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DE BUILDING OF TISSUE CULTURE SYSTEM AND POST-TRANSPLANT QUALITY COMPARISON OF 'BAIHUAYUSHIZI' POMEGRANATES (*PUNICA GRANATUM* L.)

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Abstract

The present study aimed to assess different clonal propagation methods, culture conditions and different exercising and transplanting conditions for tissue culture seedlings. Specific data, including the survival rates, growth indices, root scanning and physiological and biochemical indices of 1-year transplanted tissue culture seedlings and 1-year cutting seedlings of'Baihuayushizi'pomegranates, were collected for analysis. The present study aimed to provide theoretical and experimental bases for the production pattern and industrialized reproduction of high-quality seedlings of 'Baihuayushizi'pomegranates, so as to contribute in promoting the propagation paths of high-quality seedlings, increase the planting percentage of high-quality pomegranate varieties from Huaiyuan and improve the production efficiency of fruit farmers. The experimental results were as follows: (1) When stem tips were used as explants to build the tissue culture system, the optimal sterilization time period was 10min and required processing with 0.1% $HgCl_2$.The optimal inducing medium for proliferation of adventitious shoots was WPM+NAA 0.75 mg/l+6-BA 0.5 mg/l and the optimal inducing medium for rooting was WPM+IBA 0.8 mg/l. (2) The initial studies examining the transplanting conditions for seedling exercising demonstrated that the survival rate from seedling exercising was increased following the increase in the illumination intensity and humidity within certain limits. The survival rate was decreased when it reached the threshold, suggesting that the optimal illumination intensity for seedling exercising of 'Baihuayushizi'pomegranates was 1,000 LX and the optimal relative humidity for the transplant was 70%. The survival rate of the transplanted tissue culture seedlings may be significantly increased up to 85.39% if appropriate fungicides and matrix formulas are used during seedling exercising. (3) The results of the comparison of the growth, root length and physiological and biochemical indices between survived transplanted tissue culture seedlings of 'Baihuayushizi'pomegranates and cutting pomegranate seedlings in the same period indicated that tissue culture seedlings exhibited optimal root activity, growth and development activity compared with those of the cutting seedlings.In summary, the tissue culture of pomegranate seedlings can be used as a method for large-scale production and propagation of seedlings and is widely accepted by a broad scientific audience.

Key words: *Punica granatum* L.; Tissue culture; Physiological and biochemical indexes; Seedling quality.

Introduction

Pomegranates (*Punic agranatum* L.) (Bussmann *et al*., 2019) arefruits that offer substantial health benefits in various populations worldwide, whereas they providesignificantfinancial profits (Shahamirian *et al*., 2019) to several countries. They also possess high nutritional value (Bourekoua *et al*., 2018) and specific medicinal properties (Turrini *et al*., 2020). Pomegranates haverecently become popular in the global economic market (Asrey *et al*., 2020). 'Baihuayushizi' pomegranates, a fruit tree under *Punica* L., Pomegranate, is a high-quality variety of pomegranates mainly grown in Anhui Province (Qi & Qin, 2017). It is well known for its'white flowers, white fruits and white seeds'. 'Baihuayushizi' pomegranates from Huaiyuan represented by *Punica granatum* L. are popular in China and are sold to the UK, Romania, Philippines, Malaysia and other Southeast Asian countries, offering strong market competitiveness and high economic benefits. However, *Punica granatum* L. is now mainly propagated by harvesting from natural resources, which leads to variety degradation, lower seed setting rate and early aging of fruit trees.

Tissue culture is a seedling cultivation technology that clones a complete plant with part of its tissues or cells under aseptic conditions based on the principle of totipotency of plant cells in order to achieve high-quality new plants. It has obvious advantages: It significantly reduces the propagation cycle of plants, offers a high propagation coefficient, enables year-round production, speeds up propagation, enables uniform structure of the seedlings and is more suitable for the rapid propagation of varieties with a large number of seedlings. It can alsooffer clonal propagation for plants that cannot be propagated by seeds or are prone to lose their excellent characteristics following seed propagation (Pijut *et al*., 2012). Large number ofapplications in vegetables (Anbazhagan *et al*., 2008; Yang *et al*., 2004) and fruit trees (Kher & Nataraj, 2008) have proven that tissue culture can effectively solve the shortage and unsustainable utilization of wide plant resources, making it a potential high technology (Li *et al*., 2017).

Existing studies on pomegranates focus on their physiological characteristics and chemical substances (Sreekumar *et al*., 2014). However, previous studies on pomegranates (Jeong *et al.*, 2018), systematic reports on industrialized production of their seedlings and studies on relevant technologies are rare due to their short history of planting and unique physiological characteristics.

Therefore, the present study used 'Baihuayushizi' pomegranates from Huaiyun as an example and their stem tips as explants in order to build a systematic rapid-propagation tissue culture system and optimize the conditions for seedling exercising and transplant. We further wanted to examine the quality of transplanted tissue culture seedlings, built an industrialized production system for high-quality tissue culture seedling of pomegranates and optimize the pattern of the agricultural industry.

Materials and Methods

Experimental materials: The sampling site was the‘Baihuayushizi’pomegranatesplanting base of Anhui Science and Technology University and sampling was performed during the germination periods of pomegranates from March 2018 to May 2019. Notably,1-2cm tips were retrieved from the tender stems of the pomegranates. Only plants of pomegranates from Huaiyuan that were free of diseases and insect pests and well-grown were selected for the study.

Experimental materials and culture conditions: The following culture conditions were used at the culture chamber: Illumination: 3,000LX; temperature: 25±1°C; illumination time: 12h/d; relative humidity: 50%; culture time: 35-40 days.

Disinfection and inoculation of explants: For explants, collected tips of tender stems of pomegranates were rinsed with clean water in a beaker for 15 min, and subsequently rinsed with 75% alcohol for 30 sec and with sterile water 2-3 times, to remove surface dust and other impurities. Rinsed materials were placed on the clean bench and disinfected with 7.5% NaClO and 0.1% $HgCl_2$ was used as disinfectants (Table 1) for different disinfection time periods. Subsequently, they were rinsed 3-5 times with sterile water and placed in the inoculation plate for subsequent use.

Table 1. Different disinfection methods for pomegranate stem tips as explants.

Materials	Sodium hypochlorite	Treatment	Mercuric chloride	Treatment	Control group	Treatment
Stem tip	A1	5min	B1	5min	C1	5min
	A2	10min	B2	10min	C2	10min
	A3	15min	B3	15min	C3	15min
	A4	20min	B4	20min	C4	20min

After a disinfected explant was placed under a microscope, its stem tip was removed with an anatomical needle until only the growth point of the leaf primordium was left, which was approximately 0.3-0.5mm in diameter. The growth point of the stem tip was subsequently cut with a scalpel and inoculated to a prepared culture medium. In each bottle, 3 stem tips were inoculated and in each group10 bottles were used for 3 times during inoculation.

Inoculated explants were cultured at the culture chamber and following 30 days, the overall contamination and survival were observed, to compare the effects of different disinfectants and the disinfection time period required with the growth of stem tips of ‘Baihuayushizi’pomegranates. During the process, the parameter inductivity was estimated as follows:

$$\text{Inductivity} = \frac{\text{Number of induced seedlings}}{\text{Total number of inoculated explants}} \times 100\%$$

$$\text{The germination rate} = \frac{\text{Number of shoots}}{\text{Total number of inoculated explants}} \times 100\%$$

$$\text{The contamination rate} = \frac{\text{Number of contaminated seedlings}}{\text{Total number of inoculated explants}} \times 100\%$$

Selection of proliferating hormone for ‘Baihuayushizi’pomegranates

(1) Selection of culture medium: MS, 1/2MS, B5 and WPM culture media are commonly used for tissue culture of pomegranates according to existing literature and information regarding tissue culture of pomegranates. Therefore, in order to build a tissue culture system for‘Baihuayushizi’ pomegranates, the stem tips were disinfected using aseptic operations and were inoculated to pre-prepared MS, 1/2MS, B5 and WPM culture media. Subsequently, their epidermis was removed, and the stem tips were inoculated to the above mentioned 4 commonly used culture media, with 3 in each bottle and 10 bottles in triplicate in each group. Following 30 days of culture at the culture chamber, they were observed for the experiment results in order to record the stem heights and number of induced proliferated adventitious shoots. During the process, the proliferation coefficient of adventitious shoots was estimated as follows:

$$\text{Proliferation coefficient} = \frac{\text{Total number of seedlings investigated}}{\text{Total number of inoculated explants}} \times 100\%$$

(2) Selection of hormones: 0.5cm-long stems were induced and proliferated from stem tips of the same growth potential and thickness in WPM culture media containing different growth regulators at different proportions. In the experiment, the group without hormone was denoted as the control group. NAA and 6-BA hormones of 5 different concentrations were added to 25 treatment groups (Table 2). In each bottle, 3 explants were present, and 10 bottles were used in triplicate for inoculation. Following30 days of culture at the culture chamber, the number of induced shoots and heights of all stems were collected for the assessment of the inductivity. During the process, the proliferation coefficient was estimated as follows:

$$\text{Proliferation coefficient} = \frac{\text{Total number of seedlings investigated}}{\text{Total number of inoculated explants}} \times 100\%$$

Table 2. Effects of treatments with different concentrations of hormones on proliferation of stem tips of'Baihuayushizi'pomegranates.

Treatment	NAA (mg/l)	6-BA (mg/l)
1	0	0
2	0	0.25
3	0	0.5
4	0	0.75
5	0	1.0
6	0.25	0
7	0.25	0.25
8	0.25	0.5
9	0.25	0.75
10	0.25	1.0
11	0.5	0
12	0.5	0.25
13	0.5	0.5
14	0.5	0.75
15	0.5	1.0
16	0.75	0
17	0.75	0.25
18	0.75	0.5
19	0.75	0.75
20	0.75	1.0
21	1.0	0
22	1.0	0.25
23	1.0	0.5
24	1.0	0.75
25	1.0	1.0

Selection of rooting hormone for 'Baihuayushizi'pomegranates: The rooting of the tissue culture seedlings was observed following addition of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/l IBA to the basic medium WPM. Transgenerational seedlings of 'Baihuayushizi'pomegranates of the same age that were 1.5cm or more in height and exhibited almost the same growth status were used as the inoculating materials. In each bottle, 3 explants were used. A total of 10 bottles were used in triplicate for inoculation. Following 30 days of culture at the culture chamber, the rooting of the tissue culture seedlings was collected and recorded. During the process, the rooting rate was estimated using the following equation:

$$\text{Rooting rate} = \frac{\text{Number of rooted seedlings}}{\text{Total number of inoculated explants}} \times 100\%$$

Effects of different conditions on survival rate from seedling exercising in tissue culture

(1) Illumination intensity: Strong bottled tissue culture seedlings of the same growth potential and thickness and with strong and uncontaminated roots were used for seedling exercising experiments. The cultures were performed at a relative humidity of 60 to 70%, temperature of 24-26°C, illumination time of 12h/d and illumination intensity of 800, 1,000, 1,200, 1,400 and 1,600, respectively. A total of 10 bottles were used in triplicate at each illumination intensity. Their information was collected for calculation of the survival rate. During the process, the survival rate was estimated with the following equation:

$$\text{Survival rate} = \frac{\text{Number of survived seedlings}}{\text{Numbers of transplanted seedlings}} \times 100\%$$

(2) Humidity condition: Strong bottled tissue culture seedlings of the same growth potential and thickness and with strong and uncontaminated roots were used for seedling exercising experiment at an illumination intensity of 1,200LX, temperature of 24-26°C, illumination time of 12h/d and relative humidity of 50, 60, 70, 80 and 90%, respectively. A total of 10 bottles were used in triplicate at each relative humidity. The information was collected for calculation of the survival rate. During the process, the survival rate was measured by the following equation:

$$\text{Survival rate} = \frac{\text{Number of survived seedlings}}{\text{Numbers of transplanted seedlings}} \times 100\%$$

Effects of different matrices on survival rate of transplanted tissue culture seedlings: Uncontaminated bottled seedlings were collected following seedling assessment. The plants that exhibited no callus at stem bases, strong roots and lignified stems were cultured and transplanted to the four prepared matrix formulas following washing of their roots (Table 3). Prior to the transplant, the matrices were disinfected at high temperatures to prevent tissue culture seedlings from being affected by diseases and insect pests and to improve their resistance. Plastic films were applied onto the plug plates after the transplant to avoid loss of water and the relative humidity was maintained above 80% at the early stage of transplantation. Subsequently, the relative humidity was gradually reduced. Sterile nutrient solution was applied to the seedlings three times, with 30 seedlings in each treatment group. Their information was collected for calculation of the survival rate. During the process, the survival rate was calculated with the following equation:

$$\text{Survival rate} = \frac{\text{Number of survived seedlings}}{\text{Numbers of transplanted seedlings}} \times 100\% \text{ (Zhang \& Stolz, 1991)}$$

Table 3. Proportion of ingredients in each matrix.

Treatment	Matrix composition
T1	River sand
T2	Turfy soil : Perlite : Vermiculite = 1:1:1
T3	Turfy soil : Perlite : Vermiculite = 1:2:1
T4	Turfy soil : Perlite : Vermiculite = 2:1:1

Quality identification of transplanted tissue culture seedlings of 'Baihuayushizi'pomegranates.

Experimental materials: Transplanted tissue culture seedlings and cutting seedlings of 'Baihuayushizi' pomegranates were collected from the Horticultural

Laboratory of Anhui Science and Technology University. The tissue culture seedlings were transplanted on April 15, 2019 and the cutting seedlings were cut on the same day.

Measurement of growth index: The transplanted tissue culture seedlings and the cutting seedlings of 'Baihuayushizi' pomegranatesthat survived were measured for determination of their height and ground diameter on the 15th day of June, August, October and December 2019 and the information was recorded (four time points of measurement).

Scanning of root indices: Survived transplanted tissue culture seedlings and survived cutting seedlings of 'Baihuayushizi' pomegranates were scanned for root indices on the 15th day of June, August, October and December 2019 and the information was recorded.

Measurement of physiological and biochemical indices: Chlorophyll content，soluble sugar content and soluble protein content was measured as determined by the study of Wang *et al.,* (2020); The content of SOD (Kit number: A001-3-2) and POD (Kit number: A084-3-1:) activity in cells was detected by assay Kitmethod produced by Nanjing Jiancheng Bioengineering Institute.

Data processing and analysis: The EXCEL 2010 software was used for the basic processing of the experimental data. The SPSS19.0 was used to analyze significant differences.

Result

Effects of different disinfection treatments on inoculation of stem tips: As shown in Fig. 1, following inoculation, the stem tips of 'Baihuayushizi' pomegranates began to exhibit green color within 3 to 5 days. On day 15th following inoculation, apparent differentiation of shoots was observed in the media. On day 30th, it was observed that the stem tips were completely differentiated into tissue culture seedlings of 'Baihuayushizi' pomegranates of 0.5cm or more in height, available to be used for subsequent experiments.

The contamination rates (between 22.67% and 10.03%) of the explants disinfected with 7.5% NaClO for over 15 min and with 0.1% $HgCl_2$ for over 10 min were significantly lower than those of the other treatments. Therefore, the optimal method included the disinfection of the explants with 0.1% $HgCl_2$ for 10 min (Table 4).

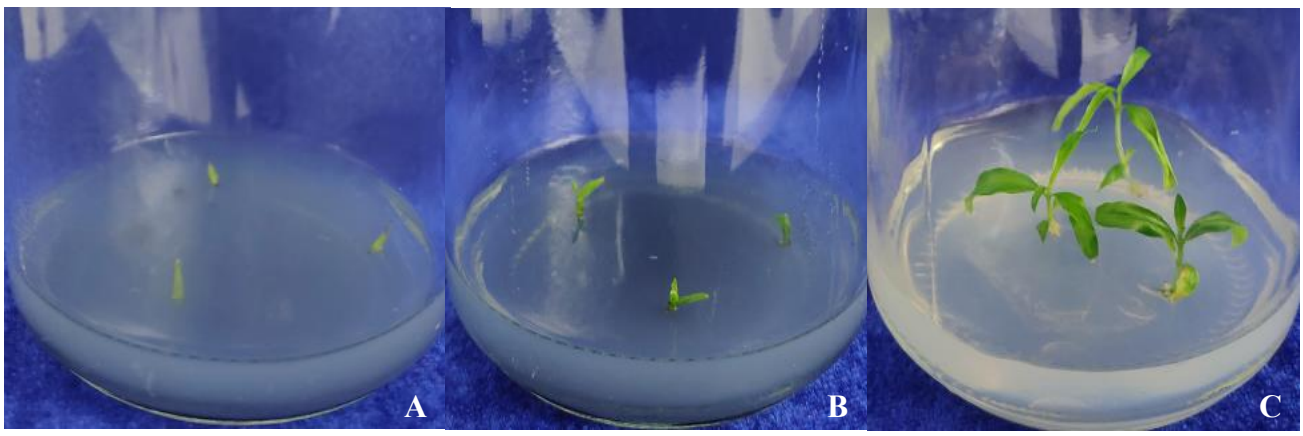

Fig. 1. Growth of in-vitro rapid-propagation stem tips of 'Baihuayushizi' pomegranates following inoculation.
A: Growth of stem tips 5 days following inoculation; B: Growth of stem tips 15 days following inoculation; C: Growth of stem tips 35 days following inoculation

Table 4. Effects of different disinfectants and of the disinfection time on inoculation of stem tips of 'Baihuayushizi' pomegranates.

Treatment	Contamination rate (%)	Survival rate (%)
A1	62.17 ± 0.99 c	33.70 ± 1.40 e
A2	41.63 ± 0.90 d	47.93 ± 2.06 d
A3	22.23 ± 1.44 e	55.63 ± 2.27 c
A4	15.63 ± 1.14 f	19.47 ± 1.22 f
B1	43.93 ± 2.42 d	53.93 ± 1.55 c
B2	22.67 ± 2.32 e	80.40 ± 1.21 a
B3	11.03 ± 1.51 g	67.73 ± 2.1 2b
B4	10.03 ± 0.42 g	46.40 ± 1.08 d
C1	92.83 ± 1.72 a	12.07 ± 1.46 g
C2	92.53 ± 1.72 a	12.93 ± 0.93 g
C3	82.20 ± 0.87 b	13.50 ± 1.11 g
C4	81.57 ± 0.99 b	14.03 ± 0.70 g

Note: Numbers in the table are indicative of the mean ± standard deviation and different lowercase letters indicated the level of significance ($p<0.05$)

Selection of proliferation conditions for 'Baihuayushizi' pomegranates

(1) Selection of culture medium: According to Table 5, stem tips cultured in WPM media exhibited improved effects than those noted following culture in MS, 1/2MS and B5 media.

(2) Selection of hormone: According to Table 6, the explants grew a higher number of adventitious shoots when treated with higher concentrations of NAA and 6-BA. The explants treated with 0.75 mg/l NAA and 0.5 mg/l 6-BAexhibited the highest induction rate of the adventitious shoots (90.77%).

Selection of rooting hormone for 'Baihuayushizi' pomegranates: 'Baihuayushizi' pomegranates exhibited a significant response to IBA and different concentration levels of IBA promoted the rooting of pomegranates to different extents. In particular, 0.6 mg/l IBA exhibited the

optimal promoting effect, allowing single seedling to grow 4 to 5 roots on average. However, the root-induction rate was reduced when the stem heights were significantly lower than 0.6 mg/l and the concentration of IBA reached 1.0 mg/l. When IBA was used at higher concentrations, it was able to promote a higher number of roots in pomegranate seedlings. Therefore, 0.6 mg/l IBA was the optimal rooting hormone concentration for tissue culture seedlings of 'Baihuayushizi' pomegranates (Figs. 2, 3).

Quality identification of the transplanted tissue culture seedlings of 'Baihuayushizi' pomegranates

Measurement of growth index: The height of the tissue culture seedlings of 'Baihuayushizi'pomegranateswas rapidly increased. The cutting seedlings between June and August exceeded the height of those in December at the time of measurement, while the growth of the ground diameters of the two was nearly the same. However, the height of the tissue culture seedlings was similar to but did not exceed that of the cutting seedlings at the 4 time points of measurement, indicating that the tissue culture seedlings of 'Baihuayushizi' pomegranate exhibited strong vitality and growth following survival of the transplant (Fig. 4).

Analysis of root scanning results: The growth of tissue culture seedlings and cutting seedlings of 'Baihuayushizi' pomegranates exhibited significant differences. The tissue culture seedlings exhibited apparent taproots and had longer total root length, additional root bifurcations and higher number of total root tips than those of the cutting seedlings. In contrast to these observations, the cutting seedlings at the same period of time exhibited larger surface area and shallower root distribution than those of the tissue culture seedlings, which were inhibitory to the absorption of nutrients and water and caused lodging, indicating that the tissue culture seedlings of 'Baihuayushizi' pomegranates exhibited better root indices than those of the cutting seedlings (Fig. 5).

Table 5. Effects of different media types on stem height and germination rate of 'Baihuayushizi' pomegranates.

Type of culture medium	Stem height (cm)	Number of induced adventitious shoots	Growth
MS	0.3 ± 0.15 c	0.5 ± 0.75 d	Yellowish leaves and poor growth
1/2MS	1.2 ± 0.21 b	0.6 ± 0.83 c	Tender green leaves and normal growth
B5	1.5 ± 0.27 b	1.8 ± 0.54 b	Normal leaves and similar growth
WPM	2.3 ± 0.18 a	3.5 ± 0.79 a	Dark green leaves and strong growth

Note: The numbers in the table are indicative of the mean±standard deviation and the different letters indicate the different level of significance ($p<0.05$)

Table 6. Effects of treatments with different concentrations of hormones on the induction rate of adventitious shoots of 'Baihuayushizi' pomegranates.

Treatment	NAA (mg/l)	6-BA (mg/l)	Stem height (cm)	Proliferation rate of adventitious shoots (%)
1	0	0	0.60 ± 0.10 k	21.53 ± 1.55 n
2	0	0.25	1.47 ± 0.15 i	64.07 ± 0.90 gh
3	0	0.5	2.10 ± 0.10 fg	21.37 ± 1.40 n
4	0	0.75	1.20 ± 0.10 j	62.11 ± 0.85 h
5	0	1.0	0.73 ± 0.06 k	72.67 ± 2.52 ef
6	0.25	0	0.77 ± 0.06 k	24.41 ± 1.22 m
7	0.25	0.25	1.90 ± 0.20 gh	30.87 ± 1.86 l
8	0.25	0.5	2.40 ± 0.10 de	74.67 ± 1.53 de
9	0.25	0.75	1.40 ± 0.10 ij	39.67 ± 1.53 j
10	0.25	1.0	1.20 ± 0.10 j	83.27 ± 2.11 b
11	0.5	0	1.20 ± 0.10 j	62.13 ± 1.03 h
12	0.5	0.25	2.03 ± 0.15 gh	71.67 ± 1.15 f
13	0.5	0.5	2.43 ± 0.12 de	71.03 ± 1.05 f
14	0.5	0.75	1.87 ± 0.15 h	76.17 ± 0.97 d
15	0.5	1.0	1.43 ± 0.15 ij	71.83 ± 1.61 f
16	0.75	0	1.37 ± 0.12 ij	75.77 ± 0.49 d
17	0.75	0.25	2.73 ± 0.06 b	83.53 ± 0.50 b
18	0.75	0.5	3.07 ± 0.15 a	90.77 ± 0.68 a
19	0.75	0.75	2.50 ± 0.17 cd	84.83 ± 1.32 b
20	0.75	1.0	2.10 ± 0.20 fg	80.03 ± 1.05 c
21	1.0	0	1.20 ± 0.10 j	74.77 ± 1.57 de
22	1.0	0.25	2.27 ± 0.15 ef	71.77 ± 1.54 f
23	1.0	0.5	2.67 ± 0.06 bc	64.73 ± 1.42 g
24	1.0	0.75	2.07 ± 0.15 fgh	59.37 ± 1.18 i
25	1.0	1.0	1.53 ± 0.15 i	34.71 ± 1.54 k

Note: The numbers in the table are indicative of the mean±standard deviation and the different letters indicate the different level of significance ($p<0.05$)

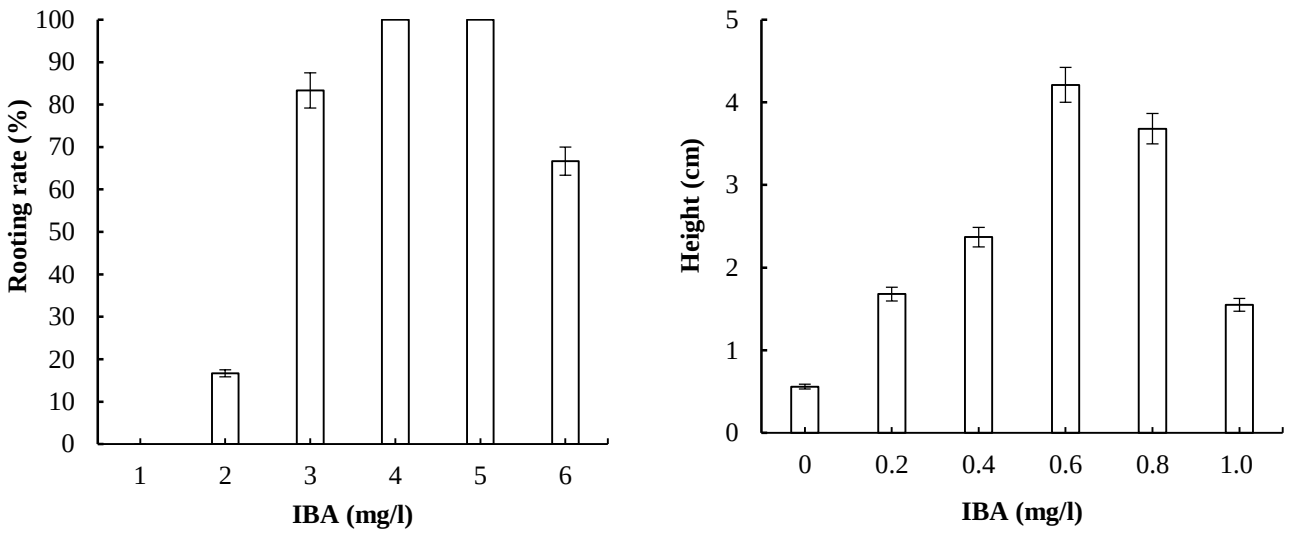

Fig. 2. Effects of IBA of different concentrations on the rooting rate and height of tissue culture of 'Baihuayushizi' pomegranates
Note: The numbers in the table are indicative of the mean ± standard deviation and the different lowercase letters indicate the different level of significance ($p<0.05$).

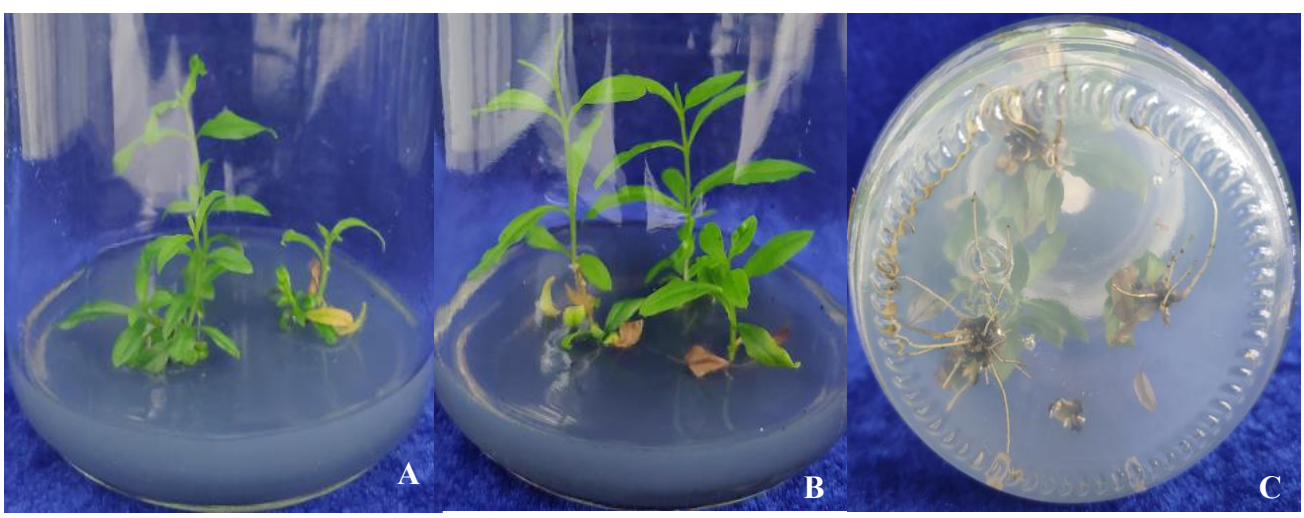

Fig. 3. Growth of 'Baihuayushizi' pomegranates 30 days following culture with 0.6 mg/l IBA hormone.

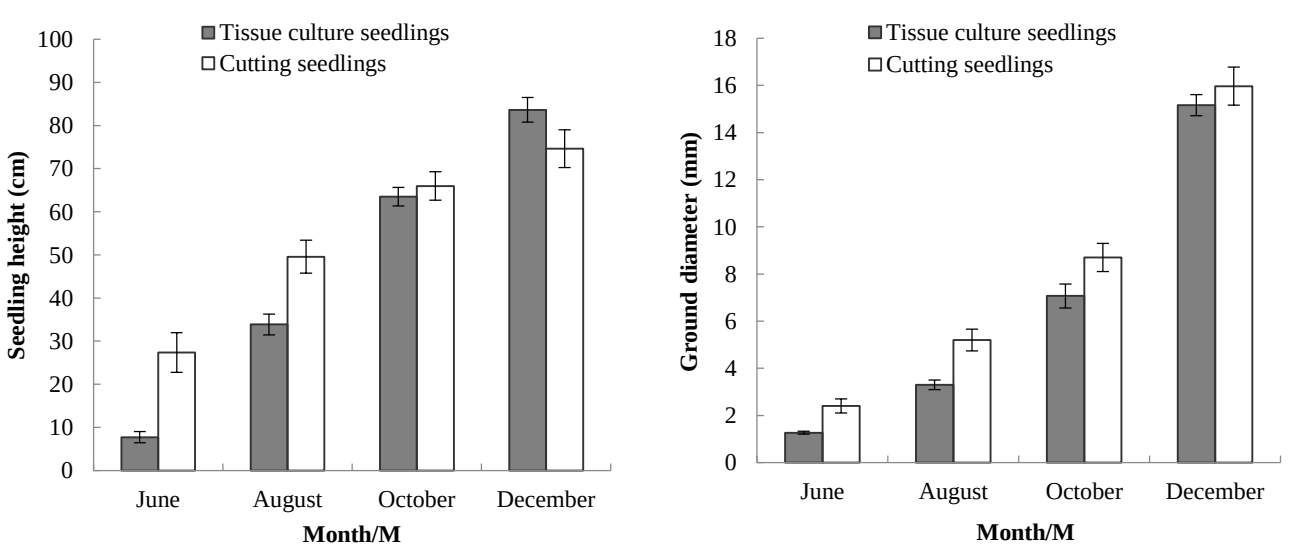

Fig. 4. Comparison of seedling height and ground diameter of tissue culture seedlings and cutting seedlings in different months.

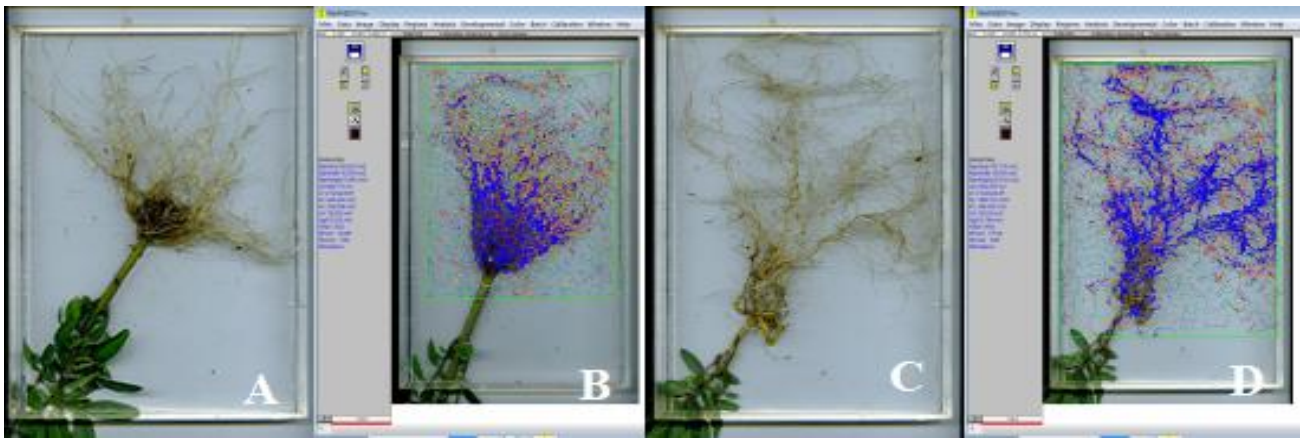

Fig. 5. Images of root scanning. A: Image of root scanning of cutting seedlings; B: Image of root analysis of cutting seedlings; C: Image of root scanning of tissue culture seedlings; D: Image of root analysis of tissue culture seedlings.

The root scanning results of the tissue culture seedlings and cutting seedlings of 'Baihuayushizi' pomegranates indicated that the total length and total surface area of the roots of the tissue culture seedlings were significantly higher than those of the cutting seedlings at the 4 time points of measurement (Fig. 6). This suggested that the tissue culture seedlings of 'Baihuayushizi' pomegranates exhibited optimal root vitality and root growth than those of the cutting seedlings.

Physiological and biochemical indexes: Physiological indices of tissue culture seedlings and of the cutting seedlings were measured with trees of 'Baihuayushizi' Pomegranates in rich seasons, which were used as the control group.

Comparison of chlorophyll content: The chlorophyll content in the transplanted tissue culture seedlings was significantly higher than that in the cutting seedlings and trees collected during the rich seasons. In particular, the chlorophyll content in the transplanted tissue culture seedlings was 2.18 times higher than that in the cutting seedlings and 1.88 times higher than that in the trees of the rich seasons, indicating that the transplanted tissue culture seedlings exhibited better photosynthetic ability to synthesize organics (Fig. 7).

Comparison of content of soluble sugar and soluble protein: The content of soluble sugar and soluble protein in the transplanted tissue culture seedlings was higher than that in the cutting seedlings and trees grown during the rich seasons. In particular, the content of soluble sugar in the transplanted tissue culture seedlings was 1.36 times higher than that in the cutting seedlings and 1.07 times higher than that in the trees grown during rich seasons. The content of soluble protein in the transplanted tissue culture seedlings was 1.08 times higher than that in the cutting seedlings, indicating that the transplanted tissue culture seedlings exhibited improved storage of nutrients, cell activity and growth and development (Fig. 8).

Comparison of protective enzyme content: It was observed by comparing the measurement results of protective enzymes in the transplanted tissue culture seedlings and cutting seedlings of the 'Baihuayushizi' pomegranates that the former exhibited higher SOD and POD enzymatic activity than the latter. In particular, the SOD activity of the tissue culture seedlings of 'Baihuayushizi' pomegranates was 18.2% higher than that of the cutting seedlings, indicating that the tissue culture seedlings of 'Baihuayushizi' pomegranates exhibited improved growth and metabolism (Fig. 9).

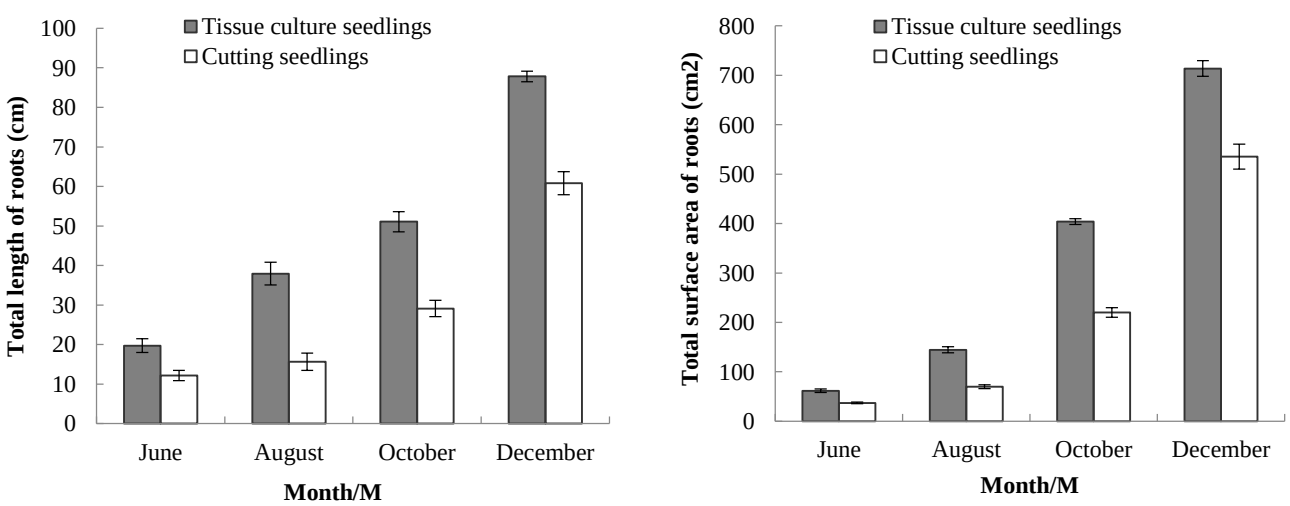

Fig. 6. Comparison of length and surface area of the roots of the tissue culture seedlings and cutting seedlings in different months.

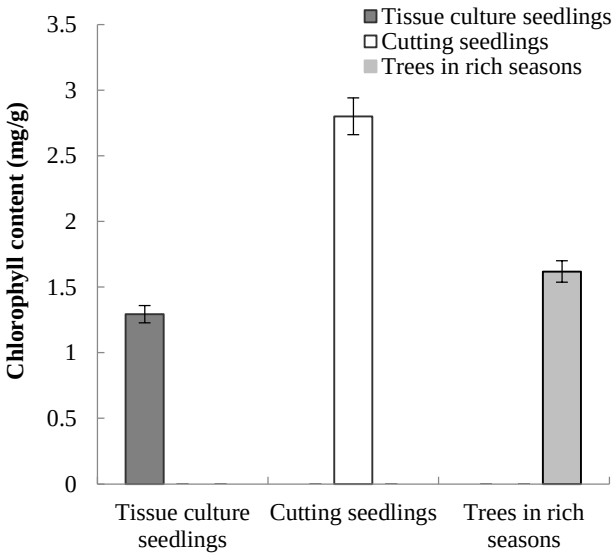

Fig. 7. Chlorophyll content of tissue culture seedlings and cutting seedlings of 'Baihuayushizi' Pomegranates.

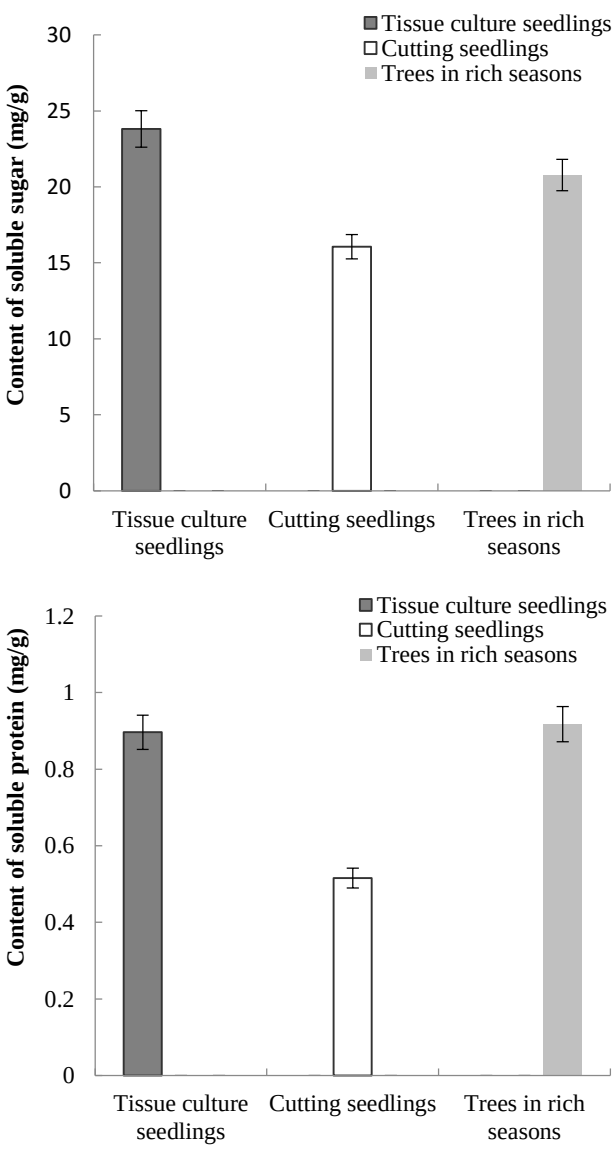

Fig. 8. Comparison of soluble sugar and soluble protein content in the transplanted tissue culture seedlings and cutting seedlings of 'Baihuayushizi' pomegranates.

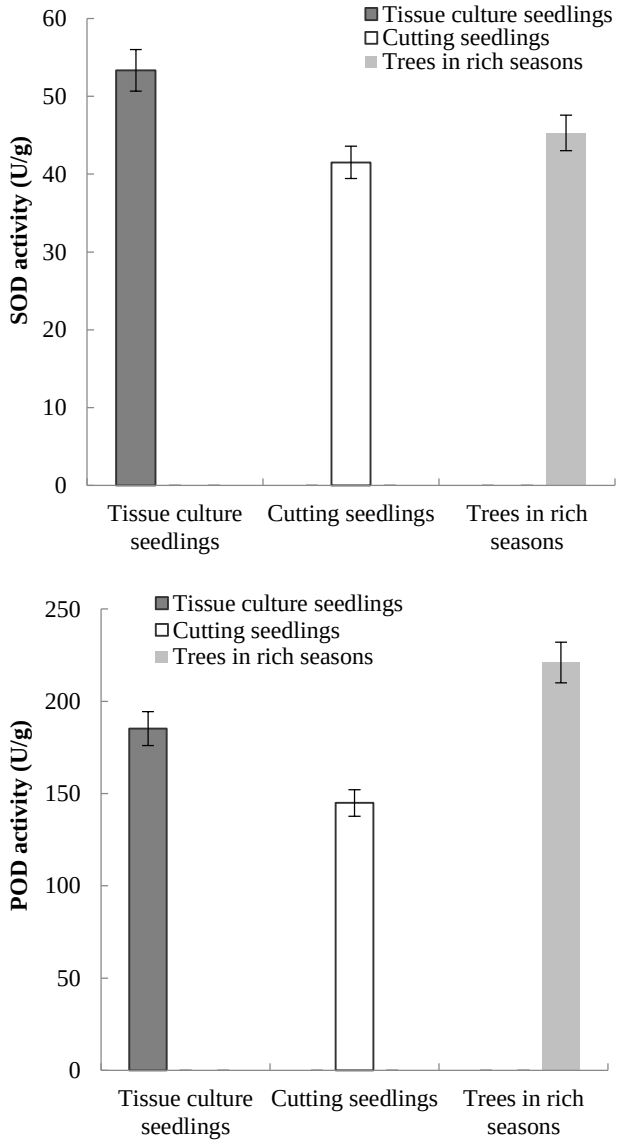

Fig. 9. Comparison of SOD and POD enzymatic activity levels of the transplanted tissue culture seedlings and of the cutting seedlings of 'Baihuayushizi' pomegranates.

Discussion

Tissue culture, or cloning of plants is a newly-developed *In vitro* vegetative propagation technology based on the totipotency of plant cells. Due to their high nutritional value and healthcare effects, pomegranates are popular among consumers and pomegranate products are in short supply in the market. Traditional ways of propagation, such as cutting, division and layering, have long been far from satisfactory in meeting the demands of consumers in the market (Soumendra & Pradeep, 2011). Therefore, the advantages of tissue culture are highly significant. Tissue culture of plants is characterized by rapid propagation, high propagation efficiency and large amounts of seedlings in a short time. Therefore, it is increasingly applied to the culture of pomegranate seedlings, which has laid the foundation for the generation of standardized demonstration plantations of pomegranates.

In the present study, successful experiment and research of tissue culture of 'Baihuayushizi' pomegranates have revealed a new path for the rapid propagation of seedlings of high-quality pomegranate varieties. In addition, they have provided solutions for variety degradation and uniformed quality of seedlings incurred in traditional propagation methods, such as cutting as well as theoretical support and technical bases for industrialized production of high-quality pomegranate seedlings, which is essential in order to further improve the optimization of the tissue culture system for woody plants. This addition has enabled the production of the top propagating high-quality seedlings that has significant implications in the development of the pomegranate industry.

The present report performed a comparative study on the quality of 1-year transplanted tissue culture seedlings and 1-year cutting seedlings of 'Baihuayushizi' pomegranates by analyzing their growth index, assessing their roots and measuring their physiological indices. The results of these analyses indicated that tissue culture seedlings of 'Baihuayushizi' pomegranates exhibited improved vitality and environmental adaptability and superior root indices than those of the cutting seedlings. This is essential for the further improvement and optimization of the tissue culture system for woody plants, to lay a theoretical and technical foundation for further theoretical studies and to enable its application in large-scale production (Dinesh *et al*., 2019). It was intuitive to observe the height, ground diameter and root indices of the transplanted tissue culture seedlings and cutting seedlings of 'Baihuayushizi' pomegranates. The 1-year tissue culture seedlings exhibited significant advantages over the 1-year cutting seedlings and the ground diameter of the former was very likely to exceed that of the latter. This was based on the trend of differences indicating that the ground diameter of the former was 50%, 34%, 22% and 6% shorter than that of the latter in the four time points of measurement. In terms of SOD and POD enzymes that protect plants against stress, the higher content indicated the higher stress resistance of the seedlings. In the present study, the contents of SOD and POD enzymes in the transplanted tissue culture seedlings were significantly higher than those in the cutting seedlings, indicating that tissue culture seedlings of pomegranates exhibited a broader application prospect based on the fact that drought is a common disadvantage encountered frequently in the agricultural industry.

In conclusion, the development of the tissue culture of pomegranates is of great significance to the development of the pomegranate industry. By comparing the quality of 1-year transplanted tissue culture seedlings and cutting seedlings with 'Baihuayushizi' pomegranates, which is a variety that grows in the Anhui Province, the study has laid a foundation for the future industrialized production of tissue culture seedlings of 'Baihuayushizi' pomegranates.

Conclusion

The present study to provide theoretical and experimental bases for the production pattern and industrialized reproduction of high-quality seedlings of 'Baihuayushizi'pomegranates, so as to contribute in promoting the propagation paths of high-quality seedlings. The experimental results were as follows: (1) When stem tips were used as explants to build the tissue culture system, the optimal sterilization time period was 10min and required processing with 0.1% HgCl2.The optimal inducing medium for proliferation of adventitious shoots was WPM+NAA 0.75 mg/l+6-BA 0.5 mg/l and the optimal inducing medium for rooting was WPM+IBA 0.8 mg/l. (2) The initial studies examining the transplanting conditions for seedling exercising demonstrated that the survival rate from seedling exercising was increased following the increase in the illumination intensity and humidity within certain limits. The survival rate was decreased when it reached the threshold, suggesting that the optimal illumination intensity for seedling exercising of 'Baihuayushizi'pomegranates was 1,000 LX and the optimal relative humidity for the transplant was 70%. The survival rate of the transplanted tissue culture seedlings may be significantly increased up to 85.39% if appropriate fungicides and matrix formulas are used during seedling exercising. (3) The results of the comparison of the growth, root length and physiological and biochemical indices between survived transplanted tissue culture seedlings of 'Baihuayushizi'pomegranates and cutting pomegranate seedlings in the same period indicated that tissue culture seedlings exhibited optimal root activity, growth and development activity compared with those of the cutting seedlings.

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