (\text{fruit cavity width (cm)})/2 \times (\text{fruit cavity width (cm)})/2 \times 3.14159 \times 3/4$$

$$\text{fruit volume (cm}^3\text{)} = (\text{fruit height (cm)})/2 \times (\text{fruit width (cm)})/2 \times (\text{fruit width (cm)})/2 \times 3.14159 \times 3/4$$

$$\text{fruit cavity volume (cm}^3\text{)} = (\text{fruit cavity (cm)})/2 \times (\text{fruit cavity (cm)})/2 \times (\text{fruit cavity (cm)})/2 \times 3.14159 \times 3/4$$

Fruit pulp total soluble solid (TSS) content: after longitudinal sectioning of the fruit, a section slice was taken

from 0.5–3 pieces, approximately 20–30 g. After weighing, water at 4 times the weight was added to make juice (diluted 5 times), and the sugar content was measured using a sugar meter (PR-101, ATAGO), with °Brix (%) as the unit. That reading was then multiplied by 5 to determine the TSS concentration of the actual pulp.

2.2.5. Fruit Carbohydrate Analysis

Fruit of the winter squash was dried and ground into a powder. Then, 0.1 g of the dried sample was placed in a 30-mL centrifuge tube, 10 mL of distilled water were added, and the mixture was shaken for 3 hours in a constant-temperature water bath at 30°C. The mixture was then centrifuged at 13,000 rpm for 15 minutes at room temperature (25°C), and the supernatant extracted to determine the TSS content. After centrifugation, the lower layer of the precipitate (the supernatant having been completely removed) was placed in an oven at 70°C for 24 hours for starch content determination [12].

2.2.6. Total Soluble Sugar Content

Five ml of the supernatant were removed after centrifugation, and 1 mL of HCl (6N) was added, followed by shaking in a water bath at 70°C for 15 minutes. The sample was then removed from the water bath and cooled quickly using ice water. Then, 0.1 mL of the extract was added to 1.9 mL of deionized water and the mixture shaken evenly, followed by the addition of 0.1 mL of liquid phenol and 6 mL of concentrated sulfuric acid. After shaking evenly, the mixture was allowed to stand for 30 minutes to allow the color to change to orange. The absorbance at 490 nm was then measured using a spectrophotometer (U-2900, HITACHI). A standard solution was prepared at 0.5 $\mu\text{mol/ml}$ D-glucose to calculate the total soluble sugar content of the sample in units of mg/g DW.

2.2.7. Starch Content

To dried sample powder was added 2 mL of deionized water, and the mixture was placed in a water bath at 100°C for 15 minutes. After being removed, it was quickly cooled using ice water, and then, after shaking for 2 minutes following the addition of 2 mL of HClO_4 (9.2 N), 6 mL of deionized water were added. After centrifugation at 15,000 rpm for 10 minutes at room temperature (25°C), 0.1 mL of the supernatant was added to 1.9 mL of deionized water and the mixture shaken evenly, followed by the addition of 0.1

mL of liquid phenol and 6 mL of concentrated sulfuric acid, and further shaking evenly. The mixture was allowed to stand for 30 minutes to allow the color to change to orange. The absorbance at 490 nm was then measured using a spectrophotometer (U-2900, HITACHI). A standard solution was prepared at 0.5 $\mu\text{mol/mL}$ D-glucose to calculate the starch content of the sample in units of mg/g DW.

2.3. Statistical Analysis

The six varieties of winter squash tested were analyzed using a completely randomized design (CRD), with 4 replicates per process and 7 replicates per plant. The data for each variety were analyzed by analysis of variance (ANOVA) using the SAS 9.4 software package (SAS Institute, Cary, NC, USA). Variant analysis was performed, and analysis of significant differences in the means between processes was performed using Fisher's least significant difference (LSD) test.

All data were subjected to ANOVA, and significant differences in the means were evaluated using Fisher's least significant difference test (Fisher's LSD test) or Student's t-test. Data are presented as the mean \pm standard error ($n = 3$).

3. Results

3.1. Diurnal Variation of Photosynthesis in Different Varieties and in Leaves of Different Ages

We analyzed 1–3 mature leaves from above the fruit on a sunny day from 9:00–10:00 and compared the photosynthetic parameters in the six varieties of winter squash (Table 1). The photosynthesis rate was significantly higher in 'Black lamp', 'East elite', 'Lyric' and 'Tastemaker', at 14.02, 13.06, 12.33 and 12.19 $\mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$, respectively, while the rate in 'Sugar' and 'Julius' was significantly lower, at 10.08 and 8.74 $\mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$, respectively. By performing the tests at 9:00–10:00 a.m. on sunny days, differences in the photosynthesis rate between varieties were effectively distinguished. There were no significant differences in stomatal conductance or evapotranspiration, while the intercellular carbon dioxide concentration decreased as the photosynthesis rate increased, 'Julius' having the highest concentration, followed by 'Sugar', 'Tastemaker', 'East elite', and 'Lyric', while 'Black lamp' had the lowest concentration, with values of 328, 285, 278, 273, 265 and 254 ppm, respectively.

Table 1. Photosynthetic characteristics of the six cultivars of winter squash.

| Cultivar | Net photosynthetic rate $\mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$ | Stomatal conductance $\text{mmol H}_2\text{O}/\text{m}^2\cdot\text{s}$ | Intercellular CO_2 concentration ppm | Evapotranspiration rate $\text{mmol H}_2\text{O}/\text{m}^2\cdot\text{s}$ |
|---------------------|---|---|--|--|
| Black lamp | 14.02 a ^z | 0.276 a | 254 c | 5.36 a |
| Sugar | 10.08 b | 0.188 a | 285 b | 3.81 a |
| Julius | 8.74 b | 0.192 a | 328 a | 3.33 a |
| Tastemaker | 12.19 a | 0.229 a | 278 b | 4.22 a |
| East elite | 13.06 a | 0.244 a | 273 bc | 5.12 a |
| Lyric | 12.33 a | 0.238 a | 265 bc | 4.70 a |
| LSD _{0.05} | 1.98 | 0.125 | 21 | 2.34 |

^zMeans with the same letters in a column were not significantly different according to Fisher's least significant difference (LSD) test at the 5% level. Investigation date: 2017/03/27; light intensity: 600–700 $\mu\text{mol}/\text{m}^2\cdot\text{s}$.

The photosynthetic characteristics of winter squash 'Black lamp', 'Tastemaker', 'East elite', 'Julius', 'Sugar', and 'Lyric' were compared. The daily change of the photosynthesis rate for each cultivar is shown in Figure 1. Testing was conducted on a sunny day 70 days after transplant, and analysis was performed every hour from 6 a.m. to 3 p.m. The photosynthesis rate was less than $1 \mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$ at 6 a.m.; then, from 6 a.m. to 8 a.m., the rate of each variety increased in two stages. From 9 a.m. to

10 a.m., the photosynthesis rate of each variety reached a peak; 'East elite' and 'Sugar' reached a peak at 9 a.m., with values of 14.4 and $7.5 \mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$, respectively, while 'Black lamp', 'Tastemaker', 'Lyric', and 'Julius' reached a peak at 10 a.m., 'Black lamp' having the highest rate at $15.4 \mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$. After 11 a.m., the photosynthesis rate of the six varieties of winter squash decreased rapidly, and at 1 p.m., the rate of each variety was lower than $3 \mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$.

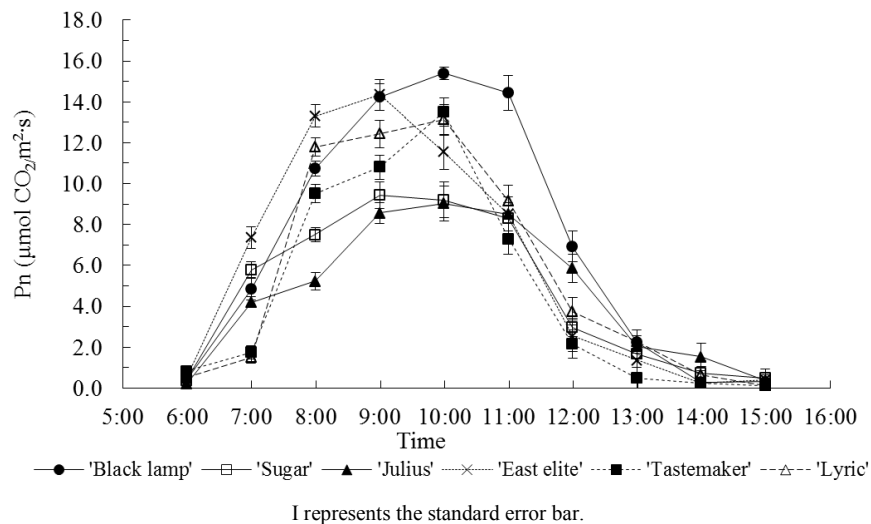


Figure 1. Diurnal patterns of the photosynthetic rate of the six cultivars of winter squash.

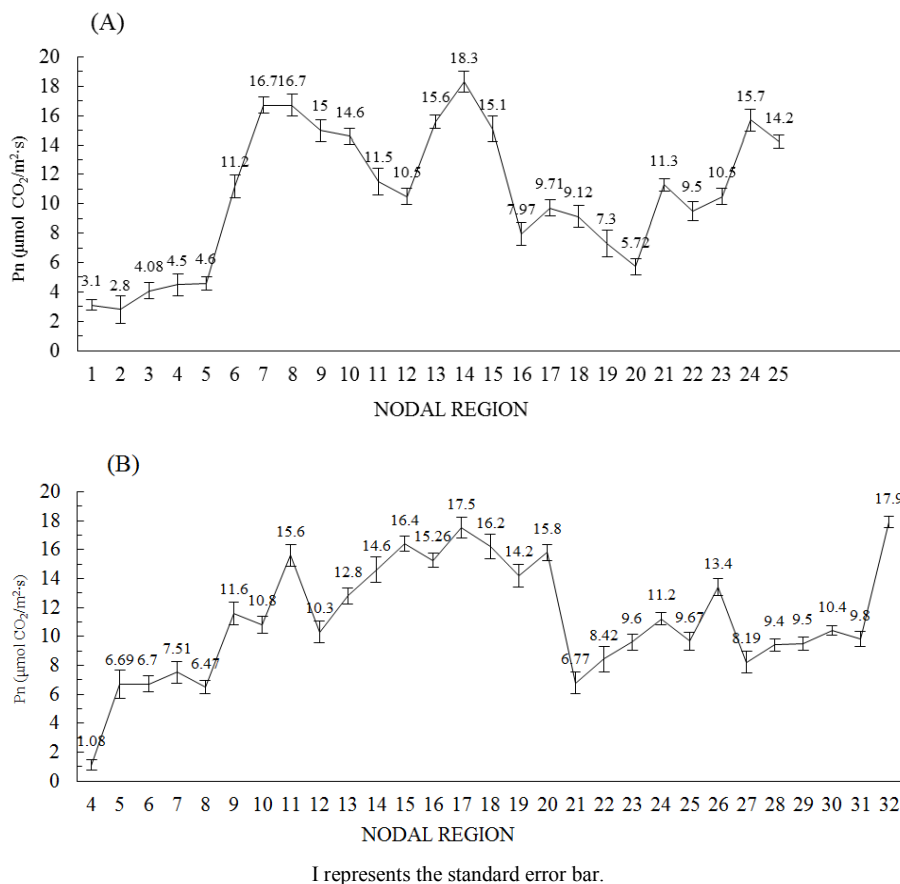


Figure 2. Photosynthetic rates of 'East elite' (A) and 'Black lamp' (B) at different nodes.

The experiment was conducted 70 days after transplant, and the results of net photosynthetic efficiency evaluation of leaf productive forces at different nodes are shown in Figure 2. The photosynthesis rate of 'East elite' for the 1st–5th leaves was significantly low, at between 3.1 and 4.6 $\mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$, while for the 4th–5th leaves, which was when the PAR increased, the photosynthesis rate did not increase, a phenomenon that may be related to the age of the leaves. The 'East elite' leaf photosynthesis rate was increased significantly for the sixth leaf, and the 7th–10th and 12th–15th leaves had the maximum photosynthesis rate, at 10.5–18.3 $\mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$, at which time the photosynthesis rate and the PAR curve were the closest. For leaves on the 16th–23rd nodes, the photosynthesis rate decreased to 5.72–10.5 $\mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$, and at nodes 21–23, the photosynthesis rate was far away from the PAR curve, and the rate for the leaves on the 24th node was significantly increased at 15.7 $\mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$.

The photosynthesis rate at the different nodes of winter squash 'Black lamp' exhibited the same pattern as that of 'East elite' (Figure 2). Leaves on nodes 5–8 had a lower

photosynthesis rate at 6.47–6.69 $\mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$, while leaves on node 9 exhibited a significantly higher photosynthesis rate, and leaves on nodes 10 and 14–20 had the maximum photosynthesis rate at 10.8–17.5 $\mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$. There were also significant decreases in the PAR and photosynthesis rate for leaves of nodes 21–31, at 8.19–11.2 $\mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$, and the photosynthesis rate recovered at node 32, at 17.9 $\mu\text{mol CO}_2/\text{m}^2\cdot\text{s}$.

3.2. Comparison of Plant Growth and Fruit Quality of Different Varieties

The characteristics of the six varieties of winter squash are shown in Figure 3. The peel color of 'Tastemaker', 'East elite' and 'Black lamp' is green, and 'Black lamp' has the deepest green peel color; 'Julius' and 'Sugar' have orange peel, and 'Lyric' has white peel. In terms of fruit shape, for 'Lyric' and 'Tastemaker', the upper edge of the fruit and fruit shoulder are slimmer, and the fruit bottom is bulging, and belongs to rear high circle, while the fruit of 'East elite', 'Julius', 'Black lamp' and 'Sugar' are oblate in shape.

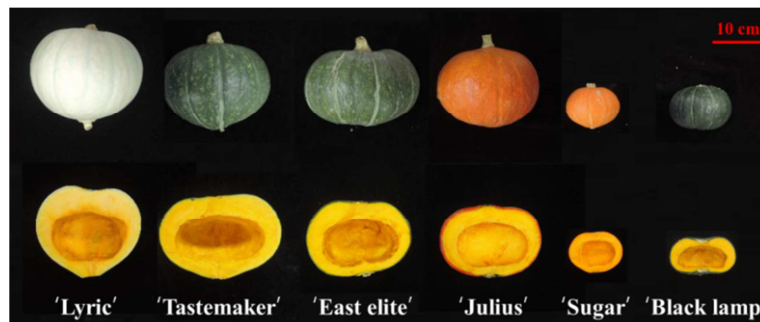


Figure 3. Images of fruit of the six cultivars of winter squash.

Harvesting was carried out approximately 90 days after transplant. The plant and fruit traits are presented in Table 2. Under the same cultivation height, there were significant differences in the number of nodes among varieties. 'Black lamp' and 'Julius' had significantly greater numbers of nodes, at 31.6 and 30.2, followed by 'East elite', 'Julius' and 'Lyric', at 22–25.4 nodes. The node length decreased as the number of nodes increased; that of 'Black lamp' and 'Sugar' was shortest at 10.2 and 9.9 cm, respectively, and the node length of the other varieties was between 12.8 and 13.6 cm. Regarding the total leaf area, 'East elite' had the highest area, followed by 'Black lamp' and 'Julius' at second, 'Tastemaker' and 'Lyric' at third, and 'Sugar' had the lowest area at 25406, 23639,

22655, 20599, 20286 and 16988 cm^2 , respectively. Based on single leaf area, varieties can be divided into four groups: 'Tastemaker' and 'Julius' had the greatest area, second was 'Lyric', third was 'East elite' and 'Black lamp', and 'Sugar' had the lowest area, at 1046, 994, 920, 808, 748 and 560 cm^2 , respectively. With regards to the first female flowering day, 'East elite' and 'Black lamp' exhibited their first female flowering about 30 days after transplant, while the other varieties required around 35–37 days. Regarding the number of fruit per plant, 'Black lamp' had a significantly higher fruit number than the second-highest, 'Sugar', at 3 and 2 fruit per plant, respectively; there were no significant differences between the other varieties, at 1.0–1.2 fruit per plant.

Table 2. Vegetative and reproductive growth of the six cultivars of winter squash.

| | Leaf area cm^2/plant | Node no. | Internode length cm | Days to flowering days | Number of fruits No./plant |
|------------|---|-------------|------------------------|---------------------------|-------------------------------|
| Black lamp | 23639 ab | 31.6 a | 9.9 b | 30 b | 3.0 a |
| Sugar | 16988 c | 30.2 a | 10.2 b | 36 a | 2.0 b |
| Julius | 22655 ab | 22.8 b | 13.6 a | 37 a | 1.1 bc |
| Tastemaker | 20599 bc | 25.4 b | 12.8 a | 30 b | 1.0 c |
| East elite | 25406 a | 23.5 b | 13.0 a | 35 a | 1.2 bc |
| Lyric | 20286 bc | 22.0 b | 13.6 a | 37 a | 1.0 c |

^aMeans with the same letters in a column were not significantly different according to Fisher's least significant difference (LSD) test at the 5% level.

Fruit quality parameters, such as fruit weight, pulp rate and inclusions, are presented in Table 3. With regards to fruit weight, 'Lyric' was of the heaviest weight, second were 'East elite' and 'Tastemaker', third was 'Julius', and 'Sugar' and 'Black lamp' were lightest, with fruit weights of 1752, 1428, 1413, 1032, 359 and 312 g, respectively. The six varieties could be divided into three categories based on fruit weight: 'Black lamp' and 'Sugar' are small fruit, with a fruit weight of 312–359 g; 'Tastemaker', 'East elite' and 'Julius' are medium fruit, with a fruit weight of 1032–1428 g; while 'Lyric' is a large fruit, with a fruit weight of 1752 g.

In order to assess the edible quality of the six varieties, the soluble sugar and starch contents were measures as important factors related to the quality of the fruit (Table 3). 'East elite' had the highest soluble sugar content at 630.6 g/kg, followed by 'Black lamp', 'Sugar', 'Tastemaker' and 'Julius', at 385.7–513.3 g/kg; while 'Lyric' had the lowest content at 326.8 g/kg. Regarding starch content, 'Lyric' had the highest starch content

at 193.7 g/kg, followed by 'Tastemaker', 'Sugar', 'Black lamp' and 'East elite', and 'Julius' had the lowest starch content. The crude fiber content can be used as a reference for dietary fiber: the higher the crude fiber content, the higher the nutritional value. 'Sugar' had the highest crude fiber content at 7.31%, followed by 'East elite' and 'Julius', at 6.1–6.17%, while 'Black lamp', 'Lyric' and 'Tastemaker' had significantly lower contents than 'Sugar', at 4.76–5.28%. With regards to the total soluble solid content and dry matter rate of the six varieties. 'East elite' had the highest total soluble solid content and 'Lyric' the lowest, at 14.6% and 8.3%, respectively. Regarding dry matter rate, from high to low, the six varieties can be divided into three groups: the first included 'Tastemaker' and 'Black lamp', with the highest dry matter rates at 24.2% and 23.8%, respectively; in the second group were 'Lyric' and 'East elite', at 21.1% and 20.0%, respectively; while the third group consisted of 'Julius' and 'Sugar', at 15.6% and 15.4%, respectively.

Table 3. Fruit characteristics of the six cultivars of winter squash.

| | Fruit weight | PRV | TSS | Dry matter | Soluble sugar | Starch |
|------------|--------------------|---------|--------|------------|---------------|---------|
| | g | % | °Brix | % | g/kg DW | g/kg DW |
| Black lamp | 312 d ^z | 81.5 ab | 13.3 b | 23.8 a | 513.3 b | 61.7 c |
| Sugar | 359 d | 75.7 c | 12.4 b | 15.4 c | 482.5 c | 64.4 c |
| Julius | 1032 c | 80.3 b | 10.9 c | 15.6 c | 385.7 e | 54.9 c |
| Tastemaker | 1413 bc | 84.8 a | 12.3 b | 24.2 a | 630.6 a | 59.9 c |
| East elite | 1428 b | 74.5 c | 14.6 a | 19.9 b | 452.2 d | 118.2 b |
| Lyric | 1752 a | 81.5 ab | 8.25 d | 21.1 b | 326.8 f | 193.7 a |

^zMeans with the same letters in a column were not significantly different according to Fisher's least significant difference (LSD) test at the 5% level. PRV, pulp rate of volume; TSS, total soluble solid.

A comparison of production efficiency between the varieties according to fruit yield and plant biomass was presented in Figure 4. Under vertical cultivation at the same planting density, the higher the yield per plant, the higher the yield per unit area. Among the cultivars, 'Lyric' and 'Tastemaker' had the highest yields, at 1.8 and 1.7 kg/plant, respectively, followed by 'East elite' and 'Julius', at 1.4 and 1.1 kg/plant, respectively, while 'Black lamp' and 'Sugar' had the lowest yields, at 0.9 and 0.7 kg/plant, respectively. The leaf area and fruit weight ratio demonstrated the relationship between total leaf area per plant and fruit yield,

and the results followed a pattern similar to that of the yield per plant. 'Lyric' was the highest at 0.90 kg/m², followed by 'East elite' and 'Tastemaker', at 0.69 and 0.67 kg/m², respectively, while 'Sugar' and 'Black lamp' were the lowest, at 0.42 and 0.40 kg/m², respectively. A comparison of the daily carbon fixation of the six varieties was shown in Figure 5. 'East elite' had the highest daily carbon fixation, and there were no significant differences between 'East elite', 'Tastemaker' and 'Lyric', while 'Sugar' had a significantly lower daily carbon fixation.

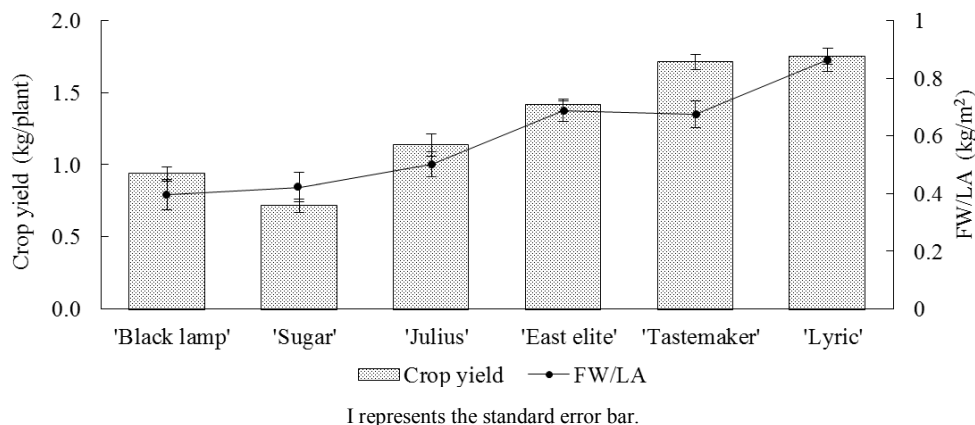


Figure 4. Crop yield and leaf area to fruit weight ratio (FW/LA) of the six cultivars of winter squash.

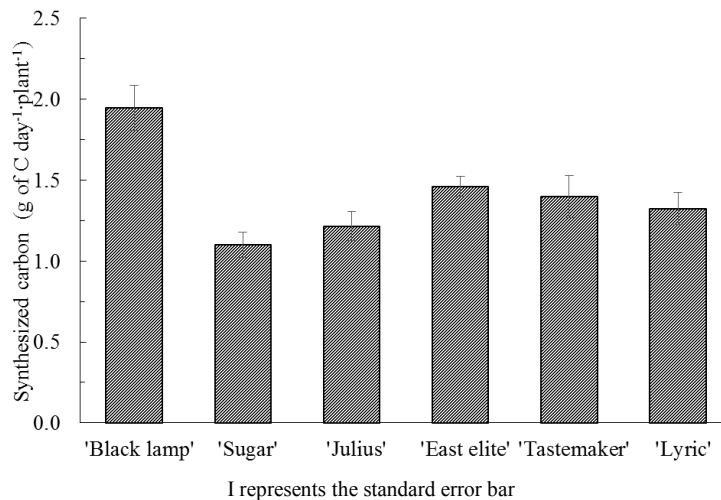


Figure 5. Daily synthesized carbon of the six cultivars of winter squash.

4. Discussion

4.1. Vegetative Characteristics of Six Squash Cultivars

On the 70th day of planting, the lower leaves of the plants were aged and yellow, and the differences in photosynthetic activity at each node of the plants were investigated (Figure 2). Leaves at the base of the internodes were shaded by leaves above, and leaf photosynthesis was compared. The rate of photosynthesis of the base leaves was significantly lower than of the top leaves, and as the light intensity increased, the photosynthetic rate of the older leaves did not increase, resulting in a lesser contribution to growth and a poorer overall production efficiency of the plant. These results showed that leaves from lower areas of the plant contributed less to fruit development, and therefore should be removed before they become old (approximately 15~20 days after pollination) in order to reduce pests and diseases, while increasing the air circulation between individual plants. Mature leaves, as compared with old leaves, had a better carbon-assimilating ability under same lighting conditions, and were therefore more beneficial for fruit production if located at a fruiting node of the plant [14]. Thinning of leaves of Chinese squash plants by 25% during female flower anthesis has been shown to improve the photosynthetic rate of leaves near fruiting nodes and increase the fruit dry weight [13].

Diurnal variation in the photosynthetic rate of the winter squash was shown as a single peak; the six squash varieties in this experiment reached a photosynthetic peak at around 9~10 am (Figure 1), the rate then decreasing rapidly after 11am to 39~78% by around 11~12 pm. The results indicated that severe midday depression of photosynthesis (MDP) was present in this experiment, and no sign of recovery was observed in the afternoon. However, the study of Liu et. al. reported no sign of MDP in squash seedlings in their experiment, the photosynthetic rate reaching its highest peak at noon on sunny days. This difference in photosynthetic rate could be due to differences in temperature at the leaves, as the temperature change in the leaves was in the range of 16~28°C in the experiment of Liu et. al, while the temperature change in the

leaves in our experiment was higher, the temperature reaching 38°C at 12 pm. In these two experiments, the photosynthetic rate decreased rapidly when the leaf temperature was highest, and the leaf temperatures of seedlings and mature plants during the photosynthetic rate peak were 28.1°C and 38.3°C, respectively. Therefore, leaves on mature plants were speculated to have a better ability to maintain normal photosynthetic activity under higher temperatures.

The contribution of unit area per leaf to fruit weight was demonstrated by FW/LA (Figure 4), and varietal difference was contributed to by leaf assimilating ability, nutrient reserving ability, fruit weight and composition. Compared with the leaf-to-fruit ratio, FW/LA is a better calculation method by which to represent the leaf contribution to fruit production. Among the different varieties in the experiment, the yield per plant had a similar trend to FW/LA, with the green-peel, large-fruit variety having a higher yield than small-fruit varieties. This showed that fruit weight was more accurate than number of fruit in terms of assessing the yield of winter squash, which was in agreement with previous results of cytokinin experiments in peach [10] and apple plants [11]. However, the assimilated production per plant in green-peel, small-fruit variety 'Black lamp' was significantly higher than that of other varieties (Figure 5). Although the assimilate production did not have a direct effect on the yield, the soluble solids and dry matter rate of the fruit were significantly higher, which showed that the assimilate production of the plant had more effect on the qualitative characteristics of the fruit than the quantitative characteristics.

4.2. Reproductive Characteristics of Six Cultivars

After 82 days of planting in facilities, the six varieties in the experiment were sorted based on the reproductive state of the plant. In terms of flowering days, 'Black lamp' and 'East elite' were early-flowering and early-production varieties. Sorting the varieties based on production cumulation type, 'Black lamp' and 'Sugar' were high-fruit-number varieties, in which increasing fruit numbers was an important factor in order to increase the fruit yield; whereas 'Julius', 'East elite',

'Tastemaker' and 'Lyric' were high-fruit-weight varieties, in which an increase in fruit weight results in an increased fruit yield per plant. In addition, during the vegetative stage of high-fruit-number plants, an increased number of leaves was an important factor affecting the increase in leaf total area, and the increased leaf total area was associated with an increase in the area per leaf (Tables 2 and 3).

Total soluble solids content in winter squash is an important classification criterion. 'Black lamp' and 'East elite' had the highest amounts of soluble solids in this experiment, and thus were better-tasting varieties. Culpepper and Moon analyzed fruits of 36 pumpkin varieties, and found that the total soluble solids content of the pumpkin ranges from 6.4-15.7%. With a total soluble solids content of 10% as the threshold for culinary and processing varieties, all squash in this experiment, with the exception of 'Lyric', with a TSS of 8.25%, met the standard for culinary varieties. Using flesh percentage and soluble solids content as fruit quality standards, of the six varieties, 'Tastemaker', 'Black lamp', 'Sugar' and 'East elite' had significantly higher soluble solids contents (Table 3), suitable for classification as culinary varieties, while 'Lyric' had a higher yield per plant and a greater flesh percentage, and was more suitable for classification as a processing variety.

Dry matter percentage is an important factor contributing to fruit texture. 'Julius' and 'Sugar' had lower dry matter percentages, and hence a moister and mushier flesh texture, while 'East elite' and 'Black lamp' had soft but not mushy flesh, with dry matter percentages of about 20%. Zhou et al. identified that Chinese squash had a dry matter percentages of around 10-16% [15]. In addition, the starch content in the flesh also affected the flesh texture (Table 3). 'Tastemaker' had the highest soluble sugar content, while 'Lyric' had the significantly highest starch content. Although flesh texture cannot be interpreted from the data, 'Tastemaker' had sweeter flesh, and 'Lyric' had flesh that was silkier in texture. Cumarasamy et. al. used cooked Kabocha squash in a taste test to assess its sweetness and texture, and compared its physicochemical properties, and found that dry matter weight and starch content were highly-correlated with fruit quality, while sweetness was correlated with the total sugar content in the fruit.

5. Conclusion

"Black Light" was a fruit-number type, and the increase in fruit number could increase the yield per plant; "East elite" was a fruit-weight type, and the yield per plant is related to the weight of a single fruit. The photosynthetic capacity of the two varieties was similar, but there was no same trend between nodes, which should be affected by different fruit number. The "Black Lamp" produced photosynthetic carbon every day significantly the highest among six varieties. However, if the fruit fresh weight was considered, the affect of the unit leaf of "Black Lamp" on the fruit fresh weight was the lowest performance in the reproductive growth. For, increasing productivity of small fruit such as 'Black lamp' western pumpkin, the balance between the vegetative growth and the reproductive growth was necessary to be improved.

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