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Advances in Research of Cutting Breeding Technology of *Xanthoceras* sorbifolium Bunge

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Abstract Cutting breeding is an important method of asexual reproduction of *Xanthoceras sorbifolium* Bunge. At present, it has been found that the factors influencing the cuttings of *X. sorbifolium* mainly include cutting material type, substrate type, cutting season, cutting material length and thickness, mother tree age, cutting material pretreatment method, hormone species concentration and soaking time. Besides, different regions, control conditions, and germplasm types have different cutting rooting rates. This paper introduced some of the problems in the cuttings of *X. sorbifolium*, and came up with some recommendations to provide a reference for the future research and technical promotion of *X. sorbifolium* cuttings.

Key words Xanthoceras sorbifolium Bunge, Cutting, Rooting rate, Asexual reproduction, Research advances

1 Introduction

Xanthoceras sorbifolium Bunge is a deciduous shrub or small tree of Xanthoceras Bunge in the family Sapindaceae Juss. It is widely distributed in Xinjiang, Qinghai, Tibet, Ningxia, Gansu, Shaanxi, Shanxi, Hebei, Liaoning, Inner Mongolia, Henan, Shandong, northern Jiangsu, and northern Anhui. It is an excellent woody oil tree species unique to China. In recent years, it has been cultivated in large quantities. X. sorbifolium is resistant to drought, barrenness, sand-dust, and can grow in stony mountains, loess hills, calcareous alluvial soil, and fixed or semifixed sandy areas. Its seed oil content is 45% - 50%, and the kernel oil content is as high as 70% [1]. The oil is yellow and transparent, edible and delicious, has medicinal effects. The oil cake can be used as feed and fertilizer. X. sorbifolium has beautiful plant shape, excellent flowers and leaves, large inflorescence and dense flowers, and the flowering period can last for more than 20 days. It has high ornamental value, is an excellent greening tree species, and its production scale is getting larger and larger. As an important method of asexual reproduction, cutting can maintain the excellent traits of the variety, and have early flowering, fast growth, and large reproduction volume. Compared with other asexual reproduction methods, it has such advantages as high efficiency, lower cost, and easy promotion. In this study, we sorted out technologies related to cutting of X. sorbifolium, in order to provide guidance for X. sorbifolium production.

2 Effects of cutting materials on cuttings

Effects of cutting material types on cuttings According to the current reports, the cutting material types used in X. sorbifolium cuttings are root, hard branch and tender branch. The earliest report of cutting research can be found in 1978, that is the air culture method to study the rooting of X. sorbifolium cuttings by Research Institute of Forestry, Chinese Academy of Forestry^[2]. Affected by the differences in regions and germplasm types, the three types of cutting materials have slightly different effects on rooting rate. The rooting rate of root cuttings is the highest. For example, Wang Yilin^[3] and Mo Baoru^[4] found that X. sorbifolium root cuttings were better than hard branch cuttings and tender branch cuttings. However, the rooting rates of hard branch and tender branch cuttings are different. The study of Zhao Guojin et al. [5] on different cutting material types in Binzhou of Shandong in 2006 showed that the rooting rate of tender branches was slightly higher than that of hard branches. In 2013, Song Qunyan found that the cutting rooting rates of roots, tender shoots and hard branches differed significantly in different seasons in Daqing, Heilongjiang, and the cutting rooting rate of tender branches was better than the hard branch cuttings^[6]. Through analyzing the maximum value of cutting rooting rate of different types of cutting materials obtained in the literature (macroscopically, without considering the difference of time, region and germplasm type), it is found that the rooting rate of root cuttings is the highest, and the difference between hard and tender branches is extremely significant, the maximum rooting rate is 95.3% [7], the average is 84.61%. The difference between hard branches and tender branches is not significant, followed by rooting rate, the maximum value is 93.3% [8], the average is about 58.60%. The cutting rooting rate of tender branches is the lowest, the maximum value is

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sources. E-mail: zhaoyj3308@163.com Editorial Office E-mail: asiaar@163.com 94.9% [9], the average is about 56.42%. These are indicated in Table 1 and Table 2, and the data source is seen in Table 3.

Variance analysis of rooting rate of cutting material types Table 1

Group	Number of observations	Sum	Mean value	Variance
Root	16	1 353.75	84.61	50.41
Tender branch	12	677.05	56.42	371.86
Hard branch	18	1 054.72	58.60	585.27

Significance of the effect of cutting material type on rooting Table 2

Difference source	df	MS	F	P	F crit
Inter-group	2	3 787.81	11.01	0.000 138	3.21
Intra-group	43	344.10			
Total	45				

2.2 Effects of cutting material depth and length on cuttings

In the study of the effects of different cutting material length and thickness on the cuttings, Dong Yunlan et al. [8] compared the different cutting material thickness (0.4, 0.6, 0.8 cm) and found that the thickness was directly proportional to the healing and rooting amount. Song Qunyan^[6] and Xing Helong^[10] used cutting materials with thickness of 3, 6 and 9 cm, and made a comparative analysis and found that 6 cm was the best. Using root cuttings with different lengths in Jining, Shandong, Cheng Ran found that the cutting material with 10 cm length was slightly better than the cutting material with the length of 5 and 15 $\mathrm{cm}^{[11]}$. In 2010, a hard branch cutting experiment was conducted in Yangling. Shaanxi Province. Sun Bingyin^[12] found that the rooting rate of cutting materials with a length of 10 cm was significantly higher than that of cutting materials with a length of 5 and 15 cm (P < 0.05), and the difference in the rooting rate of cutting materials with a length of 10 and 15 cm was not significant, the rooting rate of cuttings with the diameter of 0.5 - 0.8 cm is significantly higher than that of 0.2 - 0.5 cm. According to analysis of Mo Baoru et al. [4] on the root length, the rooting rate of 9 cm cutting material was significantly higher than that of 6 and 12 cm treatment, which was 64.9%. Wang Junxi analyzed the effects of rooting quantity and root length on roots of different lengths in Hengren County, Liaoning, and pointed out that the effect of 8 cm cutting was better than that of 5 and 11 cm^[13]. According to Wang Huilin's research on the number of roots and the length of roots in Fuxin, Liaoning, when the root length was set at 3, 6, and 9 cm, the rooting rate of the 6 cm root was the highest (90.5%), and the average rooting number and average root length were higher than the that of the control group [14].

No.	Year	Region	Time month/day	Hormone	Hormone concentration	Treatment time//h	Material length // cm	Material type	Substrate	Rooting rate // %	Remarks
1[2]	1978	Beijing	9/1	IBA	250	Mud quick	15 - 20	Hard branch	Air culture	50	
						soaking					
2[23]	1980	Pu County, Shanxi	4/23	IBA	150	6	18 – 20	Hard branch	Sand and soil 45 cm	60.9	
3[48]			9/29	IBA	50	24	15 – 20	Hard branch,	Vermiculite, middle 5-6 cm fine	56.7	
								upper $10-15~\mathrm{cm}$	sand, lower $-6~\mathrm{cm}$, small pebbles		
4[8]	1982	Shan County, Henan	2/20	IAA	200	16	12 – 15	Hard branch	Fine sand 25 cm	93.3	0.6-1.0 cm thick
				IBA		16			Sawdust		
									Soil		
2	2003		9/16	NAA + ABT	500 + 100	15 (s)	20	Hard branch	Sand and soil layering	35	Sand covered to 1/2-1/3 height of cutting material
5[11]		Jining, Shandong	6/5	IBA	100	15 (s)	10	Tender branch	Sand, vermiculite, perlite	29	
			10/16	NAA, IBA, ABT	500	15 (s)	10	Root	Sand and soil layering	92	Sand wholly covered
6[15]	2005	Makehe, Qinghai	4/1	ABT6			13 – 15	Tender branch	Humus: Perlite: Vermiculite 7:3:1	94.9	
	2004		9/25	ABT	250		10	Hard branch	Upper sand 5 cm, lower soil 5 cm	38	Cutting depth is 1/2-2/3 of the cutting material length
7[5][49]		Binzhou, Shandong									
	2005	-	5/25				10	Tender branch	Peat and vermiculite	47	
	2004		10/15	ABT	250	30 (s)	10	Root		94	
8[22]	2010	Shenmu County, Shaanxi	4/21	NAA	150	5-8 (s)	10	Root	Cow dung: soil 1:2	70	
9[12]	2010		Middle of March	ABT1	400	2		Hard branch		88.6	
	2010	Yangling, Shaanxi	Early of October	IBA	600	4	12 - 15	Hard branch	River sand	66.11	
	2000		10/25	ABT1	150		6	Hard branch		34	
			10/25				6	Tender branch		44	
			5/20				6	Root		88	
11[51]		Daqing, Heilongjiang					6	Root		87.6	One-year old mother tree
									Peat: vermiculite: perlite: sand 4: 2: 2: 1	89.1	

(Continued)

No.	Year	Region	Time month/day	Hormone	Hormone concentration	Treatment time//h	Material length//cn	Material type	Substrate	Rooting rate // %	Remarks
			month/ day	IAA	100	1		Root	Vermiculite: sand: humus 6:2:1	80	
12[20]	2013	Dingxi, Gansu	8/22	NAA	100	6	13 – 15	Root	Vermiculite: sand: humus 6:2:1	80	
		,	Early of April	IBA	100			Hard branch		38.89	
13[4]		Dingxi, Gansu	Middle of August	ABT6	100			Tender branch		58.57	
			Middle of August	NAA	100			Root		80.1	
			6/10	ABT2	250	1 (min)	8	Tender branch		51.08	With 2 and half leaves
14[3]	2014	Zhangye, Gansu		ABT2	250	1 (min)	12	Hard branch		37.42	Cutting depth 4-6 cm
				ABT2	250	1 (min)	12	Root		94.24	Fully covered with sand
15[52]		Jianping County, Liaoning		ABT2	125	6		Hard branch		90	
16[21]	2016	Kazuo County, Liaoning	4/1	IBA + NAA (1:1)	125	6	10 - 15	Hard branch	River sand	90.67	
			Spring	NAA	100	4		Root	Peat: vermiculite: perlite 1:1:1	80.1	
17[13]							8	Root		82.3	
			Summer	ABT6	100	2	10	Tender branch		58.57	
18[16]	2016	Yanbian University	June	IBA	1 000	1 (min)	10 – 15	Tender branch		87.4	Yellowing of the base
				ABT			6	Hard branch		34	
						6	Tender branch		39		
19[10]		Fuxin, Liaoning					6	Root		78	
								Root		88.9	One-year old mother tree
20[7]				NAA or ABT	250			Root		95.3	
		Laiwu, Shandong						Hard branch	Vermiculite	31.2	
	21[13]						Tender branch		42.7		
21[13]			AB6				Root		80.24		
			ABT6				Tender branch		57.23		
						8	Root		82.97		
22[29]	2018	Yangqu County, Shanxi	Early and middle of June	IBA	200	2	12 – 15	Tender branch		67.6	Cutting depth is 2/3
		Spring	IBA	200	4				82.5		
23[17]		College of Forestry, Shanxi Agricultural University	April					Hard branch	Peat: perlite 7:3	38.33 ± 10.4	1 Covered with sand in the cellar for wintering

Note: Ranked according to the publication (test) time; The blank area means no description in the original literature.

2.3 Effects of mother tree age on cuttings Song Ounvan selected X. sorbifolium cuttings of different tree ages for root cutting experiments, and statistics of the seedling height, ground diameter, average rooting number, and average rooting rate of root cuttings showed that there was significant difference in the average rooting rate between 1, 2, 3, 5, and 20-year-old mother trees^[6]. Ma Mingcheng et al. [15] used cutting materials with different ages of 1-3 years for experiment, and found that as the age of the mother tree increased, the cutting rooting rate and seedling growth decreased, the number of roots decreased, and the ground diameter decreased. The experimental results of Wang Huilin [14] and Xing Helong [10] showed that there was no significant difference between the average root number and rooting rate of 1, 2, and 3 year old mother trees after cutting, but their average root number and rooting rate were significantly higher than that of 10 and 20 year old mother trees.

3 Effects of pretreatment and hormone treatment on cuttings

3.1 Effects of pretreatment on cuttings In Yanbian University, Jin Xianghua yellowed the tender branches of *X. sorbifolium*,

and found that compared with complete yellowing and non-yellowing treatments, base yellowing of cutting materials would significantly increase the rooting rate of cuttings^[16]. Li Xiang *et al.* ^[17] set up three kinds of cutting material pretreatment methods for natural overwintering, cellar sand storage overwintering, and refrigerator low temperature storage, and found that the average rooting rate of different pretreatment methods was significantly different. The average rooting rate of overwintering cutting materials in the cellar sand storage was significantly higher than other methods, with a rooting rate of 38.33%.

3.2 Effect of different hormones and treatment time on cuttings Through comparing the literature of *X. sorbifolium* cuttings, it was found that the hormones used were rooting powder ABT1, ABT2, ABT3, ABT4, ABT6, indoleacetic acid (IAA), indolebutyric acid (IBA), naphthaleneacetic acid (NAA), and various hormone compounds. For hard branch cuttings and tender branch cuttings, the rooting rate of cuttings can be increased by varying degrees, but the survival of cuttings was quite different. In 1982, Dong Yunlan *et al.* reported that cuttings were treated with 200 mg/L of IAA was used in Shan County of Henan for 10 – 12 h, or 100 mg/L of IBA for 10 h, the rooting rate was 93.3%. In the

Oinghai greenhouse. Ma Mingcheng et al. [15] adopted ABT 6 to treat hard branch cuttings, and obtained a rooting rate of 94.9%. In the experiment carried out in Yangling, Shaanxi, Zong Jianwei et al. [18] found that, compared with IAA and ABT1, IBA had the best rooting effect on X. sorbifolium cutting materials, and IBA 600 and 800 mg/L had a higher rooting rate, which was significantly higher than the rooting rate of IBA 1 000 and 1 200 mg/L. As the concentration of IBA treatment increased, the total root length, total root surface area and total root volume all showed an increasing trend and then decreased, and under 800 mg/L treatment, the total root length and total root surface area reached the maximum^[19]. In the experiment in Dingxi, Gansu, Jiao Qiang et al. ^[20] found that 100 mg/L IAA and 100 mg/L NAA had higher rooting rates. Wang Yilin et al. [3] performed a cutting comparison experiment and a cutting propagation experiment on different types of materials of X. sorbifolium in Zhangye, Gansu, and found that ABT2 250 mg/L was the best for rooting of tender and hard branches, and the average rooting rate was 48.96% and 35.00%. At the same system concentration, the average rooting rate of root cuttings was 43.90% and 57.86% higher than that of tender and hard branch cuttings, respectively. In a study in Kazuo County, Liaoning, Tang Xin et al. [21] found that the hormone IBA + NAA (1:1), hormone concentration 125 mg/L, cutting material root treatment time was 6 h, the rooting rate of X. sorbifolium cuttings reached 90.67%.

4 Effects of different substrates on cuttings

Substrate is an important factor influencing the rooting rate of cuttings. Dong Yunlan et al. [8] explored the cutting substrate of X. sorbifolium in the greenhouse, and proposed that it should be carried out directly in the soil; sand and loam were slightly different; if the ground temperature and soil humidity are well grasped, the experiment also can be carried out outdoors. Wu Hongxue carried out cutting experiments of different substrate types on the roots of X. sorbifolium from 24 provenances in 16 provinces and cities, using formula 1 abandoned farmland topsoil, formula 2 sheep dung/topsoil (1:2), formula 3 cow dung/topsoil (1:2) and formula 4 humic acid/topsoil (1:2), made a comparative analysis of the impact of various factors on the seedling emergence rate of the root of X. sorbifolium, and found that the optimal substrate in the selected substrate type may be formula 3^[22]. Li Xiang et al. ^[17] set up 5 substrate formulas at Shanxi Agricultural University, formula 1 grit/peat/perlite (1:7:2), formula 2 grit/peat/perlite (5:3:2), formula 3 grit/peat (5:5), formula 4 peat/perlite (7:3), formula 5 grit, and found that different substrate formulas have a significant effect on the cutting rooting rate, and the rooting rate from high to low is formula 4 > formula 1 > formula 3 > formula 2 > formula 5, and formula 4 had the highest rooting rate (36.67%). Although other literatures mentioned various substrates and ratios (Table 3), there are relatively few reports and no comparative analysis of various substrate types.

Effects of season on cuttings

The cutting season has a significant effect on the rooting rate

of cutting materials. At present, the months used in cuttings are considerably different. There are reports from mid-February to late October. The distribution of cutting months is shown in Fig. 1, and a considerable part of the cutting experiments are conducted in the greenhouse. Feng Zhiqiang et al. [23] performed a hard branch cutting experiment of X. sorbifolium in Puxian Forest Farm, Shanxi Province, and proposed that in addition to hormone treatment, substrate temperature is also an important factor influencing rooting. Dong Yunlan et al. [8] believed that it is suitable to conduct X. sorbifolium cutting in early spring, control the ground temperature above the air temperature, and the higher the ground temperature (not higher than 37 °C), the faster the injury will be healed. Similar reports have been made in Sichuan - Tibet poplar, Actinidia chinensis Planch, and Pinus sylvestris var. mongolica Litv., etc., mainly because the ground temperature is directly related to the formation of cuttings callus tissue^[24-27]. According to the experiment data, Cheng Ran recommended that the root cutting time should be October 16 (September 16, October 1, October 16)^[11]. Ma Mingcheng et al. [15] pointed out that when the temperature of greenhouse cuttings is controlled not higher than 25 °C, the effect is generally better than field cuttings. The root cutting experiment performed by Song Qunyan in Daqing, Heilongjiang Province showed that the cutting rate on May 20 was higher than that on October 20^[6]. According to Zhao Guojin's research in Binzhou, Shandong, the best cutting time for roots was late winter (rooting rate reached 82.9%), and the cutting effect was the worst in autumn^[5]. Kang Guosheng et al. ^[9] found that the suitable season for X. sorbifolium in Qinghai is mainly concentrated in July, August and September. Tang Guihui et al. [28] recommended that the spring shoots in Chaoyang, Liaoning, stopped growing in late June, and the use of full-light mist tender branch cuttings had a higher rooting rate in early June. Wang Limin conducted experiment in Taiyuan, Shanxi on June 18, 2018, November 8, 2018, December 20, 2019, and February 11, 2019, respectively, and the cutting materials were treated with 200 mg/L IBA for 4 h. The results indicate that in spring (February), the cutting rooting rate was the highest, up to 82.5% [29]. According to the current literature, X. sorbifolium has a broad natural distribution, and the cutting month in each region should be appropriately selected according to the conditions of different regions and cuttings.

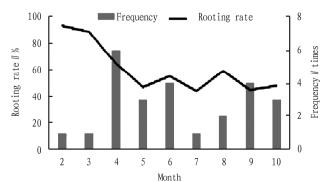


Fig. 1 Month distribution and rooting rate statistics of *Xanthoceras* sorbifolium Bunge cuttings

6 Problems and recommendations

- Influence of cutting material type and specification on cutting effect According to literature analysis, the rooting rate of root cuttings is usually better than other materials, which is consistent with most difficult-to-root tree species such as Catalpa bungei^[30] and Toona sinensis^[31-32]. However, it is difficult to obtain materials for root reproduction and the reproduction coefficient is relatively low. According to the statistical analysis of the current literature reports, the difference between the hard branch cutting and the tender branch cutting of X. sorbifolium is not significant. As far as the current main technology is concerned, hard branch cutting materials are still the mainstream means in X. sorbifolium production. With reference to the general law of cuttings of other tree species, the rooting rate of semi-lignified cutting materials should be higher. For the definition of tender branch or hard branch, the main meaning is not to distinguish the old and young branch, but to find the best time stage and the best position for cutting materials in order to increase the rooting rate. Factors such as the thickness and length of the cutting material directly affect the accumulation of nutrients in the material, which may have a direct impact on the rooting of the cutting material in the early stage. According to the current reports, although the cutting material lengths are not the same, they all show the rule with the best length, that is, too long or too short will affect the rooting rate. As for the influence of the age of the cutting material mother tree on the cutting effect, it shows a strong age effect on hard and young branches, which is consistent with the cutting of many coniferous trees. At present, in the research of X. sorbifolium cutting, the type, size and age of spikelets still need further research and improvement.
- **6.2** Influence of substrates on cutting effect The ideal substrate should have good water retention and ventilation and rich nutrients. By comparison, the composite substrate can directly affect its physical and chemical composition by changing the distribution ratio, and improve the rooting rate of plant cuttings. At present, the comparative experiments of cutting substrates are mostly based on light substrates such as soil, sand, peat and perlite, or a combination of several materials. These materials can be obtained locally, have low cost and are suitable for extensive management. The future research should be to increase the substrate type, refine the ratio scheme, and define the pH range of the substrate formula. At present, the cutting breeding measures have been greatly improved. From the perspective of large-scale production, it is recommended to learn from the successful experience of other tree species in open tissue culture [33-37], water culture [38-42], etc., to expand the cutting breeding methods of X. sorbifolium.
- **6.3** Cutting temperature and humidity control Temperature and humidity control is a key factor for survival of cuttings. According to the research literature, controlling the cutting substrate temperature higher than the air temperature is an important means to increase the cutting rooting rate. The effect of facility-assisted cutting and seedling raising is better than that of open-cutting. The main reason is that the degree of precise control of external conditions is higher, and the cutting rooting time can be shortened and

the efficiency can be improved. As an important means to increase the rooting rate of cuttings, full-light mist cuttings can be combined with auxiliary facilities such as greenhouses to improve cutting efficiency.

- Comparison of hormone type and concentration, soaking time The role of hormones is to cause plants to change their original state of differentiation. Hormone is an important means of regulation for breaking dormancy and promoting germination of hard-to-root tree species. According to the current literature of X. sorbifolium cuttings, basically all experiments are carried out with a fixed ratio of single hormone or ABT. The regulation of plant growth state is the result of the influence of multi-hormonal factors. Different kinds of auxins have different promoting effects on adventitious roots. The experiments of Guo Sujuan and Ling Hongqin prove that the use of a certain endogenous hormone alone to promote rooting of Pinus bungeana cuttings is not obvious. The rooting of cuttings mainly depends on the joint action of IAA and abscisic acid (ABA); the greater the IAA/ABA concentration ratio, the higher the rooting rate of cuttings, and vice versa [43-44]. Therefore, in the future research, it is recommended to focus on the study of the mixed effects of various hormones, the selection of hormone type, concentration and other parameter indicators, and combine the type and evaluation of the germplasm resources of X. sorbifolium.
- **6.5 Influence of cutting material pretreatment on cutting effect** At present, there are relatively few reports on the pretreatment of spikelets, limited to a fewstudies such as Jin Xianghua and Li Xiang. With reference to the treatment of hard-to-root trees such as *Catalpa bungei*, they tried to use a variety of different pretreatment methods to improve the cutting effect [45-46]. In future, it is recommended to learn from the experience of other tree species and explore new models.
- **6.6** Comparison between different strains At present, there are few systematic researches on the differences between different varieties and strains of *X. sorbifolium*. The genetic diversity of *X. sorbifolium* resources varies greatly among regions, and there is a lack of systematic collection and collation. As a key link in the development and utilization of *X. sorbifolium*, systematically collecting *X. sorbifolium* resources, conducting multi-year and multi-point systematic evaluations, and carrying out targeted adjustment of the factors affecting the rooting rate of *X. sorbifolium* cuttings, forming technical system of different strains and different formulas (treatments) are of great significance to the future development of *X. sorbifolium* industrialization.
- **6.7** Research on rooting mechanism At present, the domestic research on cutting rooting mainly focuses on the physiology and anatomy, few studies focus on the mechanism of forest cutting rooting from the molecular level^[47]. Most of the studies on *X. sorbifolium* cuttings remain at the preliminary exploration stage. The results obtained from the treatment of various experimental factors are mostly descriptions of the results. There is no in-depth analysis to study the interaction between the various factors and the factors that cause difficult to root. It is recommended to summarize and draw on the previous research results to further understand the molecular mechanism affecting rooting,

so as to realize the efficient and large-scale reproduction of the asexual reproduction of *X. sorbifolium*.

7 Prospects

Cutting is an important means of forestry technology promotion and industrialization due to their advantages of easy operation and low cost. In forestry production, cutting is also more easily accepted and popularized by forest farmers, and is still widely applied in production. Through sorting out the existing studies, we summarized the researches and shortcomings of *X. sorbifolium* cutting breeding. Besides, we learned from seedling container, new substrate, new root promoting agent and other improvements and innovations, mother plant cultivation, cutting production, cutting method, cutting seedling management carried out for other species and research to eliminate matters influencing rooting, *etc.*, we are intended to develop and improve the cutting technology system, promote the development of *X. sorbifolium* breeding technology.

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cessed as by-products and combined with market prices to decide whether to plant in these two sites. In the four experimental sites, *A. melanocarpa* planted in Xiqing 2 Experimental Site not only has relatively short plants, but also has low yield, small fruit and poor taste, the planting effect is the worst in the four experimental sites.

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