EFFECTS OF GRAFTING ROOTSTOCKS, SEASONS AND METHODS ON SPROUTING RATE AND SHOOT GROWTH OF SUGAR APPLE LINE Na16 (*Annona squamosa*) IN THE NURSERY STAGE

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ABSTRACT

The sugar apple line Na16 (*Annona squamosa*) selected from production by the Fruit and Vegetable Research Institute had a variety of valuable attributes. In order to improve the efficiency of the sugar apple line Na16 production, the study on the influence of grafting rootstocks, seasons and methods to the sprouting rate and growth of grafted plants in the nursery stage was conducted from January 2020 to December 2021. The research results indicated that suitable sugar apple rootstocks included the two varieties Na Dai and Na Bo, and grafting in Ha Noi from January 20 to January 25 found to be most favorable. The sprouting percentage of Na16 plants grafted by side graftingduring this period reached over 90% and the success rate ranged from 73.61 to 75.28%.

Keywords: *Sugar apple line Na16, grafting method, grafting season, rootstock, sprouting rate.*
Received: 7 June 2023; revised: 17 August 2023; accepted: 20 September 2023.

1. INTRODUCTION

The sugar apple line Na16 was selected in Chi Lang district, Lang Son province by the Fruit and Vegetable Research Institute. Originated in China and introduced through cross-border into Vietnam, the line Na16 had a variety of valuable attributes such asvigorous growth, large fruit weight (500 - 800 g/fruit), high yield, small number of seeds (<30 seed/fruit), fragrant, firm and well-structuredfleshas well as a sweet and delicate flavour.

The reproduction of sugar apple take place both sexually (from seeds) and asexually (by grafting) [1]. Vegetative propagation of sugar apple is commonly implemented with the help of patch budding, side grafting and cleft grafting [2], [3]. Besides, most species of the genus Annona can be propagated from stem and root cuttings [4]. In Vietnam, new cultivation of traditional sugar apple varieties mainly utilizes seedlings obtained from seeds (i.e. Na Dai, Na Bo). However, propagation of new varieties (such as Na Hoang Hau, Na Dua...) mainly employ vegetative methods, including grafting (i.e. side grafting and inarch grafting) and patch budding. However, each variety varies in preferable rootstocks, seasons and methods for grafting [5], [6]. There are currently limited studies on rapid multiplication and expanding cultivation area of the sugar apple line Na16.

In order to develop a propagation protocol on the sugar apple line Na16 to extend its production, the influences of factors includingrootstocks, grafting seasons and methods on sprouting rate and growth of grafted plants were studied.

2. MATERIALS AND METHODS

2.1. Materials

- Rootstocks for grafting: Rootstock plants were germinated from seeds of Na Dai, Na Bo and Na Thai varieties.

- Scions for grafting: Sugar apple line Na16 obtained from mother plants of 3 - 4 years old.

- Fertilizers and other materials: Microbiological fertilizers, superphosphate (16% P_2O_5), urea (46% N), potassium chloride (60% K_2O), pesticides, foliar fertilizers, etc. and garden tools (saws, secateurs, grafting knives, grafting strips, etc.)

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2.2. Methods

2.2.1. Experiment design

- Experiment 1: Influence of rootstock varieties on sprouting rate and shoot growth of Na16 grafted lants in nursery stage.

- The experiment 1 was conducted with 3 treatments:

+ Treatment 1 (CT1): Grafting on the rootstocks of Na Dai variety.

+ Treatment 2 (CT2): Grafting on the rootstocks of Na Bo variety.

+ Treatment 3 (CT3): Grafting on the rootstocks of Na Thai variety.

The experiment was conducted in a Randomised Complete Block Design (RCBD) with 3 replications and 120 grafted plants in each treatment of each replication. The grafting was carried out from January 20, 2020 to January 25, 2021. Apart from the experimental factor, which was the rootstocks, other non-experimental factors were maintained identical in the experiment.

- Experiment 2: Influence of grafting seasons and methods on sprouting rate and shoot growth of Na16 grafted plants in nursery stage

- The experiment 2 was designed with two experimental factors:

+ Factor 1 (P factor: Methods of grafting) including 2 grafting methods of side grafting (P1) and patch budding (P2).

+ Factor 2: (T factor: Seasons of grafting) including 3 grafting seasons in January (T1), in June (T2), and September (T3).

- The specific grafting time in the years were as follows:

+ In January: Grafting was conducted on January 20, 2020 and January 25, 2021.

+ In June: Grafting was conducted on June 16, 2020 and June 18, 2021.

+ In September: Grafting was conducted on September 18, 2020 and September 16, 2021.

- The experiment 2 consisted of 6 treatments: Treatment 1 (T1P1); treatment 2 (T2P1); treatment 3 (T3P1); treatment 4 (T1P2); treatment 5 (T2P2); and treatment 6 (T3P2).

- The experiment was laid out in a RCBD with 3 replications, with 120 grafted plants in each treatment of each replication. Apart from the experimental factors, which were grafting seasons and methods, other non-experimental factors were maintained identical.

2.2.2. Experimental indicators and methods of monitoring and evaluation

- Sprouting rate (%) = Number of sprouted grafts forming new shoots/Total number of grafted plants*100.

+ Shoot length (cm): Measured from the shoot base to the shoot tip.

+ Shoot diameter (cm): Measured with a caliper at 2 cm from the shoot base.

- Growth ability of grafted plants: The length and diameter of new shoots forming on grafted plants were monitored, with 10 plants per treatment and with 3 replications, at 60 days after grafting.

- Grafting success rate (%): Number of plants meeting the standards for transplanting/Total number of grafted plants*100. The standards of success grafts were as follows: Plant height from potting medium surface to the plant tip should be over 40 cm; the length of the new shoot from the scion should be over 30 cm.

2.2.3. Data processing

Research data were collected with the biological statistical method and processed using Excel and CropSTAT 7.2 softwares.

2.3. Time and location

The study was conductedat the Fruit and Vegetable Research Institute (FAVRI), Ha Noi, Vietnamin 2 years, from January 2020 to December 2021.

3. RESULTS AND DISCUSSION

3.1. Influence of rootstock varieties on sprouting rate and shoot growth of Na16 grafted plants in nursery stage

3.1.1. Influence of rootstock varieties on sprouting rate of Na16 grafted plants

In the production of fruit plant seedlings in general and sugar apple seedlings in particular, the proportion of grafted plants forming new shoots is always an important criterion that determines the efficiency of propagation. There are many factors affecting the sprouting rate after grafting such asscion quality, grafting technique, weather condition during and after grafting, etc. Once the above factors were the same among all treatments, the sprouting rate would be mainly affected by the compatibility between the rootstocks and the scions.

The following table describes results of monitoring the influence of rootstock varieties on the sprouting rate of Na16 line.

The data indicated that with the weather conditions in Ha Noi, grafting combination of Na16 scions on Na Thai rootstocks resulted in significantly lower rates of shoot formation (at 86.39% in 2020 and 85.28% in 2021) than those of the other rootstocks. The sprouting rate of Na16 grafted on Na Dai and Na Bo rootstocks were relatively higher, ranging from 89.72% to 91.11%, with statistically insignificant difference in the rates of these two rootstocks.

Table 1. Influence of rootstock varieties on sprouting rate of Na16 grafted plants

Treatments	Sprouting rate (2020)		Sprouting rate (2021)	
	(%)	Arcsin	(%)	Arcsin
CT1 (Na Dai rootstocks)	90.83	72.39	90.56	72.24
CT2 (Na Bo rootstocks)	91.11	72.79	89.72	71.42
CT3 (Na Thai rootstocks)	86.39	68.36	85.28	66.86
$LSD_{0.05}$		*2.69*		*3.57*
CV (%)		*1.7*		*22*

Therefore, among the rootstocks used for grafting of Na16 scions in the experiment, the treatment combination with Na Thai variety showed the lowest sprouting rates, while grafting with Na Dai and Na Bo varieties had relatively higher rates. There was insignificant difference in sprouting rates of grafting with Na Dai and Na Bo varieties.

3.1.2. Influence of rootstock varieties on shoot growth of Na16 grafted plants in nursery stage

The growth ability of the grafted plants determines the quality of the young plants for transplanting, reflected in the length and diameter of new shoots formed. The table 2 below illustrates the data of the shoot growth ability of Na16 grafted on different rootstocks monitored at 60 days after grafting.

The data presented in table 2 indicated that grafting Na16 scions on Na Thai rootstocks not only resulted in low sprouting rates, but the size of new shoots were also significantly smaller than those of the grafting combination on Na Dai and Na Bo rootstocks. The combination of Na16 and Na Thai recordedrelatively smaller shoot length and diameter, ranging in 36.70 - 38.83 cm and 0.61 - 0.62 cm.Meanwhile, the later combinations produced better new shoots. The length and diameter were insignificantly different, varying from 46.70 to 50.42 cm, and from 0.73 to 0.76 cm.

Table 2. New shoot growth of Na16 grafted plants at 60 days after grafting

Treatments	Year 2020		Year 2021	
	Shoot length (cm)	Shoot diameter (cm)	Shoot length (cm)	Shoot diameter (cm)
CT1	48.74	0.77	50.42	0.76
CT2	46.70	0.73	49.64	0.74
CT3	36.70	0.61	38.83	0.62
$LSD_{0.05}$	*6.27*	*0.04*	*2.78*	*0.03*
CV (%)	*6.3*	*2.7*	*2.6*	*1.9*

According to the formation of new shoots from the scions, the rootstocks of Na Dai and Na Bo varieties revealed to be suitable for the scions Na16.

3.1.3. Influence of rootstock varieties on the grafting success rate of Na16

The grafting success rate of Na16 scions on different rootstocks are described in the table 3.

Table 3. Influence of rootstock varieties on the grafting success rate of Na16

Treatments	Grafting success rate (2020)		Grafting success rate (2021)	
	(%)	Arcsin	(%)	Arcsin
CT1	70.83	57.33	70.28	56.98
CT2	69.44	56.45	68.33	55.82
CT3	47.50	43.56	45.28	42.27
$LSD_{0.05}$		*1.39*		*8.10*
CV (%)		*1.2*		*6.9*

The data in the table 3 showed that: among the tested rootstocks, the combination with Na Thai rootstocks led to the lowest grafting success rate for transplanting, with 47.5% in 2020 and 45.28% in 2021. Similar to the criteria of new shoots formation, the grafting success rate in the treatment 1 and 2 (CT1 and CT2) were statistically insignificantly different, ranging of 69.44 - 70.83% in 2020 and 68.33 to 70.28% in 2021.

Therefore, the combination of the Na16 scions with rootstocks of Na Dai and Na Bo varieties in the experiment resulted in better sprouting and success rates than those of the combination with Na Thai rootstocks.

3.2. Influence of grafting seasons and methods on sprouting rate and shoot growth of Na16 grafted plants in nursery stage

3.2.1. Influence of grafting seasons and methods on sprouting rate of Na16 grafted plants

Table 4 depicts monitoring data of the influence of grafting seasons and methods on sprouting rate of the line Na16 at the Fruit and Vegetable Research Institutein 2020 and 2021.

Table 4. Influence of grafting seasons and methods on sprouting rate of Na16 grafted plants

Factors	Sprouting rate(2020)		Sprouting rate (2021)	
	(%)	Arcsin	(%)	Arcsin
Grafting seasons - T				
T1 - January	85.33	68.16	85.00	67.62
T2 - June	82.50	65.43	80.56	64.07
T3 - September	78.61	62.56	76.53	61.17
$LSD_{0.05}$		*2.19*		*1.86*
Grafting methods – P				
Side grafting- P1	86.48	68.59	86.20	68.40
Patch budding - P2	78.14	62.18	75.19	60.18
$LSD_{0.05}$		*1.79*		*1.51*
Interaction effect of grafting seasons and methods				
T1P1	90.28	71.88	90.83	72.39
T2P1	86.67	68.59	86.11	68.14
T3P1	82.50	65.30	81.67	64.66
T1P2	81.39	64.45	79.17	62.86
T2P2	78.33	62.27	75.00	60.00
T3P2	74.72	59.83	71.39	57.67
$LSD_{0.05}$		*3.10*		*2.62*
CV (%)		*2.4*		*2.0*

The data in table 6 indicated that in the condition of Ha Noi in 2020 - 2021, grafting seasons and methods had a significant impact on the sprouting rate of Na16 grafted plants in the nursery stage.

- *Influence of grafting seasons:* Different grafting seasons in January (T1), June (T2) and September (T3) resulted in different effects on the rate of shoot formation of Na16 in the nursery stage. In 2020, the rate of new shoot formation in the treatment T1 (85%) was remarkably higher than those of the treatments T2 and T3, ranging from 80.56 to 82.5% and 76.53 to 78.61%, respectively; the same trend was also reported in 2021. Therefore, it can be concluded that: Grafting of the line Na16 in January resulted in the highest sprouting rate followed by that in June, and the lowest rate was in September.

Many studies on grafting seasons on other fruit trees demonstrated that the suitable season for grafting in northern provinces is autumn season (August to October) [7]. However, in regard to sugar apple trees, autumn grafting (in September in our experiment) might lead to the lowest sprouting rate. This is possibly due to their characteristics as deciduous trees. The vegetative growth begins to retard in autumn time, resulting in the formation of new shoots slowing down [8]. Meanwhile, January is the time of the year when both the rootstock and the scion varieties areswitching fromthe dormant stage to the vegetative growth stage, leading to higher sprouting rates. The findings suggest a need for further fundamental studies to serve asscientific

evidence and reference for propagation of sugar apple in Northern Vietnam.

- *Influence of grafting methods:* Analysis results revealed that in both experimental years (2020 and 2021), there was a clear difference in the sprouting rate of Na16 between the two grafting methods. In particular, the side grafting method gave significantly higher rates (reaching over 86% in both years) than the patch budding method (at 78.14% in 2020 and 75.19% in 2021). Thus, it can be initially concluded that the side grafting method was relatively suitable for grafting of the line Na16.

- *Effect of interaction between grafting seasons and methods:* The interaction between grafting seasons and methods was clearly proved in the rates of new shoot formation of Na16 grafted plants, which were dissimilar to the influence of individual factors. In 2020, different grafting methods (P1 and P2) resulted in varying sprouting rates in only two grafting seasons T1 and T3; no variation was recorded in grafting season T2. Meanwhile, the individual influence of grafting seasons (T1, T2, T3) on sprouting rate varied significantly in the experiment. In 2021, the same figure was seen in treatments utilizing side grafting technique (P1); whereas, the influence of patch budding treatments was clearly revealed since the sprouting rate in T2P2 treatment was different from that in T1P2 treatment but not that in T3P2.

From the above analysis, it can be concluded that among the experimental factors, the most suitable condition for grafting of the line Na16 was the side grafting method in January.

3.2.2. Influence of grafting seasons and methods on shoot growth of Na16 grafted plants in nursery stage

The growth ability of grafted plants determines their quality for transplanting, which is reflected in the length and diameter of the new shoots. Monitoring the main characteristics of the grafted plants in different grafting seasons and methods at 60 days after grafting revealed the results shown in table 5.

Table 5. Main characteristics of Na16 grafted plants at 60 days after grafting

Factors	Year 2020		Year 2021	
	Shoot length (cm)	Shoot diameter (cm)	Shoot length (cm)	Shoot diameter (cm)
Grafting seasons				
T1 - January	45.49	0.70	49.44	0.70
T2 - June	43.54	0.67	44.80	0.65
T3 - September	39.57	0.60	40.56	0.61
$LSD_{0.05}$	*1.29*	*0.03*	*3.08*	*0.03*
Grafting methods				
Side grafting- P1	46.25	0.73	47.87	0.75
Patch budding - P2	39.47	0.58	42.00	0.56
$LSD_{0.05}$	*1.05*	*0.02*	*2.52*	*0.02*
Interaction effect of grafting seasons and methods				
T1P1	50.48	0.79	51.59	0.80
T2P1	46.18	0.75	48.93	0.75
T3P1	42.10	0.65	43.08	0.69
T1P2	40.50	0.60	47.30	0,61
T2P2	40.89	0.57	40.67	0,55
T3P2	37.03	0.54	38.04	0,52
$LSD_{0.05}$	*1.82*	*0.03*	*4.36*	*0,03*
CV (%)	*2.1*	*3.0*	*4.9*	*3,2*

The data in table 5 indicated the significant effect of grafting seasons and methods on the growth of the line Na16 in nursery stage in the year 2020 and 2021.

- *Influence of grafting seasons:* The length and diameter of new shoots reached the highest values in the grafting season of January, followed by that of June grafting. The lowest values were recorded in

September grafting. This may be due to the fact that the young Na16 plants had favorable weather conditions for their vegetative growth after grafting in January. In regard to young plants of September grafting, the newly-formed shoots might encounter unsuitable temperature and humidity conditions for vegetative growth.This was also the time that sugar apple trees were about to enter the dormant phase.

- *Influence of grafting methods:* Different grafting methods had various effects on the growth of Na16 grafted plants. The treatment P1 of side grafting obtained significantly larger shoot size than that of the patch budding treatment (P2).

- *Effect of interaction between grafting seasons and methods:* In general, the influence of interaction between grafting seasons and methods on the length and diameter of new shoots was unclear in both years 2020 and 2021. Such vegetative growth of new shoot of Na16 grafted plants was mostly similar to that in consideration of each factor separately. Hence, there was a statistically significant difference in the length and diameter of new shoots on Na16 grafted plants in different grafting seasons and with different methods. The highest size of new shoots were obtained in January grafting treatment.

From the above results, it can be concluded that the most suitable treatment for the growth of Na16 grafted plants was T1P1 (side grafting in January), followed by T2P1 treatment (side grafting in June) and T3P1 treatment (side grafting in September).

3.2.3. Influence of grafting seasons and methods on the grafting success rate of Na16

Influence of the grafting seasons and methods on the grafting success rate for transplanting is described in table 6.

Table 6. Influence of grafting seasons and methods on the grafting success rate of Na16

Factors	Grafting success rate (2020)		Grafting success rate (2021)	
	(%)	Arcsin	(%)	Arcsin
Grafting seasons - T				
T1 - January	62.36	52.36	61.81	52.12
T2 - June	53.75	47.27	55.56	48.37
T3 - September	50.00	45.00	51.39	45.85
$LSD_{0.05}$		*1.93*		*1.68*
Grafting methods – P				
Side grafting- P1	69.26	56.38	70.74	57.33
Patch budding - P2	41.48	40.05	41.76	40.23
$LSD_{0.05}$		*1.57*		*1.37*
Interaction effect of grafting seasons and methods				
T1P1	73.61	59.09	75.28	60.19
T2P1	70.28	56.27	71.39	57.67
T3P1	63.89	53.07	65.56	54.13
T1P2	51.11	45.64	48.33	44.04
T2P2	37.22	37.59	39.72	39.07
T3P2	36.11	36.93	37.22	37.58
$LSD_{0.05}$		*2.72*		*2.38*
CV (%)		*2.8*		*2.4*

The results in table 6 exhibited that there was a clear difference in the grafting success rate of Na16 due to different grafting seasons and methods in 2020 and 2021:

- *Influence of grafting seasons:* Grafting in January resulted in the highest grafting success rate for transplanting, followed by June grafting and September grafting.

- *Influence of grafting methods:* There was a significant difference in treatments of different grafting methods. The grafting success rate in side grafting treatment (P1) was remarkably higher than that in treatment P2 (patch budding).

- *Interaction effect of grafting seasons and methods:* Influence of grafting seasons and methods in combination on the grafting success rate for transplanting was relatively similar to that in consideration of each individual factor. The highest rate of young plants available for transplanting was seen in treatment T1P1 (side grafting in January), followed by that in treatment T2P1 (side grafting in June) and lastlythat in treatment T3P1 (side grafting in September).

In summary,grafting of Na16 by side grafting in January led to the highest grafting success rate for transplanting, while the lowest was that of patch budding in September.

4. CONCLUSION AND RECOMMENDATION

4.1. Conclusion

- Among the varieties in the research, Na Dai and Na Bo rootstocks were the most suitable for the scions of Na16.

- In the experiment of grafting seasons and methods, side grafting in January was the most favourable for the growth of the line Na16, with the sprouting rate reaching over 90% and the grafting success rate ranging from 73.61 to 75.28%.

4.2. Recommendation

The research findings are suggested to be included in cultivation protocols on the sugar apple line Na16 and applied in practice in the North of Vietnam.

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