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# Analysis of growth differences between diploid and tetraploid seedlings of *Anthurium andraeanum*

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## Abstract

To clarify the growth differences between diploid and tetraploid *Anthurium andraeanum* 'Pink Champion' seedlings, their leaves, stomata, petioles, roots, root hairs, and anatomy were compared. The results showed that tetraploids had reduced leaf length, petiole length, and plant height but increased leaf width, petiole width, and stomatal length, with thicker leaf structures respectively. Tetraploid petioles, leaves, and roots had larger cross-sections and cell volumes. Root traits varied; tetraploids had decreased root number, total root length, and root hair growth rate, but increased root diameter, root area, root hair diameter, and root activity, along with higher root fresh weight and root-shoot ratio. Diploids were slender and tall, tetraploids robust and short. These indicators aided preliminary screening and cultivar selection.

[Keywords] *Anthurium andraeanum*, diploid, tetraploid, growth difference, anatomical structure

## 1. Introduction

*Anthurium andraeanum* Linden is an important ornamental plant (Lin et al., 2022). It has become a world-class flower suitable for cut flowers and potted plants due to its beautiful color and high economic value (Zhang et al., 2021a; Evelyn et al., 2023). Annual production and sales volume of *A. andraeanum* has reached more than 30 million pots, and it is increasing year by year (Mori et al., 2021). The main variety of *A. andraeanum* circulating in the market is diploids. Compared with diploids, polyploids typically produce more desirable characteristics. Polyploid breeding can improve the ornamental value of *A. andraeanum*, strengthen stress resistance of plants, increase genetic diversity (Zhang et al., 2021b; Huy et al., 2019), and bring more new vitality and opportunities to the *A. andraeanum* industry. However, polyploidy *A. andraeanum* is extremely rare, and it is urgent to breed more tetraploid anthuriums. Morphological differences in *A. andraeanum* are beneficial for the selection of polyploid breeding (Zhang et al., 2023). Understanding its morphological characteristics is the primary prerequisite for cultivating polyploid *A. andraeanum*.

Different polyploid plants have different growth differences. Compared with diploid plants, polyploidy plants have greater ornamental value and stress resistance (Corneillie et al., 2019; Zlonis et al., 2019; Zhang et al., 2020). For example, the flower organs of tetraploid *Hydrangea macrophylla* became enormous (Tränkner et al., 2020). The flowering period of polyploid *Erysimum cheiri* was extended (Fakhrzad et al., 2023) to increase its economic benefits. The thick and dwarf stems of polyploids also improve the lodging stress characteristics (Ardabili et al., 2021). In polyploid Orchids, it is prone to leaf thickening and widening, root diameter increase, stem thickening, and slow growth (Zhang et al., 2021b). Slow growth was also found in polyploids such as *Forsythia suspense* (Yang, 2024), *Echinacea purpurea* (Li et al., 2024), and *Phalaenopsis Aphrodite* (Wu et al., 2021). Polyploids have more excellent characteristics such as thick leaves and stems, rich leaf colors, big flowers, long flowering periods, strong stress resistance, and better ornamental value than diploids. Therefore, studying the differences in traits of polyploid plants is an important basis for the breeding of new varieties.

Researches on ploidies of *A. andraeanum* mainly focus on two aspects: one is the induction and growth of tissue culture seedlings and the other is the differences in the number of flower stalks, leaves, and petioles of adult plants. For example, tetraploid 'Arizona' (Chen et al., 2011) and 'Cherry Red' (Leng et al., 2014) have been studied. The research on root growth, root hair development, and anatomical characteristics of vegetative organs of *A. andraeanum* with different ploidies is little. To further explore the potential of Anthurium, taking the tetraploid (4 n = 4x = 60) and diploid (2 n = 2x = 30) seedlings of 'Pink Champion' as the experimental materials, the differences in plant characteristics and anatomy are compared to obtain the basic data on the characteristic differences of polyploid *A. andraeanum* seedlings, laying a theoretical

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foundation for polyploid breeding.

## 2. Materials and methods

### 2.1. Cultivation of 'Pink Champion' seedlings

After multiple preliminary experiments, 2.0 g $L^{-1}$ colchicine was selected for 6-hour treatment, and then transferred to the MS + 6-BA 2.0 mg $L^{-1}$ + NAA 0.5 mg $L^{-1}$ culture medium. After 75 d, a new high-quality tetraploid of 'Pink Champion' was obtained. Then, the diploid (2 n = 2x = 30) and homologoustetraploid (4 n = 4x = 60) that developed into 'Pink Champion' seedlings showed vigorous growth consistency, without pests or diseases. Clustered buds of 2 n and 4 n with growth consistency were selected and then transferred into the rooting medium under aseptic environment, 1/2 MS + NAA 0.2 mg $L^{-1}$ + sugar 30 g $L^{-1}$ + phytagel 4.5 g $L^{-1}$. Six clustered buds were transferred into each bottle of the medium and repeated this process 6 or more times. The pH of the medium was 5.8. After transfer, the cultures were placed in an environment with a temperature of 25 °C and a light intensity of 2,000 lx, cultured for 90 d before use.

### 2.2. Measurements of the main characteristics of the seedlings

The second to third leaves (counting from bottom to top) of 2 n and 4 n 'Pink Champion' seedlings were randomly selected. A vernier caliper was used to measure leaf length, leaf width, petiole length, petiole width, and plant height. The leaf area was scanned and measured with a root scanner Scan Maker i85 (Zhejiang Top Cloud-Agri Technology Co., Ltd., China). Three plants were randomly picked from each bottle for one replicate, with six or more replicates, and the same below.

After washing the root samples of *A. andraeanum*, absorbent paper was used to dry them, and the roots of similar length and thickness were selected from each group. The root scanner Scan Maker i85, scanning software Microtek Scan Wizard Pro V9.00, and its root phenotype analysis system X64 were used to scan and count root development characteristics such as the number of roots, total root area, and root length.

### 2.3. Measurements of root hair growth and root activity of the seedlings differences

The UY203i fluorescence microscope (Chongqing UOP Photoelectric Technology Co. Ltd., China) was used to observe and count the root hair growth rate per hour, as well as take pictures of the diameters of root hairs every 6 h. In addition, root activity was measured using the TTC method (Peng et al., 2025).

$$T_{rs} = \frac{T_r}{W_{fr} \times T} \quad (1)$$

Where $T_{rs}$ is the TTC reduction strength (μg·$g^{-1}$·$h^{-1}$), $T_r$ is the TTC reduction (μg), $W_{fr}$ is the fresh root weight (g), and $T$ is the time (h).

### 2.4. Anatomical observe of leaves, petioles, and roots of the seedlings

Based on the methods of Livingston et al. (2019) (with minor modifications), Safranin-O/Fast Green paraffin sections of the leaves, petioles, and roots of 2 n and 4 n 'Pink Champion' seedlings were made. The finished paraffin sections were observed under the UY203i fluorescence microscope. Ten fields of view were randomly selected for observation. Six different positions were selected in each field of view to measure parameters such as the thickness of the upper and lower epidermis, spongy tissue and palisade tissue. The lower epidermis of the leaves of 2 n and 4 n *A. andraeanum* seedlings was selected. After removing the dirt on it, colorless nail polish was applied (avoiding the midrib). After the nail polish was dried into a film, it was removed with pointed tweezers and placed on the glass slide, with the side in contact with the lower epidermis facing downward. The guard cell density was calculated after taking pictures, and then the stomata length (the length of opened stomata pores) and stomata width (the width of opened stomata pores) were measured. About 50 stomata were randomly selected in each field of view.

$$D_{gs} = \frac{N_s}{A_{fv}} \quad (2)$$

Where $D_{gs}$ is the guard cell density ($mm^{-2}$), $N_s$ is the number of stomata (-), $A_{fv}$ is the field of view area ($mm^2$).

### 2.5. Measurements in dry matter accumulation in seedlings

Dry matter accumulation was measured by the method of Han et al. (2017). 4 n and 2 n seedlings of 'Pink Champion' were selected. After removing surface moisture and dirt, the fresh weights of the shoot and underground were recorded. Then they were placed in an oven at 105 °C for 30 min. When the temperature decreased to 80 °C, they were dried to a constant weight to measure and calculate the dry matter distribution ratios.

$$DM_s = \frac{W_{ds}}{W_{dw}} \times 100 \quad (3)$$

$$DM_u = \frac{W_{du}}{W_{dw}} \times 100 \quad (4)$$

Where $DM_s$ is the percentage distribution of the shoot dry matter (%), $W_{ds}$ is the dry weight of the shoot (g), $W_{dw}$ is the dry weight of the whole plant (g), $DM_u$ is the percentage distribution of the shoot dry matter (%), and $W_{du}$ is the dry weight of the shoot (g).

### 2.6. Data Statistics

All data were replicated six times or more. The data was collected and recorded using Excel, and the IBM SPSS Statistics software version 20.0 was used for data statistical analysis. The average was expressed as mean ± SD, and '$p \leq 0.05$' indicated a significant difference.

## 3. Results and analysis

### 3.1. Differences of the main morphological characteristics of the seedlings

According to Fig. 1 and Table 1, significant changes occurred in the leaf shape, color, and petiole characteristics of 'Pink Champion' seedlings. Compared with 2 n 'Pink Champion', 4 n 'Pink Champion' had flatter leaf shapes, shorter leaf length, and slightly darker leaf colors (Fig. 1 (a)). The petioles were thicker and shorter (Fig. 1 (b)). There was no significant difference in the leaf area between 4 n and 2 n 'Pink Champion' seedlings. The leaf length, petiole length, and plant height of 4 n 'Pink Champion' seedlings were significantly shorter than 2 n, decreased by 11.83 %, 28.68 % and 11.11 % respectively. The petiole width and leaf width increased by 10.00 % and 3.48 %, respectively, and the differences were significant.

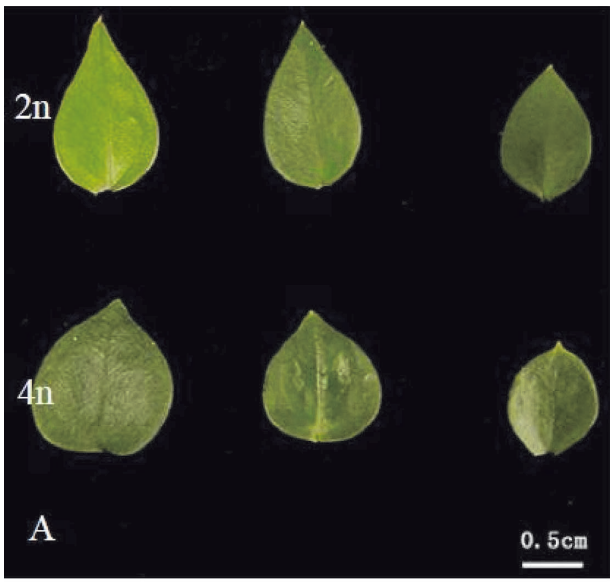
Besides, there were differences in the root morphological characteristics. Its roots were shorter and thicker (Fig. 2 (b)). The number of roots and total root length decreased significantly by 21.79 % and 20.22 % respectively, reducing the range of nutrient absorption. However, root diameter and area increased by 58.76 % and 40.84 % compared to 2 n, increasing the surface area of nutrient absorption. 4 n 'Pink Champion' had obvious root and root hair thickening, while the root number and length growth was inhibited (Table2).

### 3.2. Analysis of root hair growth and root activity of the seedlings differences

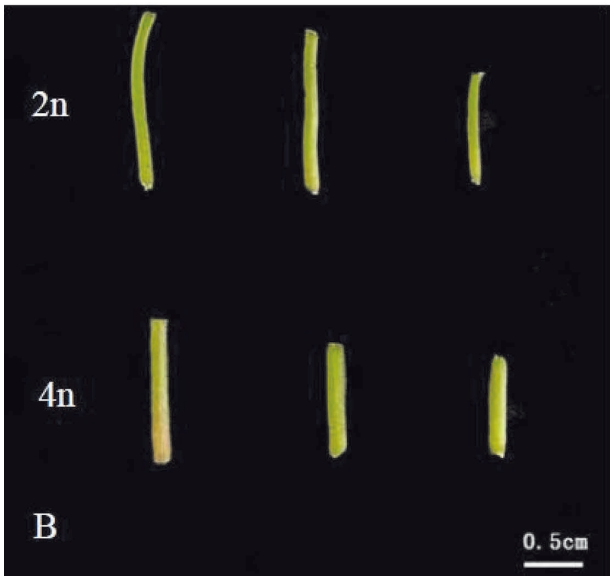
According to Fig. 3 and Table 3, the root hair diameter of 4 n was thicker than 2 n, with an increase of 21.85 %. The thickening effect of the root hairs of 4 n 'Pink Champion' was stronger (Figs. 3 (b) B and B1). In addition, measurements of root hair growth rate and root activity of the two types showed that the root hair growth rate of 4 n was significantly reduced by 25.12 % compared with 2 n, indicating slow root hair growth. indicating slow root hair growth. The root activity of 4 n significantly increased by 16.59 %, which helps to improve the growth ability of plants.

### 3.3. Anatomical differences of leaves, petioles, and roots of the seedlings

As shown in Table 4 and Figs. 4 and 5, the stomatal, leaf,




(a) Shoot leaves




(b) Petioles

The shoot leaves (A) and petioles (B) of diploid and tetraploid 'Pink Champion' are upper, middle and lower from left to right.

Fig. 1 Comparison of leaves and petioles differences on shoot between diploid and tetraploid 'Pink Champion'

Table 1 Comparison of main phenotyping characteristics on shoot between diploid and tetraploid 'Pink Champion'

| Ploidy level | Plant height (cm) | Petiole | | Leaf | | |
|---|---|---|---|---|---|---|
| | | Petiole length (cm) | Petiole width (cm) | Leaf length (cm) | Leaf width (cm) | Leaf area ($cm^2$) |
| Diploid 'Pink Champion' | 0.54 ± 0.09 [a] | 1.29 ± 0.11 [a] | 0.10 ± 0.01 [b] | 1.69 ± 0.16 [a] | 1.15 ± 0.12 [b] | 5.80 ± 2.24 [a] |
| Tetraploid 'Pink Champion' | 0.48 ± 0.11 [b] | 0.92 ± 0.14 [b] | 0.11 ± 0.01 [a] | 1.49 ± 0.23 [b] | 1.19 ± 0.11 [a] | 4.89 ± 0.99 [a] |

Number in the table is mean ± standard deviation.
Different letters in the same column meant significant difference at 0.05 level.
These are same for below tables.




(a) Roots of diploid




(b) Roots of tetraploid

The diploid (A) and tetraploid seedlings (B) of *Anthurium andraeanum* 'Pink Champion'.

Fig. 2 Observation on the roots of diploid (a) and tetraploid (b) 'Pink Champion'

petiole and root structures between 4 n and 2 n 'Pink Champion' is significantly different. The stomata of 2 n are oblong, and that of 4 n are round (Fig. 4 (b)). The density of guard cell and transverse diameter between 4 n and 2 n seedlings had no significant differences, but the stoma length of 4 n increased by 31.52 %. The leaves of 4 n 'Pink Champion' (Fig. 5 (e) (f)) are thicker, with larger cross-sectional areas of petioles and roots (Fig. 5 (a)–(d)), which increases material transport channels and cell volumes. Specifically, the thicknesses of leaf, upper epidermis, lower epidermis, palisade tissue and spongy tissue of 4 n were respectively 23.66, 9.00, 13.75, 14.65 and 41.89 % higher than those of 2 n. Thicker leaves can help increase photosynthetic pigment content, improve carbon dioxide fixation, and enhance light protection.

### 3.4. Differences in dry matter accumulation in seedlings

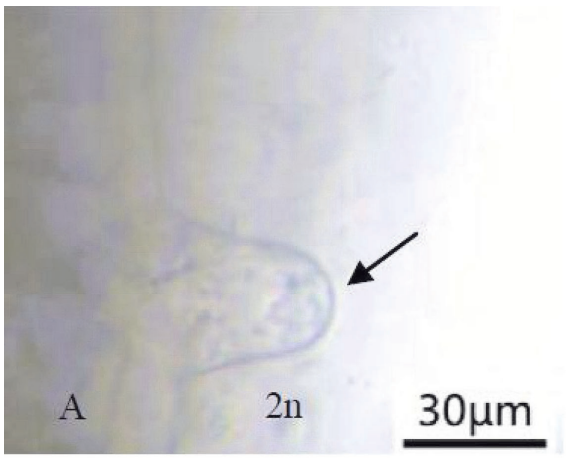
There is a difference in dry matter accumulation between 2 n and 4 n 'Pink Champion' (Table 5). Notable disparities were observed between 4 n and 2 n 'Pink Champion' in the fresh weight of roots and the root-shoot ratio. Compared with 2 n, the accumulation of fresh weight of 4 n 'Pink Champion' roots significantly increased by 21.05 %. The root-shoot ratio of 4 n 'Pink Champion' was 0.46 times that of 2 n 'Pink Champion', indicating a significant decrease. However, there was no significant difference in dry weight accumulation and dry matter distribution ratio of 4 n 'Pink Champion'.

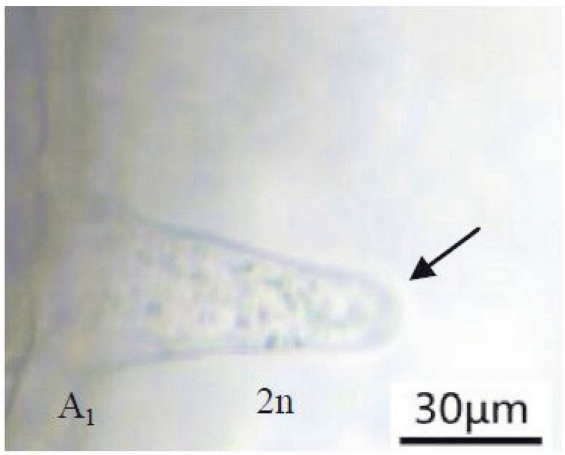
## 4. Discussion

Polyploid breeding refers to the cultivation of excellent plants through the doubling of chromosome sets. Currently, the direction of polyploid breeding of flowers mainly focuses on improving the economic benefits of crops, enhancing ornamental value, and increasing resistance. For example, tetraploid lily had more resistant to Botrytis cinerea than diploid (Wang et al., 2022), with larger flowers and stronger ornamental value (Yamagishi et al., 2023). The morphological characteristics of plants were a basic method for distinguishing polyploid plants (Bora et al., 2023) and a direct source of obtaining information on the characteristics. According to the studies of Zhang et al. (2021a; 2021b; 2024), the biological mechanisms of morphological differences in polyploids were mainly originated from the differences in gene dosage. In polyploids, an increase in gene copy number led to changes in the expression levels of certain genes related to plant development. This change would directly affect the synthesis of hormones and regulatory proteins, as

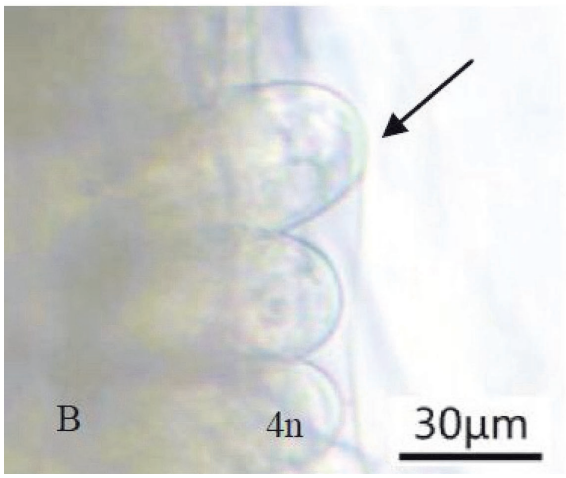
Table 2 Comparison of main phenotyping characteristics on root between diploid and tetraploid 'Pink Champion'

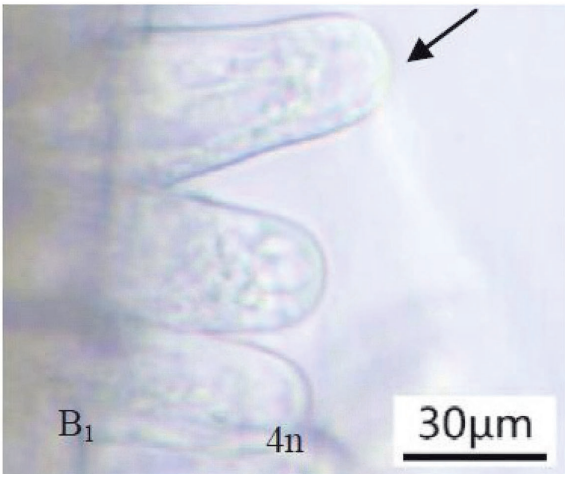
| Ploidy level | Number of roots per tissue culture plantlet | Root total length (cm) | Root diameter (mm) | Root area ($mm^2$) |
|---|---|---|---|---|
| Diploid 'Pink Champion' | 7.80 ± 2.20 [a] | 12.02 ± 1.39 [a] | 0.97 ± 0.10 [b] | 338.07 ± 95.04 [b] |
| Tetraploid 'Pink Champion' | 6.10 ± 1.52 [b] | 9.59 ± 2.01 [b] | 1.54 ± 0.25 [a] | 476.14 ± 164.81 [a] |




(a) Growth of diploid




(b) Growth of tetraploid

The diploid (A and $A_1$) and tetraploid seedlings (B and $B_1$) of *Anthurium andraeanum* 'Pink Champion' following six hours of continuous growth.

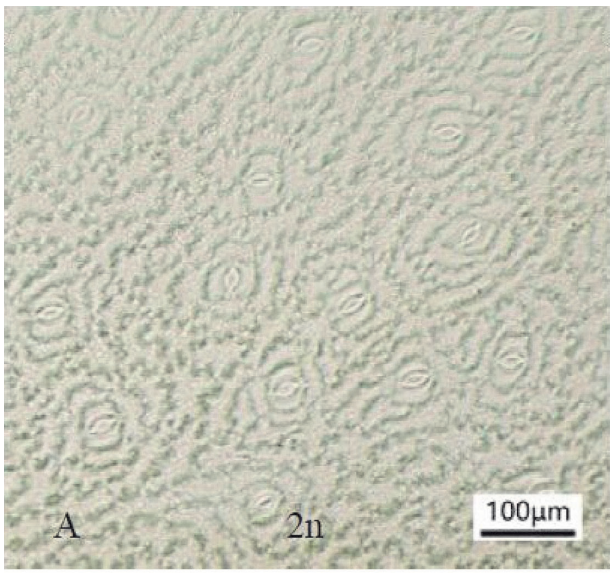
Fig. 3 Observation on the growth of diploid (A and $A_1$) and tetraploid (B and $B_1$) 'Pink Champion' root hairs

Table 3 Measurements of root activity and growth rate of roots on shoot between diploid and tetraploid 'Pink Champion'

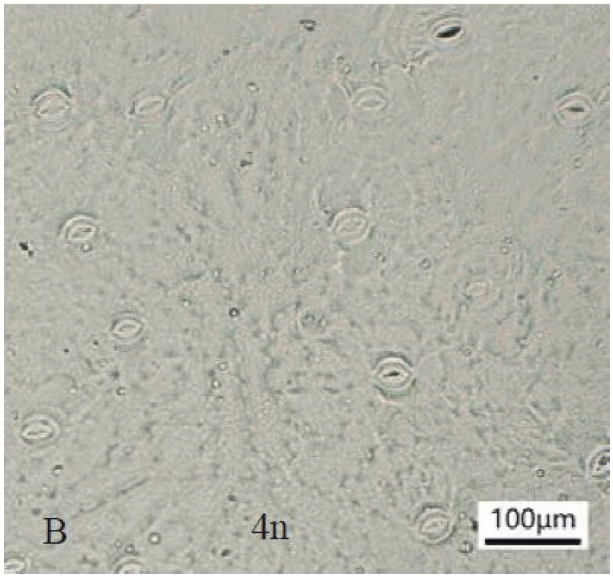
| Ploidy level | Root hair diameter (μm) | Growth rate of root hair (μm/h) | Root activity (μg/g·FW/h) |
|---|---|---|---|
| Diploid 'Pink Champion' | 27.18 ± 4.39 [b] | 5.97 ± 1.82 [a] | 229.17 ± 4.03 [b] |
| Tetraploid 'Pink Champion' | 33.12 ± 3.14 [a] | 4.47 ± 1.23 [b] | 267.20 ± 20.84 [a] |

Table 4 Comparison of leaf and stomata characteristics on shoot between diploid and tetraploid 'Pink Champion'

| Ploidy level | Leaf | | | | | Stomata | | |
|---|---|---|---|---|---|---|---|---|
| | Leaf thickness (μm) | Upper epidermis thickness (μm) | Under epidermis thickness (μm) | Palisade tissue thickness (μm) | Sponger tissue thickness (μm) | Stomatal frequency (pcs/mm$^2$) | Stomata length (μm) | Stomata width (μm) |
| Diploid 'Pink Champion | 230.21 ± 6.55 [b] | 40.58 ± 5.79 [b] | 38.12 ± 4.83 [b] | 65.44 ± 9.25 [b] | 86.07 ± 13.78 [b] | 51.83 ± 9.06 [a] | 30.14 ± 3.02 [b] | 5.01 ± 0.95 [a] |
| Tetraploid 'Pink Champion' | 284.67 ± 16.92 [a] | 44.16 ± 6.55 [a] | 43.36 ± 5.88 [a] | 75.03 ± 12.41 [a] | 122.12 ± 8.12 [a] | 45.17 ± 7.03 [b] | 39.64 ± 4.57 [a] | 5.18 ± 1.04 [a] |




(a) Defense cells of diploid



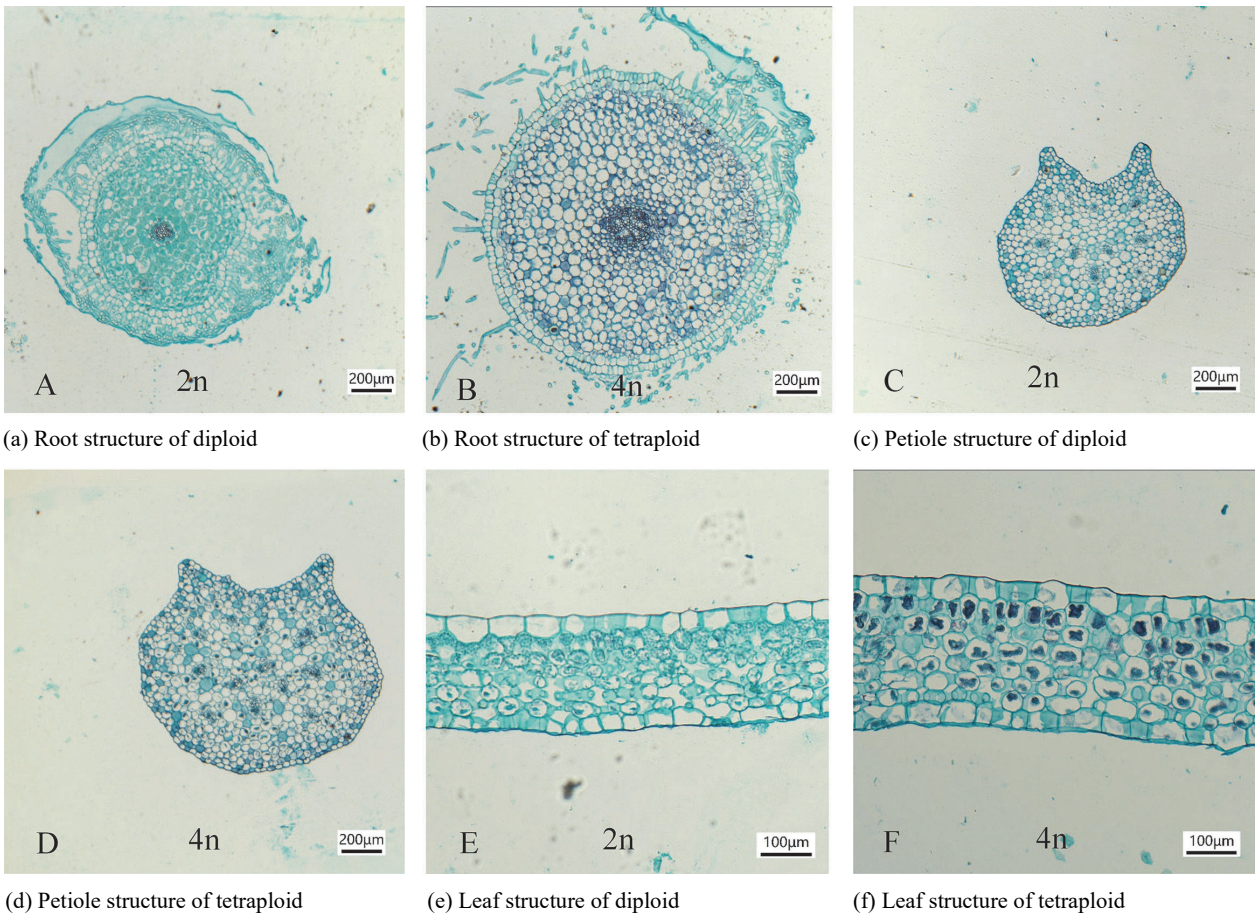

(b) Defense cells of tetraploid

Fig. 4 Observations on the defense cells of diploid (a) and tetraploid (b) 'Pink Champion'

well as the growth and morphology of plants. For example, Han et al. (2017) pointed out that the enlargement of organs may be mainly caused by the up-regulation of genes related to "cell growth". It should be noted that the enormous size of polyploid organs is due to cell enlargement, rather than an increase in the number of cells. A larger cell volume may lead to changes in the sizes of various organs, including leaves, petioles, roots, and floral organs.

The tissue structure and size of leaves would directly affect the photosynthetic rate of plants. In polyploids, thicker leaves resulting from increased cell thickness may be able to accommodate more chloroplasts (Sander et al., 2019) and improve the photosynthetic rate. For example, the leaf length and width of the tetraploid *Carexleucochlora* 'Four Seasons' are relatively large (Nie et al., 2021). The leaf width of tetraploid *Limonium sinuatum* (L.) Mill. increases (Mori et al., 2021). Similar changes in leaf shapes were also observed in polyploid plants such as *chrysanthemums* (Hoang et al., 2020), poppies (Ardabili et al., 2021), and *Iris* × *norrisii* (Ding et al., 2023). In this study, the leaf and petiole length of tetraploid *A. andraeanum* seedlings were shortened, and the leaf shape was flatter (Fig. 1). This may be that the cells may not elongate excessively. The dwarfing of plant, and the shortening and thickening of petioles are beneficial for improving the lodging resistance and ornamental value of plants, making polyploidy *A. andraeanum* more aesthetically appealing. Due to an increase in cell volumes, the thickness of the upper and lower epidermal cells (single cells) of the tetraploid was great. This change is beneficial for enhancing the protection of chloroplast photosynthetic structure. Subsequently, the thicknesses of the palisade tissue and spongy tissue were measured, and it was found that tetraploids significantly increased (Livingston et al., 2019). This is beneficial for changing the orientation of chloroplasts and reducing transmission loss of photon (Lie et al., 2021), thereby improving the photosynthetic efficiency. In addition, the increase in leaf thickness could also effectively prevent locusts from gnawing on the plants (Chapuis et al., 2023). The leaf thickening was beneficial for enhancing the resistance to harsh environments (Ayaz et al., 2024).

The growth of plants is closely related to their roots. Many studies have shown that changing polyploid chromosome sets can increase root diameter and slow growth of roots (Li et al., 2024). This was confirmed that the number of roots and total root length of tetraploids decreased sharply by 21.79 % and 20.22 % respectively. The average root diameter increased by 58.76 % (Table 2). The thickening effect of roots and root hairs was enhanced, resulting in an increase in the total root area by 40.84 %. Fresh weight of the underground part (root) increased, and the root-shoot ratio decreased. This indicates that the ecological trend, vitality, and environmental adaptability of the root system of tetraploid 'Pink Champion' have been enhanced, and it could absorb more nutrients from the soil. The TTC indicator could also reflect the growth of plant roots and root activity (Xin et al., 2025). Root activity was correlated with the shoot (Chen et al., 2011). This study found that the root hair growth rate of tetraploid was significantly reduced by 27.14 %. However, the root activity increased significantly by 16.59 %, which improved the growth ability of the plants (Wu et al., 2021) and the root resistance. Overall, these changes in the roots of tetraploid anthurium seedlings have both advantages and disadvantages in terms of their ability to absorb water and nutrients. The positive effects brought about by the increase of root diameter, root area, and root hair diameter may to some extent compensate for the negative effects caused by the decrease in the number of root number, total root length, and




(a) Root structure of diploid (b) Root structure of tetraploid (c) Petiole structure of diploid

(d) Petiole structure of tetraploid (e) Leaf structure of diploid (f) Leaf structure of tetraploid

Fig. 5 Observations on the root (A and B), petiole (C and D) and leaf transverse section structure (E and F) of diploid and tetraploid 'Pink Champion'

Table 5 Differences in dry matter distribution between diploid and tetraploid 'Pink Champion'

| Ploidy level | Shoot | | | Root | | | Root/Shoot |
|---|---|---|---|---|---|---|---|
| | Fresh weight (g) | Dry weight (g) | Dry matter partitioning (%) | Fresh weight (g) | Dry weight (g) | Dry matter partitioning (%) | |
| Diploid 'Pink Champion' | 0.10 ± 0.04 [b] | 0.01 ± 0.01 [a] | 32.42 ± 15.67 [a] | 0.19 ± 0.05 [a] | 0.02 ± 0.00 [a] | 67.58 ± 15.67 [a] | 2.54 ± 1.25 [a] |
| Tetraploid 'Pink Champion' | 0.19 ± 0.06 [a] | 0.02 ± 0.01 [a] | 47.35 ± 7.38 [a] | 0.23 ± 0.07 [a] | 0.03 ± 0.01 [a] | 52.65 ± 7.38 [a] | 1.16 ± 0.33 [b] |

root hair growth rate. However, the specific degree of impact is also comprehensively restricted by various factors such as soil environment, and nutrient and water supply, and needs to be considered comprehensively.

Morphological characteristics are an important source of reflecting differences in polyploid plants and a reliable basis for the preliminary screening of polyploid plants. The preliminary screening can be accomplished by comparing the differences in leaves, stomata, petioles, root growth and root hair characteristics. Tetraploid seedlings of 'Pink Champion' have stronger environmental adaptability and are more robust than diploid seedlings. They also have high resistance and a strong photosynthetic rate. Therefore, after introduction and testing, the tetraploid 'Pink Champion' with high resistance (strong adaptability and high survival rate) could be applied to areas with poor soil quality for agricultural seedling cultivation or urban road greening. This is of great significance for improving the economic benefits of regional agricultural production, breeding new varieties, and promoting varieties. These characteristics endow tetraploid anthuriums with enormous application potential and value for application, and are expected to have a positive impact on the actual production and promotion of *A. andraeanum*.

## 5. Conclusions

There were significant differences between tetraploid and diploid seedlings of 'Pink Champion' in leaves, stomata, petioles, root growth, and root hairs. Compared with the diploid,

the tetraploid 'Pink Champion' has thicker petioles, elliptical leaf shapes, increased leaf thickness, and oblong stomata, with significant thickening of leaf tissues. Tetraploid petioles, leaves, and roots have larger cross-sections and cell volumes. They have thick roots and root hairs, slow root growth, decreased roots, and short root length, as well as enhanced root activity.

The differences between diploid and tetraploid are of great significance for the preliminary screening of polyploidy *andraeanum*, and provide more new germplasm resources for plant breeding.

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**Author's contributions**

Rongya Gong: Designed and performed the experiments, work.

Yongxu Qiao and Yongping Zhang: Designed experiments and provided technical guidance.

Jiayi Zhang, Jinxiu He, Lihong Gao: Participated to collect the materials related to the experiment.

**Declarations**

The authors declare that they have no competing financial interests or personal relationships that appear to influence this work. The corresponding author affirms that all of the authors have read and approved the manuscript.

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