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COMPARISON OF THE EFFECTS OF SEVEN HYBRID COMBINATIONS OF PUMPKIN ROOT STOCKS ON THE GROWTH AND ANTIOXIDANT ENZYME ACTIVITY OF GRAFTED CUCUMBER

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Abstract

At present, melon crops suffer significant losses due to long-term continuous cropping, which can be overcome in production by selecting excellent rootstocks for grafting. This study used seven different hybrid combinations of pumpkin rootstocks as materials. We measured growth physiological indicators by grafting with cucumbers, including: Measuring plant height with a tape measure, which is the length from the root stem junction to the growth point. Measuring scion stem thickness with a vernier caliper, which is the thickness at 1 cm above the grafting junction parallel to the cotyledon development direction. Manually counting the number of leaves, starting from the bottom and counting them one by one, each time confirming the position of the previous leaf to avoid duplication or omission. A portable chlorophyll meter (SPAD-502) was used to measure the relative chlorophyll content of grafted seedlings. The determination of antioxidant enzyme activities (SOD, POD, and CAT) was carried out in a physiological laboratory, following the method of Nakano and Asada (1981) with slight improvements. The purpose of this study was to compare the differences in plant growth and antioxidant enzyme activity of grafted cucumbers, and to screen for good cucumber rootstock materials, providing a basis for the breeding and utilization of cucumber grafted rootstocks. This study used the membership function value method to analyze the comprehensive evaluation of the effect of pumpkin rootstock hybrid combination grafting on cucumber growth. Based on the research results, it was found that the Yanbian-3×360-3 grafted seedlings had the highest plant height, stem thickness, chlorophyll content, leaf number, and antioxidant enzyme activity indicators, with a total membership function value of 3.734, which was the highest. This indicates that, as a rootstock for grafting, it can improve the growth and antioxidant capacity of cucumber seedlings. Therefore, it can be validated and promoted as an excellent rootstock for cucumber grafting in production, providing a basis for screening excellent commercial cucumber rootstocks.

Key words: Pumpkin root stock, Hybrid combinations, Grafting, Cucumber, Growth and development, Antioxidant enzyme activity.

Introduction

Grafting is one of the most convenient methods to increase crop yield, improve crop quality, and overcome continuous cropping obstacles (Wang Jianan *et al.*, 2018). Grafting technology has been widely used in agricultural production to increase crop yield, improve crop quality, overcome obstacles to continuous cropping, and improve the stress resistance of vegetable crops, such as resistance to salt (Yang Xiuling, 2015), temperature (Gao Junjie *et al.*, 2009), moisture (Zhang Zhihuan, 2016), copper (Tan Mingming *et al.*, 2014), and a series of other issues. Lin Lijin (2005) and Huang Xingxue *et al*., (2013) mainly focused on improving the quality traits of bitter gourd; while Wu Zhuangsheng *et al*., (2014) and Li Dazhong *et al*., (2008) mainly focused on improving the stress resistance of bitter gourd. In addition, it was mentioned that Stegemann & Bock (2009) provided preliminary progress in their research on grafted tobacco, and Nakamura *et al*., (2016) made progress in their research on heat-resistant tomato rootstocks, both contributing to further understanding the molecular mechanisms by which grafting alters plant resistance.

Cucumber (*Cucumis sativus* L.), an annual vine or climbing herbaceous plant in the gourd family, is one of the main vegetables. With the continuous improvement of cultivation facilities, the annual production of cucumbers has increased significantly. However, long-term continuous cropping has also led to serious occurrences of diseases and insect pests, causing significant losses to production (Bai Quanjiang *et al.*, 2012; Gu Duanyin *et al.*, 2005). Grafting cucumbers can overcome continuous cropping obstacles, improve stress resistance and pest and disease resistance, and promote above-ground growth and yield, thus achieving yield increases, disease prevention, and stress resistance (Liu Qing *et al.*, 2016; Yu Xianchang & Wang Lijiang, 1998; Rivero *et al.*, 2003; Sun Yan *et al.*, 2002). Choosing a good rootstock is crucial for grafting cultivation. Pumpkin, with its developed root system, strong disease resistance, and stress resistance, is widely used as a rootstock for melon grafting (Li Han *et al.*, 2013). Different pumpkin rootstocks have different characteristics, and after grafting, they exhibit different effects on the resistance, growth potential, yield, and quality of the scion plants (Tao Meiqi *et al.*, 2018). Previous studies have shown that grafting can enhance the growth potential of cucumber plants, significantly prolong the harvest period of cucumber fruits, and increase yield (Jiao Zigao, 2000). In production practice, selecting cucumber seedlings and pumpkin seedlings with coordinated stem thickness for paired grafting is beneficial for the nutrient growth of the above-ground parts of the plant, prolonging the growth period, and increasing the total yield of cucumbers (Zeng Yi'an *et al.*, 2004, 2005; Mo Yunbin *et al.*, 2005).

This study used seven different hybrid combinations of pumpkin as rootstocks and grafted them onto cucumbers. By comparing the differences in plant growth potential and antioxidant enzyme activity between the grafted cucumbers and those without grafting (or perhaps more specifically, between cucumbers grafted onto different pumpkin rootstocks), we aim to screen for superior rootstock materials and provide a basis for the selection and utilization of cucumber grafting rootstocks.

Material and Methods

Materials. Test materials. The tested pumpkin rootstock hybrid combinations include 'Hetoua2×041-1', 'Yanbian-3×Lingchuanc1', 'Yanbian-3×360-3', 'Hetoua2×360-3', 'Yanbian-4×Lingchuanc1', 'Yanbian-2×041-1', and '360-3×041-1'. All of these were provided by the School of Horticulture and Landscape Architecture, Henan Institute of Science and Technology (see Table 1 for details). Based on the seed germination rates of these different pumpkin hybrid combinations, seven hybrid combinations were selected for grafting experiments. The cucumber scion variety used was Jinyun 301, which was purchased from Tianjin Gengyun Seed Industry Co., Ltd.

Test method. The trial will be conducted in an open field in Huojia, Henan Province, from April to October 2023. Huojia County is situated in the northern part of Henan, within the western region of Xinxiang City. It is bordered by the Taihang Mountains to the north and the Yellow River to the south, with geographical coordinates ranging from 113°39′42″E to 113°44′20″E and from 35°9′34″N to 35°15′37″N (note: the original coordinates for the northern latitude seem to have a typo; I assumed it should be 35° rather than repeated 35° followed by numbers that don't fit typical latitude format). The terrain is flat, and the climate is warm temperate continental monsoon. The average frost-free period is 221.2 days annually, with an average annual rainfall of 557.2 millimeters, an average of 14.1 snowy days, and an average of 2,058.4 hours of sunshine.

To prepare the pumpkin rootstock seeds, disinfect them in boiling water at 55°C for 10 minutes, stirring continuously throughout the process. Once the water cools to room temperature, soak the seeds for 8 hours and then place them in a constant temperature incubator at 28°C for germination. After approximately 24 hours, when the embryo has grown 2-3 mm, sow each hybrid combination with 50 seeds. Cucumbers should be sown 4 days after the pumpkins, with a total of 400 seeds sown.

On April 28th, when the first true leaf of the pumpkin has unfolded and the cucumber cotyledons have just flattened, perform the grafting. Use a blade to remove the growth point of the pumpkin to ensure a clean cut. Then, insert a bamboo stick at an angle of 30-40° (note: the original angle of 3-4mm seems incorrect for an angle; I assumed it should be degrees) from the main leaf vein on the right side of the pumpkin cotyledon, towards the opposite side, with a thickness that matches the young stem of the scion. This prevents the scion's young stem from rupturing and promotes wound healing. Cut the cucumber 1 cm below the cotyledon into a wedge shape and insert it securely into the hole in the pumpkin seedling, forming a "cross" with the cucumber cotyledon facing south.

After grafting, provide shade and moisture for 7 days, gradually increase light exposure, manage normally after 10 days, and measure the survival rate of the grafted seedlings 14 days post-grafting.

Measurement Items and Methods (1) Grafting survival rate. The grafting survival criterion is that the interface heals well (Fusion and differentiation of wound gaps to form new thin-walled callus tissue) and the grafted seedlings grow normally (The scion does not wilt and new leaves grow) (Zhou Junguo *et al.*, 2010).

$$\text{Grafting survival rate (\%)} = \frac{\text{Number of surviving plants}}{\text{Total number of grafted plants}} \times 100 \qquad (2)$$

Table 1. Resources of different pumpkin hybrid combinations.

Pumpkin hybrid combinations	Hundred-grain weigh/g	Seed color	Number of seeds per melon
Hetoua 2×041-1	8.75	yellow and white	352
Yanbian-3×Lingchuanc1	10.31	yellow and white	304
Yanbian-3×360-3	12.296	grey	246
Hetoua2×360-3	9.69	yellow and white	303
Yanbian-4×Lingchuanc1	8.85	yellow and white	434
Yanbian-2×041-1	9.57	white	135
360-3×041-1	12.648	white	290

Determination of growth physiological indicators. Plant height refers to the length from the junction of roots and stems to the growth point; The thickness of the scion stem is measured at 1cm above the grafting junction parallel to the direction of the cotyledons, we test 9 plants per group, set up 3 replicates, and take the average value as the final result using a vernier caliper; count the number of leaves one by one from the bottom, and confirm the position of the previous leaf during each count to avoid repetition or omission. For the convenience of counting, a side view angle is used for counting, so that the number and arrangement of blades can be seen more clearly. Measure the relative chlorophyll content of grafted seedlings using a portable chlorophyll analyzer SPAD-502. The determination of antioxidant enzyme SOD, POD, and CAT activities was conducted with slight modifications based on according to the methods of (Nakano & Asada, 1981). (3) Membership function. The comprehensive evaluation of the effect of pumpkin root stock hybrid combination grafting on cucumber growth was conducted using the

membership function value reference method, which was calculated using the method of referenced in (Song Hongyuan *et al.*, 1998) and the formula. The larger the membership function value of the hybrid combination of pumpkin root stocks and grafted seedlings, the better the growth potential of the grafted cucumber.

Data processing: Use Excel 2003 to analyze the experimental data and calculate the mean, and SPSS 27 for analysis of variance. Compare the systematic errors resulting from a specific controlling factor to other random errors to determine whether there are significant differences between each group of samples, in order to assess whether the factor has a significant impact on the population.

Results and Discussion

Effects of different pumpkin root stock hybrid combinations on the survival rate of cucumber grafted seedlings.

As can be seen from Fig. 1, the survival rates of the Yanbian-3×360-3 and Yanbian-2×041-1 pumpkin rootstock grafted seedlings are the highest, both exceeding 90%, specifically 96% and 94%, respectively. Hetua2×360-3, Yanbian-3×LinghuanC1, and Yanbian-4×LinghuanC1 all exceeded 85%, with rates of 88%, 86%, and 86%, respectively.

Effects of different pumpkin root stock hybrid combinations on the growth indicators of cucumber grafted seedlings.

From Table 2, it can be seen that the four growth indicators of the Yanbian-3×360-3 and Yanbian-2×041-1 pumpkin rootstock hybrid combination grafted seedlings are higher than those of other pumpkin rootstock hybrid combination grafted seedlings. Specifically, there is a significant difference in these indicators when compared to the others. However, there is no significant difference in the plant height index between Hetoua2×360-3 and the two aforementioned varieties.

Effects of different pumpkin root stock hybrid combinations on membrane lipid peroxidation in leaves of grafted cucumber seedlings.

From Table 3, it can be seen that the four growth indexes of the grafted seedlings from the Yanbian-3×360-3 and Yanbian-2×041-1 pumpkin rootstock hybrid combinations were higher than those of other pumpkin rootstock hybrid combinations, indicating significant differences. However, there was no significant difference in plant height indexes between the Hetoua2×360-3 grafted seedlings and those of the aforementioned two hybrid combinations.

Membership function values of different pumpkin root stock hybrid combinations on related indicators of cucumber grafted seedlings.

From Table 4, it can be observed that the total value of the membership function for Yanbian-3×360-3 is the largest, at 3.734, while the total value for 360-3×041-1 is the smallest, at 0.060. This indicates that cucumbers grafted with Yanbian-3×360-3 exhibit better growth, whereas those grafted with 360-3×041-1 show poorer growth. Based on the membership function values, the order of growth potential for cucumbers grafted with hybrid combinations of pumpkin rootstocks is as follows: Yanbian-3×360-3, Yanbian-2×041-1, Hetoua2×360-3, Yanbian-3×LingchuanC1, Yanbian-4×Lingchuanc1, Hetoua2×041-1, 360-3×041-1.

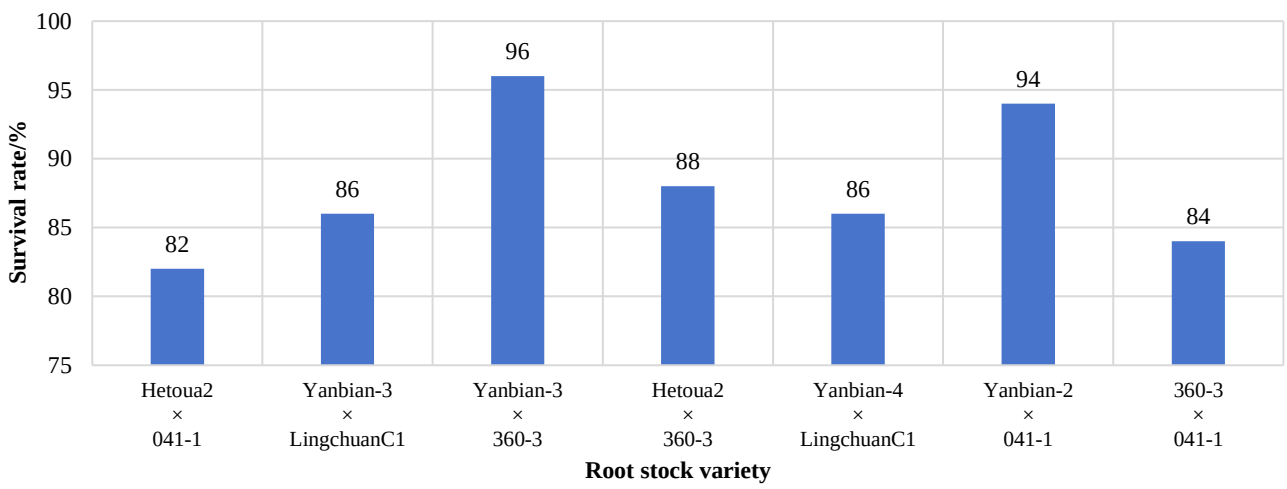

Fig. 1. Effects of different pumpkin root stock hybrid combinations on the survival rate of cucumber grafted seedlings.

Table2. Effects of different pumpkin root stock hybrid combinations on the growth indicators of cucumber grafted seedlings

Root stock variety	Plant height	Stem diameter	Chlorophyll	Number of blades
Hetoua2×041-1	99.33 ± 13.43d	8.73 ± 0.53cd	52.77 ± 1.68d	12.67 ± 1.53c
Yanbian-3×LingchuanC1	98.67 ± 3.21cd	9.05 ± 0.59bc	56.00 ± 0.78bc	13.67 ± 0.58bc
Yanbian-3×360-3	115.67 ± 8.50a	10.91 ± 0.11a	57.93 ± 1.22a	15.00 ± 1.00a
Hetoua2×360-3	113.33 ± 6.66ab	9.68 ± 0.301b	55.80 ± 2.54b	14.00 ± 1.00ab
Yanbian-4×Lingchuanc1	102.67 ± 3.21bc	9.45 ± 0.88bcd	53.03 ± 1.95cd	12.00 ± 1.00bc
Yanbian-2×041-1	115.33 ± 10.50a	10.36 ± 0.50a	58.30 ± 1.01a	16.00 ± 1.00a
360-3×041-1	94.00 ± 11.00d	8.73 ± 0.53d	53.10 ± 0.89d	11.00 ± 1.00c

Table 3. Effect of different pumpkin root stock hybrid combinations on membrane lipid peroxidation in leaves of grafted cucumber seedlings.

Root stock variety	SOD/U·g^{-1}(FW)	POD/U·g^{-1}·min^{-1}	CAT/U·g^{-1}·min^{-1}
Hetoua2×041-1	50.01 ± 1.66d	449.62 ± 3.54e	347.76 ± 9.64d
Yanbian-3×LingchuanC1	53.72 ± 0.97bc	495.78 ± 5.66d	392.41 ± 3.81c
Yanbian-3×360-3	61.75 ± 2.05a	605.83 ± 8.23a	440.93 ± 9.28a
Hetoua2×360-3	56.13 ± 1.36b	523.98 ± 5.68c	426.51 ± 6.67b
Yanbian-4×Lingchuanc1	53.14 ± 2.64c	487.59 ± 11.66d	388.8 ± 6.19c
Yanbian-2×041-1	60.54 ± 1.05a	538.34 ± 7.92b	434.28 ± 5.68a
360-3×041-1	51.25 ± 1.35cd	453.98 ± 5.67e	354.97 ± 7.18d

Table 4. Membership function values of different pumpkin root stock hybrid combinations on related indicators of cucumber grafted seedlings.

Root stock variety	Plant height	Stem diameter	Chlorophyll	Number of blades	Sum	Order
Hetoua2×041-1	0.246	0.002	0.000	0.333	0.581	6
Yanbian-3×LingchuanC1	0.215	0.148	0.584	0.533	1.480	4
Yanbian-3×360-3	1.000	1.000	0.934	0.800	3.734	1
Hetoua2×360-3	0.892	0.434	0.548	0.872	2.746	3
Yanbian-4×Lingchuanc1	0.400	0.331	0.048	0.200	0.979	5
Yanbian-2×041-1	0.985	0.745	1.000	1.000	3.730	2
360-3×041-1	0.000	0.000	0.060	0.000	0.060	7

Results and Discussion

Generally, materials with good grafting affinity tend to heal better and exhibit a higher grafting survival rate (Zhou Junguo, 2008). This study concludes that the grafting seedlings of Yanbian-3×360-3 and Yanbian-2×041-1 pumpkin rootstocks have the highest survival rates, exceeding 90% with 96% and 94% respectively. This indicates that when used as rootstocks, Jinyun 301 cucumber exhibits better grafting compatibility with Yanbian-3×360-3 and Yanbian-2×041-1. The impact of grafting on plant growth is largely dependent on the selection of rootstocks, and suitable rootstocks can effectively enhance the biomass of grafted seedlings (Tan Mingming *et al.*, 2014; Yuan Junwei *et al.*, 2019). The results of this study further show that the grafting seedlings of Yanbian-3×360-3 and Yanbian-2×041-1 pumpkin rootstock hybrid combinations exhibit the highest values in plant height, stem diameter, chlorophyll content, and leaf number indicators, suggesting that both have the best growth potential after grafting cucumber.

There are two types of protective systems in plants: the first is the enzymatic protective system, which includes SOD, POD, CAT, APX, GR, and DHAR; the second is the non-enzymatic protective system, which comprises antioxidant substances such as glutathione (GSH) and reduced ascorbic acid (AsA) (Liu Fengjiao *et al.*, 2017). In the enzymatic protection system, SOD serves as the first line of defense, protecting plant antioxidant systems by converting reactive oxygen species to H2O2 (Asada, 2000). POD and CAT can directly scavenge H2O2 in plants, decomposing it into H2O and O2 to reduce the damage caused by reactive oxygen species (Zhu *et al.*, 2008). The results of this study indicate that Yanbian-3×360-3 grafted seedlings have the highest antioxidant enzyme activity index, suggesting that grafting can improve the antioxidant capacity of cucumber seedlings and inhibit the production and accumulation of reactive oxygen species. Additionally, the total value of the membership function for Yanbian-3×360-3 is the largest, at 3.734, indicating that Yanbian-3×360-3 grafted cucumbers have the best growth performance.

Conclusion

The results of this study show that Yanbian-3×360-3 grafted seedlings exhibit the highest plant height, stem diameter, chlorophyll content, leaf number index, and antioxidant enzyme activity index. Additionally, their total membership function value is the largest, indicating their potential as a rootstock for improving cucumber growth. This suggests that using Yanbian-3×360-3 as a rootstock for grafting can enhance the growth and antioxidant capacity of cucumber seedlings. Therefore, it can be validated and promoted as an excellent rootstock for cucumber grafting in production, providing a basis for screening superior rootstocks for commercial cucumbers.

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