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Improving the grafted-plant rate in triploid watermelons by optimizing the water moisture in substrates and the usage of scion

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ABSTRACT

The cost of growing triploid watermelon seedlings has increased due to their low-efficiency grafting. The first priority in growing seedless watermelon seedlings is increasing the grafted-plant and seed-utilization rates. This study aimed to improve the grafted-plant rate by screening the most suitable substrate formulation, optimizing water moisture in the substrate, and evaluating the effect of different scions to improve seed-utilization rate. Five combinations of substrate (S1 to S5) and seven relative humidity levels (45%, 50%, 55%, 60%, 65%, 70%, and 75%) were used. Three types of scions (yellow bud, Ts-1; two cotyledons did not unfold, Ts-2; and first true leaf appeared, Ts-3) were tested. Results showed that the combination of S1 exhibited the best seed-utilization rate which was 71.6%. Moreover, the most suitable water moisture in the substrate ranged from 50% to 55%. The usage of the scion from Ts-3 significantly increased the grafting survival, grafted plant and seed-utilization rates by 13.7%, 10.1% and 22%, respectively, compared with the conventional method. Our study suggested that the best time to use the scion and the rootstock was during the seedling stage when the first true leaf unfolded. The proposed method decreased the production cost of seedlings and significantly improved the efficiency of grafting procedures. The results of this work are applicable to the technique of growing seedlings and can thus guide growers of high-quality grafted plants of triploid watermelon.

Keywords: grafting technique, rootstock, seedless watermelon, substrate formulation, water content

INTRODUCTION

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. en Nakai) is one of the favorite fruits at present and belongs to the family Cucurbitaceae (Edwards et al., 2003). Watermelon, as recorded, is initially native to the Kalahari Desert of Africa, but currently, it is also cultivated in tropical regions of the world. To date, watermelon is an important summer crop of high economic and nutritional value worldwide and an influential specialty crop, accounting for 7% of the agricultural area devoted to vegetable

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crops (Ren et al., 2012). With the great need for watermelon, seedless watermelons are popular with the consumers. For instance, triploid watermelons have increased in popularity since the early 1990s, and the demand for seedless fruits remains on the rise (Beaulieu and Lea, 2006; Levi et al., 2004). At present, seedless watermelons constitute a high proportion of the grown watermelons and a continuous need to consume seedless watermelons is observed. The portion dedicated to fresh cuts has been increasing and now constitutes 25% of the total fresh-cut fruit market (Beaulieu and Lea, 2006). Consumers desire seedless watermelons, especially when conveniently packaged as fresh cuts in many countries, including China.

Presently, China has become the largest consumer of this crop, followed by Turkey, the United Sates, Iran, and Korea (Naz et al., 2014; Ren et al., 2012). China is currently the leading global producer of watermelons with approximately 1.8 million ha under cultivation at 2013 (Mo et al., 2016). Currently, more than 1,000,000 ha of watermelon is grown annually in China. Seedless watermelons constitute an important part of the watermelon market, accounting for 20% of the total watermelon production acreage (Wenge et al., 2006). Seedless watermelon has become increasingly popular in recent years. Presently, the total area of planted watermelons is 160,000 ha in Anhui Province. Approximately 26,667 ha is planted with seedless watermelons, accounting for 17% proportion. Now, seedless watermelons are highly prized by consumers because they are sweeter than seeded diploid cultivars and seedlessness is also a desirable trait (Shi et al., 2013; Thayyil et al., 2016). However, the production of seedless watermelons is more expensive than that of diploid watermelons, so the priority tasks in the growing enterprises are to reduce the cost and increase the yield. Grafting of watermelon scions on the bottle gourd (*Lagenaria* spp.) rootstocks is practiced in the majority of watermelon-growing areas worldwide now (Davis and Perkins-Veazie, 2005; Hassell et al., 2008; Schwarz et al., 2010). Recently, the practice of grafting watermelons has become popular in China due to its advantage of preventing soilborne diseases (Miguel et al., 2004), improving nutrient absorption and fruit quality and obtaining high yield from grafted plants (Lopez-Galarza et al., 2004; Proietti et al., 2008; Rouphael et al., 2010; Shi et al., 2013; Wang et al., 2015). Despite the increasing interest in using grafted transplants for field watermelon production in disease management, the majority of users of grafted plants in Anhui Province are currently greenhouse watermelon growers. Therefore, grafted plants have higher costs compared with the non-grafted plants, especially for the triploid watermelon.

However, although a consistently significant need for triploid watermelon is observed, various limitations still exist in the production of triploid watermelon. Several thresholds have been reported in cultivating grafted plants of triploid watermelon due to its genetic characteristics (Islam et al., 2015). The triploid watermelon seed capsule is thick and un-fleshy which results in germination difficultly and the need to "break shell" to improve germination (Sihui et al., 2012). These characteristics of the seed lead to low germination, emergence, and grafted-plant rates. Our former results showed that breaking the shell of seed would improve the seed germination rate up to 90% (data not shown). However, the key steps are not only to obtain a large amount and high quality of grafted triploid watermelon seedling but also to increase the seed-utilization rate. These steps are the most important prerequisites for the seedling breeding enterprise. Notably, the cost of growing triploid watermelons has become increasingly expensive because its seeds do not germinate well and its growth in the grafting process is poor. Presently, seed germination and survival rates of grafted plants are regarded as two vital factors in the production of triploid seedlings. The first priority in the production of seedless watermelon is how to increase the efficiency of grafted-plant and seed-utilization rates.

Nevertheless, to our knowledge, limited studies have focused on these unsettled issues. The main objectives of this study were to screen the most suitable substrate formulation, optimize the water content in the substrate, and evaluate the effect of the usage of grafting scions on the survival of grafted plants to improve the grafted-plant and seed-utilization rates of triploid watermelons.

MATERIAL AND METHODS

Location and experimental period

This study was conducted at the experimental field of the Institute of Horticulture at Anhui Academy of Agricultural Sciences, Hefei City, China, from March 3, 2016, to April 1, 2017.

Plant and substrate materials

Triploid watermelon (*C. lanatus* (Thunb.) Matsum. en Nakai) cultivar Zhengkang No. 5 was used in this work. Zhengkang No. 5 is one of the widely grown cultivars in the commercial production area of watermelons in China. Pumpkin (*Cucurbita moschata* Duchesne) named Wanzhen No. 2 was used as rootstocks. Wanzhen No.2 is the most popular rootstock available for watermelon grafting in Anhui Province. Seeds were sown in trays containing 50 holes. Substrates were constituted with peat and perlite.

Seed preparation and sowing

After the triploid watermelon seeds were soaked in water for 12 h, scrubbed and dried, the shells were broken. Subsequently, the seeds were sown in a seedling plate with a concentration of 3,000 seeds m^{-2} under greenhouse conditions. The seeds of the rootstocks were germinated and sown in 50-cell styrofoam trays under greenhouse conditions. The trays were filled with the substrate. The environmental conditions for germination were 24°C-28°C and 50%-70% relative humidity (Mohamed et al., 2014).

Screening of substrate formulation

Five combined substrates compounded of peat and perlite were prepared in accordance with the volume ratio of peat to perlite: 1:1, 2:1, 3:1, 1:2, and 1:3 for S1, S2, S3, S4 and S5, respectively. Seeds were sown in the mixed substrates. The seedling boxes were filled with these five matrixes and then, water was poured (regular method used in the production). The germination temperature was 33°C. Hole insertion grafting was used for each of the 100 seeds and the process was repeated three times in this study. The rootstock was grafted shortly after seed germination. The specific process was described in a previous report (Mohamed et al., 2014).

Detection of the substrate's porosity

Total porosity (TP) of the substrate, including aeration porosity (AP) and water-holding porosity (WHP), was determined. In brief, a cutting ring with a volume of 100 cm^3 was used. The substrate was placed in the cutting ring, weighed (W_1), and then placed in water for 24 h. Subsequently, the cutting ring was removed and the substrate was weighed (W_2). The cutting ring was converted and water leaked out freely. Then, the substrate was weighed (W_3). The following equations were used to calculate the substrate's porosity: $TP = W_2 - W_1$; $AP = W_2 - W_3$; $WHP = TP - AP$ (Ling et al., 2012). The procedures were repeated at least three times.

Optimization of the substrate's moisture

On the basis of the former experimental results, the volume of peat and perlite was set at 1:1 to prepare the growing seedling substrate. Seven moisture contents were arranged as follows: 45%, 50%, 55%, 60%, 65%, 70% and 75%. The seedling boxes were filled with the matrixes (peat and perlite mixes in 1:1 v/v). Prepared seeds (100) were sown. Water was poured after covering with matrixes (regular method). The germination temperature was 33°C. Procedures were repeated three times. For the control treatment, the germination rate of seedless watermelon was tested in the growing box spread with filter paper. After more than 50% of the seeds were geminated, water was poured to the substrate through tidal irrigation.

Preparation of scion

Seeds were prepared and germinated as previously described. Three types of scions were prepared (Fig. 1). In treatment 1 (Ts-1), after the emergence of seedlings from the substrate, two cotyledons did not unfold. We defined Ts-1 as the conventional method of yellow bud. In treatment 2 (Ts-2), two cotyledons unfolded completely but the first true leaf did not grow. In treatment 3 (Ts-3), the first true leaf appeared and unfolded. We called this treatment as the older seedling method (Tab. 1). Grafting method was

Figure 1. Three types of scions were used in this study: (A) after the emergence of seedlings from the substrate, two cotyledons did not unfold; (B) two cotyledons unfolded completely and the first true leaf did not grow; (C) the first true leaf appeared and unfolded; (D) rootstock with the first true leaf appeared and unfolded

Table 1. Description of three types of scions used in this study

Scion	Treatment		
	Ts-1	Ts-2	Ts-3
	Yellow bud	Cotyledon	First true leaf
Growth descriptions	After sowing, the seeds' shells were removed	Two cotyledons did not unfold	Two cotyledons unfolded and first true leaf grew
Growing time after sowing seeds (day)	4-5	7-8	12-15

used in accordance with the previous description. A total of 500 seeds were used, and the process was repeated three times. The following measurements were recorded: seedling-emergence, scion-usage, grafted-plant, and commercial seedling rates (high-quality seedling rate). Seed usage efficiency was calculated using these four measurements (Tab. 2 shows the specific definitions). Germination index was calculated for each treatment in accordance with the previous methods (Ahmad et al., 2012; Sun et al., 2014).

Management of grafted plants under greenhouse conditions

Grafted plants and treatments were transferred to the greenhouse following the regular management. The cultivation was performed through surface irrigation and normal fertilization with the day and night temperatures at 24°C-28°C and 15°C-20°C, respectively.

Statistical analysis

Data are presented as the mean ($n \geq 5$). The statistical analysis was accomplished using one-way analysis of variance (ANOVA). Means were separated through Fisher's protected Least Significant Difference (LSD) test at $p < 0.05$ followed by Duncan's test at $p < 0.05$ by using the SPSS software (version 19.0). Values are the means ± standard deviations.

RESULTS AND DISCUSSION

In this work, the methods of improving the seedling growth rate were studied. We focused on the three key questions involved in the production of seedless watermelon. Our research was centered on screening the optimal substrate formulation, isolating the best water moisture in the substrate and evaluating the effect of different stages of scions on the grafted-plant and seed-utilization rates.

Screening the optimal matrix formulation

To improve the efficiency of the grafted seedless watermelon, five groups of the growing substrates were set by using the volume ratio of peat and perlite. The AP and WHP are important factors in the seedling substrate (Harp et al., 2015; Vo and Wang, 2014). The AP represents the pores that provide aeration to the substrate without retaining water (Barreto and Testezlaf, 2015). Results showed that the combination of S3 had the largest AP which was 52.8%, among the five groups, whereas S5 showed the smallest AP which was only 40.8% (Fig. 2A). However, S5 had the largest WHP which was 20.8%, whereas S3 showed the smallest WHP which was 11.5%. Thus, the substrate of S3 had poor water-holding capacity. The AP value was slightly higher and WHP was lower in the current mixed substrate compared with the previous report (Hernandez-Zarate et al., 2014). Nevertheless, we found that S1, S4 and S5 were beneficial for seed germination compared with other groups. Previous studies have shown that the most optimal value of AP/WHP in the substrate is between 2 and 4 (Ling et al., 2012), which will be suitable to grow the seedlings. We found that S1, S4 and S5 were qualified to these standards (Fig. 2B), indicating

Table 2. Specific definition of tested items used in this study

Tested items	Definition in this study
Seedling-emergence rate	The seedling grew out of the substrate
Germination index	The calculation was in accordance with the previous reports (Ahmad et al., 2012; Sun et al., 2014)
Scion-utilization rate	The total number of scions can be used in the experiment
Grafting survival rate	The seedlings can survive from the all grafted plants
Grafted-plant rate	The seedlings from the survival ones and qualified as commercial seedlings
Seed-utilization rate	The seeds were needed to produce the grafted plant

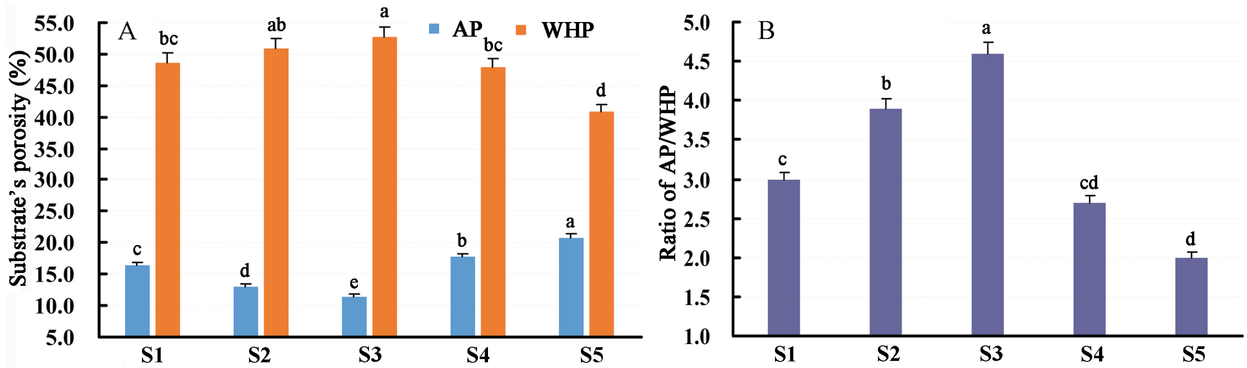

Figure 2. Porosity of the substrate tested in this study: (A) aeration porosity (AP) and water-holding porosity (WHP); (B) ratio of AP/WHP. Five combinations of substrate compounded of peat and perlite were prepared (the volume ratio of peat to perlite: 1:1, 2:1, 3:1, 1:2, and 1:3 for S1, S2, S3, S4, and S5, respectively). Analysis was achieved by using ANOVA followed by Duncan's Test at $p < 0.05$, and letters (a, b, c, etc.) represent significant levels of data

their combinations are relatively fit for growing seedlings.

The germination quality of seeds is one of the most essential factors in the production system of the crop (Islam et al., 2015). In this study, the germination index of S1, S4 and S5 were 19.4%, 19.0% and 18.6%, respectively (Fig. 3). The seedling-emergence rates of S1, S4, and S5 were 85.4%, 84.8% and 83.5% (Fig. 4A) and S1 showed the largest germination index. Grafting is an alternative approach to produce seedlings with the ability to resist soil-borne diseases and to enhance plant growth (Miguel et al., 2004; Rouphael et al., 2010). Here, grafting was used to evaluate the seedling and seed-utilization rates. Hole insertion grafting is by far the most popular grafting method in watermelon at present (Mohamed et al., 2014). Our results showed that the scion-utilization rates of S1 and S4 were 90.4% and 86.6%, respectively (Fig. 4B). The grafted-plant rate of S1, which was 92.8%, was the largest whereas that of S4 was 91.4% (Fig. 4C). We found that the combination of S1 and S4 showed the best seed-utilization rates, which were 71.6% and 67.1% (Fig. 4D), respectively.

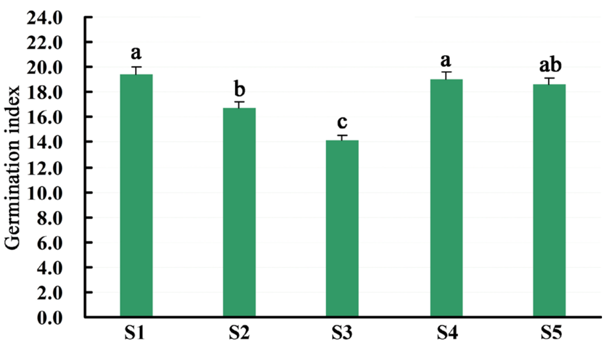

Figure 3. Germination index of triploid watermelon seeds in the five mixed substrates (S1 to S5)

Generally, combinations of S1 and S4 were suitable for grafting seedlings of triploid watermelons. The best substrate combination was S1, which was compounded of peat and perlite with 1:1 volume ratio, due to the significant improvement of the seed-utilization rate compared with other groups.

Screening the optimal water moisture in the substrate

Water is the most important factor that affects seed germination (Sevik and Cetin, 2015). Efficiency of water use has been reported related to the grafted mini-watermelon plants (Rouphael et al., 2008). On the basis of the data of the previous experiment, the combination of S1 was selected for the next research. In this section, we focused on the effect of water humidity in the substrate on the grafted plants. Seven levels of water contents were arranged from 45% to 75%. Results showed that germination indices of seeds were the largest which were 18.4% and 18.3%, when the water content was 50% and 55% (Fig. 5A), respectively. On the contrary, germination indices were only 11.6% and 10.2% in the substrate with a water content of 70% and 75%, respectively. Seed-germination rates decreased with increased water content, suggesting that elevated moisture in the substrate would reduce the germination of seeds although the seeds of triploid watermelons were broken shell. Our findings are consistent with the previous report that high water content will significantly affect the germination of seeds (Patane et al., 2013). Notably, all studies conducted until recently have proven that increasing the water content decreases the germination proportion in many plant species (Sevik and Cetin, 2015).

Seedling-emergence rates, which were 89.6% and 88.2%, were high when the moisture contents were 50% and 55%, respectively. However, the seedling-

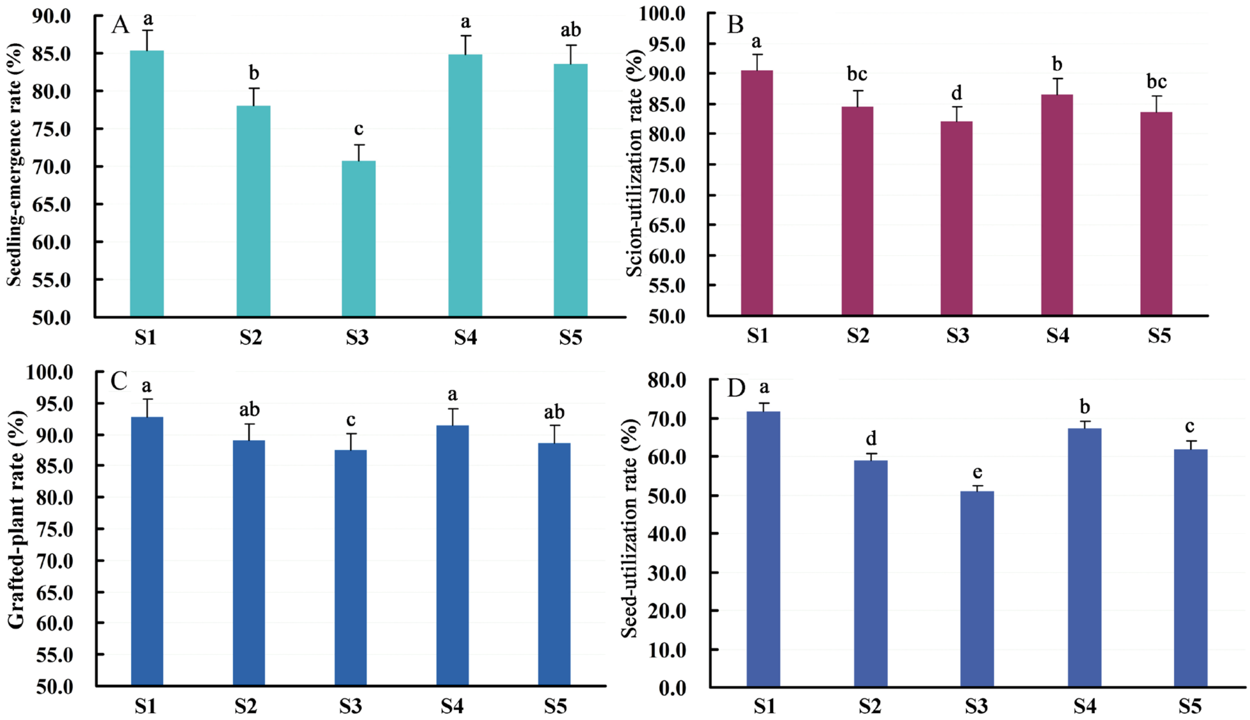

Figure 4. Tested items in the five types of substrate (S1 to S5): (A) seedling-emergence rate; (B) scion-utilization rate; (C) grafted-plant rate; (D) seed-utilization rate

emergence rate of the substrate was lower than that of the control treatment, whose seeds were germinated on the filter papers with a normal level of water (Fig. 5B). Interestingly, we found that seedlings emerged slightly slower than other treatments when the moisture contents were 50% and 55%, but the grown seedlings were stronger than the others (Fig. 5C). Thus, high humidity was inconvenient

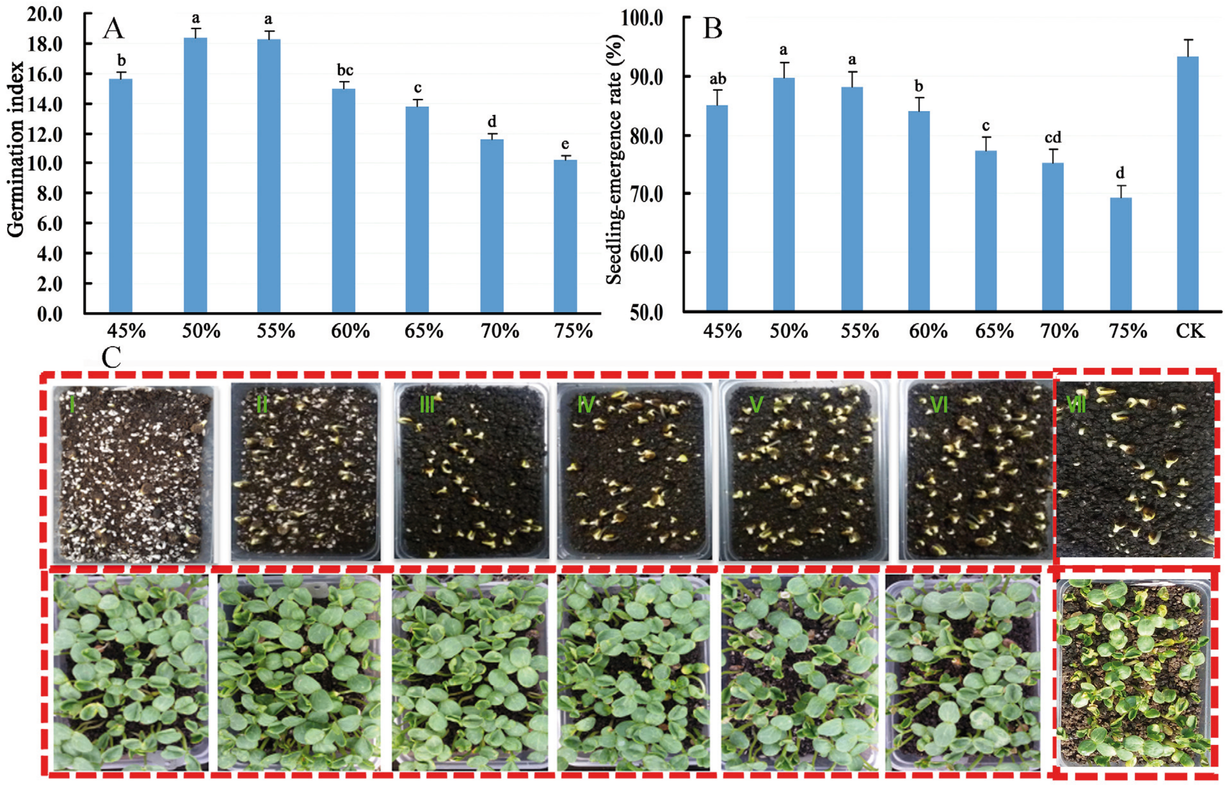

Figure 5. Screening of water moisture in the S1 combination: (A) Germination index; (B) seedling-emergence rate; (C) seed growing in the seven types of moistures. Seedling-emergence rates after 2-4 and 7-8 days

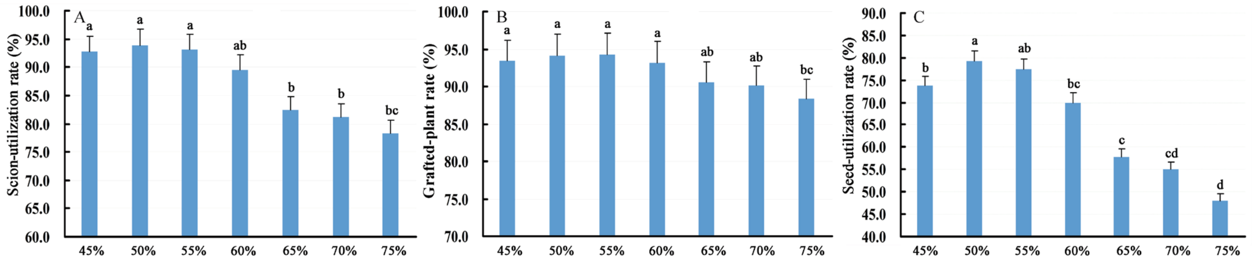

Figure 6. Tested items in the seven types of moisture: (A) scion-utilization rate; (B) grafted-plant rate; (C) seed-utilization rate

for seedling growth (Fig. 5C VII). The results of the grafting experiment showed that the scion-utilization rates which were 92.7%, 93.9% and 93.1%, were higher when the moisture contents were 45%, 50%, and 55% (Fig. 6A), respectively, compared with other treatments. The grafted-plant rates were also higher with the value ranging from 93% to 95%, when the water content was from 45% to 60% (Fig. 6B). After analyzing successfully grafted plants, the seed-utilization rate was > 75% when the substrate's moisture contents were 50% and 55% and the best ratio was 79.2% in the 50% water treatment (Fig. 6C). In this case, by using the optimized processes and proper management, one grafted seedling can be produced simply by using a 1.3 seed. With low (< 50%) and too high (> 60%) water contents in the substrate, the seed-utilization rate would be significantly reduced. A high water content for germination of seeds is harmful for seedling plant growth (Ma et al., 2016). Overall, the optimal water moisture for grafting seedless watermelons in the combination of S1 was 50%-55%.

Screening the optimal time of scion

Previous studies showed that the difference of scions affected the success of grafts (Altayeb and Ali, 2013). In this study, three types of scions were used to evaluate the best time to graft the triploid watermelon seedlings (Tab. 1, Fig. 1). Our results revealed no difference in the seedling-emergence rate in three stages of scions, which were 75.8%, 75.7% and 75.9% (Fig. 7A). Thus, the seedling-emergence rate would not change despite the long cultivation of seedlings. However, variations in the scion-utilization rate were significant. In Ts-3, the scion-utilization rate was 93.8%, which

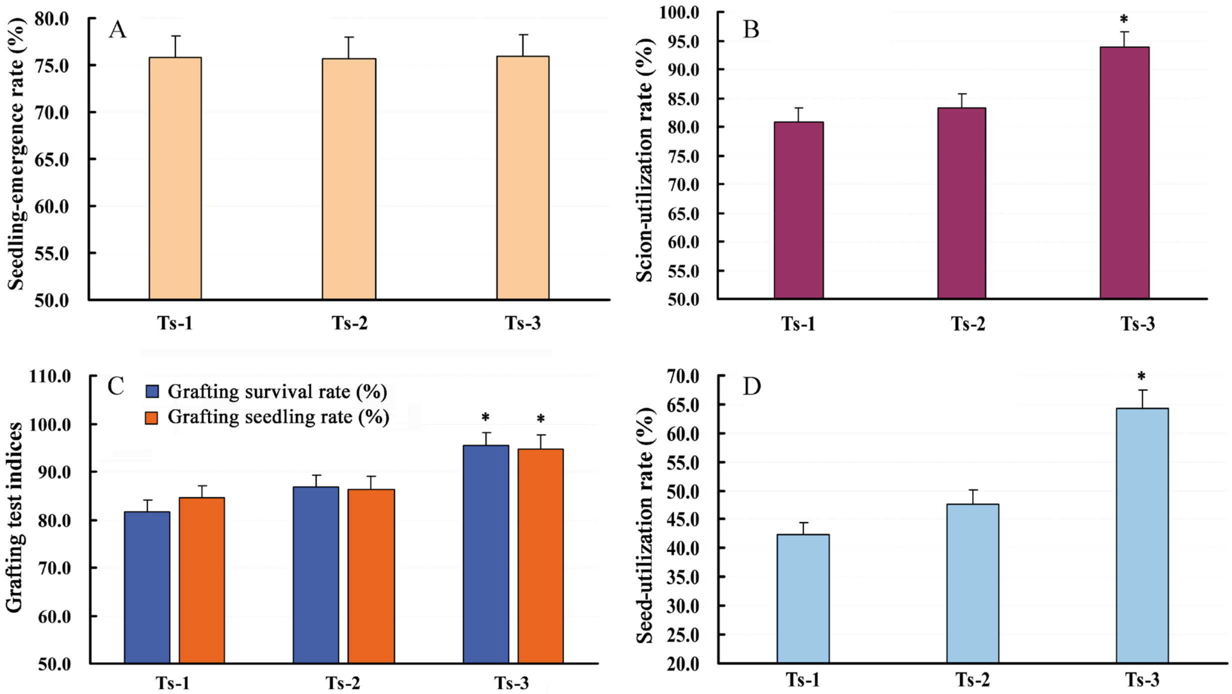

Figure 7. Tested items in the screening of the most suitable scions: (A) seedling-emergence rate; (B) scion-utilization rate; (C) grafting test indices; (D) seed-utilization rate. Three types of scions (yellow bud, Ts-1; two cotyledons did not unfold, Ts-2; and first true leaf appeared, Ts-3) were used. Analysis was achieved by using ANOVA, and means were separated through Fisher's protected LSD test at $p < 0.05$

is significantly higher than that in the other two groups (Fig. 7B), indicating that extending the scion seedling usage can be beneficial for grafting. Meanwhile, grafting survival and grafted-plant rates in Ts-3 were also higher than that in the other two groups (Fig. 7C). Similarly, Rehman et al. (2014) reported that different scions had an effect on grafting.

In comparison with the conventional methods, the usage of scion from Ts-3 significantly increased grafting survival, grafted plant, and seed-utilization rates by 13.7%, 10.1% and 22% (Fig. 7D), respectively. Our results suggest that scion can be cultivated to the stage where the first true leaf unfolded, and then it is suitable for additional successful grafts. The same observation has also been reported in the cucumber grafting experiment that the survival rate of the large scions was higher than that of the small scions (Oda et al., 1993). One possible explanation is that older scions will be easily adapted to the rootstock. M-Bhatt et al. (2013) also reported the increased success in the large scion of apple seedlings due to the better cambial contact of the scion with rootstock. Therefore, we concluded that using older scions will improve the efficiency of grafting seedlings of triploid watermelons. This is the first study to evaluate the breeding of grafted plants of seedless watermelons. However, this experiment has an unsolved issue, i.e., how to adjust the matrix density to minimize shelled seeds in the emergence of seedlings. Finally, available information from this work has illuminated the proposed methods to directly increase the utilization of triploid watermelon seeds. Nevertheless, the results of this study are applicable for the introduction of the breeding technique to the growers for the high-quality grafted seedlings of triploid watermelon.

CONCLUSIONS

1. One suitable substrate formulation was proposed, and water moisture was also optimized for seedless watermelons. Our results showed that the combination of S1, which was compounded of peat and perlite with 1:1 volume ratio, exhibited the best seed-utilization rate of 71.6%.
2. The most suitable water moisture in S1 substrate ranged from 50% to 55%. In comparison with the conventional method, the usage of scion from the Ts-3 treatment significantly increased the grafting survival, grafted-plant and seed-utilization rates by 13.7%, 10.1%, and 22%, respectively.
3. The best time to use the scion should be in the seedling stage where the first true leaf unfolded and the rootstock should grow in this stage as well. The proposed method not only reduces the production cost of the seedlings but also significantly improves the efficiency of grafting seedlings.

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AUTHOR CONTRIBUTIONS

The contributions of authors to the study are as follows: Z.J. and W.P.C. – designed the research; Z.J., W.P.C., W.Y., F.L., J.H.K., and Z.Q.A. – conducted the research; Z.J., W.P.C., T.H.M., and W.Y. – analyzed the data; Z.J., W.P.C., T.H.M. and W.Y. – wrote the paper.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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