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EFFECTS OF THE APPLICATION OF VARIOUS SUBSTANCES AND GRAFTING METHODS ON THE GRAFTING SUCCESS AND GROWTH OF BLACK MULBERRY (*Morus nigra* L.)

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Abstract. This research was conducted to determine the effects of three grafting methods (chip budding, side and splice grafting) and topical applications on graft scion of IBA (0 and 4000 ppm) and three plant growth promoting rhizobacteria (PGPR) strains (*Bacillus subtilus*-OSU142, *Bacillus megatorium*-M3 and *Burkholderia gladia*-BA7) on graft success and plant growth in black mulberry sapling production. The experiment was carried out in a randomized complete block design with three replications in Bolu, Turkey in 2014 and 2015. The black mulberry (*Morus nigra* L.) were used for grafting scions. Two-year old white mulberry (*Morus alba* L.) seedling were used for rootstocks. The results showed that in general all of the bacterial strains and IBA had significant effects on all parameters tested in graft scion compared with the control. Application of 4000 ppm IBA and *Bacillus megatorium*-M3 increased the success graft take rate (74.44 and 72.22%, respectively), graft sprouting rate (61.11 and 60.00%, respectively), graft shoot diameter (6.21 and 5.70 mm, respectively) and graft shoot length (35.50 and 35.31 cm, respectively). Grafting methods had significant effect for all parameters and grafting methods increased the graft success. The best graft take rates (75.33 and 70.67%, respectively), graft sprouting rates (62.67 and 57.33% respectively), graft shoot diameter (5.56 and 5.88 mm, respectively) and graft shoot length (34.14 and 37.86 cm, respectively) were obtained from splice grafting and chip budding methods. In conclusion, the present study showed that 4000 ppm IBA and PGPR strains (especially M3 and OSU142) increased the graft success of black mulberry graft. The PGPR application may be of benefit for grafting in mulberry cultivars, particularly for organic farming. Besides, splice grafting and chip budding were found to be successful to black mulberry plant production.

Key words: IBA, PGPR, grafting methods, black mulberry, propagation

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INTRODUCTION

Tropical and subtropical fruit species, mulberry is involved in the genus *Morus*, the tribe *Moracea*, and the family Moraceae [Özrenk et al. 2010]. There are approximately 68 mulberry species (*Morus*) in the World, and the majority of them occur in Asia. The most commonly grown species in the Morus genus are white mulberry (*Morus alba* L.), black mulberry (*Morus nigra* L.), and red mulberry (*Morus rubra* L.) [Datta 2002, Özgen et al. 2009]. The mulberry production in Turkey is significant (62.879 t) [FAO 2014].

Mulberry has a unique delicious fruit, sour and refreshing taste. Today, due to its nutritive value, the mulberry fruit is consumed both fresh and processed forms [Koyuncu 2004]. Besides, the Mulberry tree is used in forming landscapes (especially pendulous form) and ornamental plants in home gardens and parks [Güneş and Çekiç 2011].

Propagation is a common practice employed in all plants in order to obtain healthy and resistant plants. Different modes of propagation of trees are important to cultivate in large scale [Hartmann et al. 2011]. In recent years, an interest in mulberry saplings especially in black mulberry is increasing. The mulberry can be propagated by seeds, cuttings, grafting, layering and tissue culture [Lu 2002, Anis et al. 2003]. They can be easily propagated by seeds [Güneş and Çekiç 2004]. However, propagation through seed is undesirable because of enormous heterozygosis in the plants resulting from cross pollination [Anis et al. 2003]. Mulberry, cultivated for fruit, should be propagated as clone. Cutting propagation of this plant is unsuccessful due to long time taken for adventitious shoot development and low rooting potential that might be due to several factors including physiological and environmental ones [Narayan et al. 1989]. Despite the application of different plant growth regulators, rooting success rate is quite low in propagation by cuttings [Koyuncu and Şenel 2003].

Grafting or budding is usually preferred by nursery-men over cutting because of variable results. Budding has wider use than grafting because it is easy to apply and need less plant material and application time [Vural et al. 2008]. Although there are some studies conducted to evaluate the black mulberry propagation by budding or grafting [Vural et al. 2008, Güneş and Çekiç 2011], extensive works containing all such budding/grafting methods and treating bacteria strains and IBA solution on black mulberry are still limited.

Mulberry is very limited due to the difficulties in grafting. Grafting success is prevented because the emergence of the milk secretion and the space under the bud tissue emerge on mulberry [Ünal et al. 1992]. In previous studies, it was clearly shown that auxin induce formation of callus and new vascular tissue [Bonner and Galston 1952, Raven et al. 1992]. Various auxins had significant effects on fruit tree budding by affecting xylem and phloem differentiation and on lignification process which is considered as very important factors in formation of a strong unite area in grafting [Kako 2012]. Thus, several researchers have applied auxin on graft union for increasing callus formation and grafting success [Reustle et al. 1993, Köse et al. 2005, Kako 2012]. Starrantino et al. [1986] found that treatment with plant growth regulators was increased apical grafting success percentage. Studies have shown that some plant growth promoting rhizobacteria belonging to the genus of *Burkholderia* and *Bacillus* are known to have potential of producing phytohormones, particularly indole-3-acetic acid (IAA) [Köse et al. 2003, Kaymak et al. 2008]. In addition, more recent studies have demonstrated that success in rooting and basal callusing of hardwood cuttings can be achieved by bacterial applications in some plant species [Ercişli et al. 2003, Köse et al. 2003]. However, there is no attempt to study the effects of these bacterial strains on graft success of black mulberry.

The aim of this study was to investigate the effects of PGPR strains (*Bacillus subtilis*-OSU142, *Bacillus megatorium*-M3 and *Burkholderia gladia*-BA7) and IBA (0 and 4000 ppm) alone and grafting methods on the graft success of black mulberry graft.

MATERIALS AND METHODS

Study site. This study was conducted at the Abant İzzet Baysal University, Vocational Commnity College of Bolu research area and greenhouse located in Bolu Center, Turkey (North: 40°43', East: 31°33', Altitude: 768 m) in 2014 and 2015. In this study, grafts were performed in greenhouse.

Rootstocks were grown in greenhouse conditions. Specially prepared growing medium (mix of 1/1/1 sieved garden soil, peat and burned manure) were used. Soil mixture were found clayey and loamy from the analysis. Medium soil analyses were the following: pH 7.42; organic matter 8.30%; total nitrogen content 0.04%; available P_2O_5 352.6 mg kg^{-1}; exchangeable K_2O 773.6 mg kg^{-1}; total organic carbon 4.78%; EC 0.47 ms/cm; total salt 0.069%; active lime 1.5%; lime ($CaCO_3$) 3.0%.

Soil mixture is very unsalted, low lime, rich for organic matter and humus, phosphorus and potassium. Rootstocks were grown in UV stabilized polyethylene flowerpots (26.5 × 21.0 cm in size and 5 litres) full specially prepared soil mix. Cultivation factors such as irrigation, weeding and removal of suckers below graft union were done following regular intervals.

Plant Materials. The two-year old white mulberry (*Morus alba* L.) seedlings having uniform diameter (between 10.0–13.0 mm) were used as rootstocks. Plants of *Morus nigra* L. were used for scions collection. The scions (one-year old shoots) for grafting were selected in previous winter from healthy donor black mulberries trees in Bolu, Turkey. These shoots (30–35 cm long) were disease-free and lignified. The grafting scions were stored in a refrigerator at +4°C on damp paper in a plastic bag until used for grafting.

Bacterial strain and IBA treatment. Three bacterial strains (PGPB) (*Bacillus subtilis* (strain OSU142), *Bacillus megatorium* (strain M3) and *Burkholderia gladia* (strain BA7) were obtained from Yeditepe University, Department of Genetics and Bioengineering (Dr. Fikrettin Sahin, personal communication). Bacteria were grown on nutrient agar (NA) for routine use, and maintained in nutrient broth (NB) with 15% glycerol at -80°C for long-term storage. A single colony was transferred to 250 ml flasks containing NB, and grown aerobically in flasks on a rotating shaker (150 rpm) for 48 h at 27°C (Merck KGaA, Germany). The bacterial suspensions (BA7, M3 and OSU142) were then diluted in sterile distilled water to a final concentration of 10^9 CFU ml^{-1}, and the resulting suspensions were used to treat (dipping suspension for 30 min) black mulberry graft scion.

IBA solution (4000 ppm) was freshly prepared by dissolving IBA powder (Merck Chemical Co.) in an ethanol/sterile water (50/50%) solution. For IBA treatments, the basal portion of graft scion was dipped in an aqueous solution of 4000 ppm IBA (50% ethanol) for 5 min, and allowed to air dry. Grafts in the control group were treated with sterile water.

Grafting times and grafting methods. Grafting operations were done on April 18, 2014 and 2015. Chip budding, splice, and side grafting by hand were investigated (fig. 1). The procedures for three grafting were as described by Hartmann et al. [2011]. White and soft plastic tapes were used to wrap the grafting and budding. The grafting were done by expert researchers having extensive experience and researches about grafting [Zenginbal et al. 2006, Zenginbal and Dolgun 2014].

Fig. 1. The methods of grafting used in research (chip budding, splice, and side grafting)

Investigation of criteria for grafting success. Experiment carried out in greenhouse where daily mean relative humidity (%) and temperature (°C) were recorded (one hour's intervals) by mechanical data logger (HOBO U10 Temp/RH data logger) during the 90 days after grafting (April 18 to July 18, 2014 and 2015).

At the end of growing season (December 1, 2014 and 2015), the following parameters were examined to determine the effects of grafting methods and periods on grafting success:

Graft take rate (%): Percentage of grafted mulberries that have an adequate or all-around callus ring formation on the surface of the graft union. Sixty days after grafting the percentage of bud take was estimated as: Graft take rate (%) = Number of bud taken scion x 100/ Number of grafted rootstock.

Graft sprouting rate (%): Percentage of grafted mulberries that have an adequate shoot length and diameter flushed from grafted bud. After grafting the graft sprouting rate was estimated as: Graft sprouting rate (%) = Number of sprouted scion × 100/ Number of grafted rootstock

Graft shoot diameter (mm): Observations on grafting shoot diameter was measured by digital compass at a point 5 cm above the graft union.

Graft shoot length (cm): Observations on grafting shoot length was measured by meter at a point 5 cm above the graft union.

Statistical analysis. Complete randomized block design was applied with three replications and 10 grafts per replication. The experiment was evaluated as 5 (application) × 3 (grafting methods) factorial design. To analyze the data Kolmogorov-Smirnov one sample test was performed to examine the normality, test results showed that all data have not distributed normally ($P < 0.05$), so to analyse the data permutation tests was performed according to 5 × 3 factorial design [Önder 2007]. To compare the means posterior pairwise permutation tests were used. Only main effects of application and grafting methods were interpreted because application × grafting methods interaction effect was found statistically insignificant ($P > 0.05$). NPMANOVA software was used to analyse the data [Anderson 2000]. There were no statistical differences between years, therefore the data were pooled.

RESULTS AND DISCUSSION

As shown in Figure 2, daily mean temperatures varied from 12.8 to 26.9°C and mean relative humidity varied from 51.8–88.8% in 2014. In 2015, daily mean temperatures varied from 6.3 to 30.8°C and daily mean relative humidity varied from 47.7–93.6%. As a result of these findings temperature and relative humidity values were at the optimum level for grafting success. Therefore Yılmaz [1992], reported that optimum grafting success occurred under 26–28°C and this information supported our findings. Besides, the climatic data were in accordance with the long term average of Bolu [TSMS 2016], and indicating that 2014 and 2015 were an average year. Thus, generalization from the study seemed possible.

The graft success of black mulberry grafting are summarized in Table 1. In research, only main effects of applications and grafting methods were interpreted because applications (bacterial strains and IBA) × grafting methods interaction effect was found statistically insignificant ($P > 0.05$) for all parameters (tab. 1).

Data of the experiment summarized in Table 2 showed that all bacterial strains and IBA treated scion had better value than controls for all parameters. Application of PGPR and 4000 ppm IBA increased the graft take rate. The highest graft take rate (74.44%) was achieved from treatment of IBA at 4000 ppm, least graft take success percentage (63.33%) was achieved from treatment of control. Application of PGPR and

IBA also increased the graft sprouting rate. The highest sprouting rate was 61.11% for 4000 ppm IBA and 60.0% for M3, versus the 44.44% sprouting rate of untreated control. All PGPR and IBA tested had significant effects on graft shoot diameter. The best result (6.21 mm) was obtained from treatment of IBA at 4000 ppm. The lowest results (5.11 and 5.13 mm) were obtained from treatment of control and BA7, respectively. Similar to graft shoot diameter, graft shoot length was affected by PGPR applications and IBA. The best results (35.5 and 35.31 cm) were obtained from treatment of 4000 ppm IBA and M3. The lowest result (28.09 cm) was obtained from treatment of BA7.

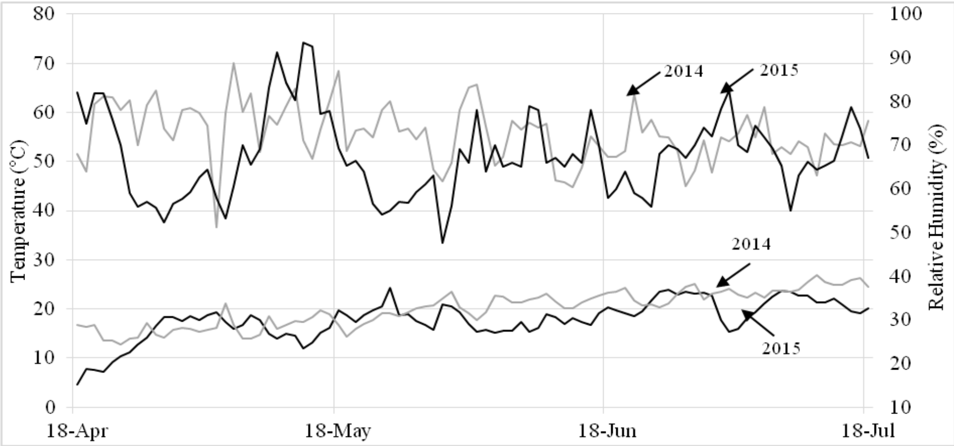

Fig. 2. The daily mean temperature and relative humidity data in greenhouse

In this study, it was observed that callus induction response was higher in grafts, treated with IBA and bacterial strains than that of control and these grafts sprouted early. According to the results from the current study indicate that 4000 ppm IBA and PGPR application significantly increases the graft success, graft shoot diameter and length of black mulberry. The best application was found from 4000 ppm IBA and M3 bacterial strain for all parameters. Generally the lowest results were obtained from treatment of control and BA7 bacterial strains for all parameters. Positive effects of IBA and bacterial applications on mulberry graft scion may be explained by auxin produced by IBA and bacterial strains. Auxin has been also known to be intimately involved in the process of callus and adventitious root formation in cuttings [Weaver 1972]. Thus, Kalyoncu et al. [2009], Kako [2012], Çekiç et al. [2013] and Husen et al. [2015] reported that treating cuttings with IBA (between 3000 and 6000 ppm) increased the percentage of rooting in mulberry. Besides, Kaymak et al. [2008] reported that treating cuttings with M3 bacterial strains increased the percentage of rooting in mint. The present study showed that IBA and PGPR strains (especially M3 and OSU142) increased the graft success of mulberry graft. This findings are in accordance with the findings of Kako [2012] showed that IBA and kinetin application increased the graft success in mulberry.

Likewise, in research of Köse et al. [2005] reported that in general all of the bacterial strains (*Pseudomonas* BA8, *Bacillus* BA16 and *Bacillus* OSU142) was significant effects on all parameters tested in all rootstock-scion combinations compared with the control in grapevine.

Table 1. The effect of IBA, bacteria stains and grafting methods on the grafting success of black mulberry (average of 2014 and 2015 years)

Application	Grafting methods	Graft take (%)	Graft sprouting (%)	Graft shoot diameter (mm)	Graft shoot length (cm)
Control	chip budding	63.33 ±3.33	46.67 ±3.33	5.43 ±0.18	37.83 ±2.66
	splice graft	73.33 ±3.33	53.33 ±3.33	5.09 ±0.23	33.66 ±2.75
	side graft	53.33 ±3.33	33.33 ±3.33	4.79 ±0.21	24.42 ±1.95
OSU142	chip budding	70.00 ±0.00	56.67 ±3.33	5.66 ±0.43	37.15 ±5.52
	splice graft	73.33 ±3.33	60.00 ±0.00	5.25 ±0.47	34.36 ±7.46
	side graft	56.67 ±3.33	36.67 ±3.33	4.84 ±0.18	25.41 ±1.47
M3	chip budding	76.67 ±3.33	66.67 ±3.33	6.17 ±0.38	43.04 ±2.00
	splice graft	80.00 ±0.00	70.00 ±0.00	5.95 ±0.56	36.04 ±5.34
	side graft	60.00 ±0.00	43.33 ±3.33	4.99 ±0.31	26.85 ±3.18
BA7	chip budding	63.33 ±3.33	50.00 ±0.00	5.33 ±0.08	29.24 ±1.88
	splice graft	70.00 ±0.00	56.67 ±3.33	5.18 ±0.25	29.61 ±2.63
	side graft	60.00 ±0.00	40.00 ±0.00	4.89 ±0.14	25.42 ±0.49
4000 ppm IBA	chip budding	80.00 ±0.00	66.67 ±3.33	6.81 ±0.22	43.03 ±2.36
	splice graft	80.00 ±0.00	73.33 ±3.33	6.30 ±0.32	37.02 ±4.42
	side graft	63.33 ±3.33	43.33 ±3.33	5.52 ±0.54	26.45 ±1.61
P volume		0.124	0.242	0.928	0.813

Table 2. The effect of IBA and bacteria stains on the grafting success of black mulberry (average of 2014 and 2015 years)

Application	Graft take (%)	Sprouting (%)	Shoot diameter (mm)	Shoot length (cm)
Control	63.33 ±3.33 c	44.44 ±3.38 c	5.11 ±0.14 b	31.64 ±2.24 ab
OSU142	66.67 ±2.89 bc	51.11 ±3.89 b	5.25 ±0.22 b	32.31 ±3.24 ab
M3	72.22 ±3.24 ac	60.00 ±4.41 a	5.70 ±0.28 ab	35.31 ±3.01 a
BA7	64.44 ±1.76 c	48.89 ±2.61 bc	5.13 ±0.11 b	28.09 ±1.16 b
4000 ppm IBA	74.44 ±2.94 ab	61.11 ±4.84 a	6.21 ±0.27 a	35.50 ±2.86 a
P volume	<0.01	<0.01	<0.01	<0.05

The means ± standard error followed by the same letter were not significantly different

The effects of grafting methods on the grafting success of black mulberry are summarised in Table 3. Grafting methods had significant effect ($P < 0.01$) for all parameters. The best graft take rates (75.33 and 70.67%, respectively) were obtained from splice grafting and chip budding (no significant difference between splice grafting and chip budding). Grafting method also increased the graft sprouting. The highest result (62.67%) was obtained from splice grafting. The lowest result (39.33%) was obtained from side grafting. Grafting method had significant effects on graft shoot diameter. The best result (5.88 mm) was obtained from chip budding. The lowest result (5.01 mm) was obtained from side grafting. Similar to graft shoot diameter, graft shoot length was affected by grafting method. The best result (37.86 cm) was obtained from chip budding. The lowest result (25.71 cm) was obtained from side grafting.

Table 3. The effect of grafting methods on the grafting success of black mulberry (average of 2014 and 2015 years)

Grafting methods	Graft take (%)	Sprouting (%)	Shoot diameter (mm)	Shoot length (cm)
Chip budding	70.67 ±2.06 a	57.33 ±2.48 b	5.88 ±0.18 a	37.86 ±1.82 a
Splice graft	75.33 ±1.33 a	62.67 ±2.28 a	5.56 ±0.20 a	34.14 ±1.96 a
Side graft	58.67 ±1.33 b	39.33 ±1.53 c	5.01 ±0.14 b	25.71 ±0.77 b
P volume	<0.01	<0.01	<0.01	<0.01

The means ±standard error followed by the same letter were not significantly different

According to the results from the current study indicate that grafting methods significantly increases the graft success, graft shoot diameter and length of black mulberry. Splice grafting and chip budding were found to be successful, side grafting was found to be unsuccessful to black mulberry plant production. It was also observed that rootstock could not soak water sufficiently in April, 18 when grafts were performed. This phenomenon affected the success rate of side grafting. So, success depends on easily separation of bark from wood tissue which is possible effective water soaking of plant tissues. But, there is no need water soaking for splice grafting and chip budding applications. If water presence is weak between bark and wood tissues, as observed in the present study, barks have cracked causing the expansion of wounded tissue and not cohering of graft. Besides, excessive cutting surface affected graft success negatively as observed by Hartmann et al. [2011]. On the contrary of side grafting, cutting surface is less than that of splice grafting and chip budding, thus rootstock and graft scion was cohered early. Thus, mean values of graft take, graft sprouting, graft shoot diameter and length were higher in these grafting methods as a result of early cohering of rootstock and graft scion. Several researchers [Czarneck 1990, Chandel et al. 1998, Çelik et al. 2006, Chauhan et al. 2007, Zenginbal and Dolgun 2014, Zenginbal 2015] reported that splice, tongue grafting and chip budding produced higher results in terms of graft success, graft diameter and length in different fruits. This finding conflict with the results in study conducted by Vural et al. [2008] reporting that side grafting produced the highest success of graft when compared to T and patch budding of black mulberry. The results are not in accordance with those of us, probably deriving from different grafting dates. Because, the grafting were performed between May 1 and August 27 by the aforesaid authors. As reported by Hartmann et al. [2011] grafting time has an important effect on graft success. Our results for graft take, graft sprouting, graft shoot diameter and length are supported by Vural et al. [2008], Güneş and Çekiç [2011] who studied on mulberry production via grafting.

CONCLUSION

The present study showed that IBA and PGPR strains (especially M3 and OSU142) increased the graft success of mulberry graft. The PGPR application may be of benefit for grafting in mulberry cultivars, particularly for organic farming. Besides, splice grafting and chip budding were found to be successful to black mulberry plant production.

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WPŁYW APLIKACJI RÓŻNYCH SUBSTANCJI I METOD SZCZEPIENIA NA POWODZENIE SZCZEPIENIA I WZROST MORWY CZARNEJ (*Morus nigra* L.)

Streszczenie. Badanie przeprowadzono w celu ustalenia wpływu trzech metod szczepienia (okulizacja na przystawkę, szczepienie boczne i w klin) szczytowej aplikacji IBA (0 i 4000 ppm) i trzech szczepów ryzobakterii (PGPR) wspierających wzrost roślin (*Bacillus subtilus*-OSU142, *Bacillus megatorium*-M3 i *Burkholderia gladia*-BA7) na powodzenie szczepienia i wzrost roślin w produkcji sadzonek morwy czarnej. Doświadczenie przeprowadzono w miejscowości Bolu w Turcji w latach 2014 i 2015 w układzie bloków losowych w trzech powtórzeniach. Czarna morwa (*Morus nigra* L.) była użyta do cięcia. Dwuletnie sadzonki białej morwy (*Morus alba* L.) użyto jako podkładki. Wyniki wykazały, że ogólnie wszystkie szczepy bakterii oraz IBA miały istotny wpływ na wszystkie badane parametry cięcia w porównaniu z kontrolą. Użycie 4000 ppm IBA oraz *Bacillus megatorium*-M3 zwiększało wskaźnik powodzenia szczepień (odpowiednio 74,44 i 72,22%), wskaźnik kiełkowania szczepów (odpowiednio 61,11 i 60,00%), średnicę pędów szczepów (odpowiednio 6,21 i 5,70 mm) oraz długość pędów szczepów (odpowiednio 35.50 i 35.31 cm). Metody szczepień miały istotny wpływ na wszystkie parametry i zwiększały powodzenie szczepienia. Najlepsze wskaźniki powodzenia szczepień osiągnięto w przypadku metod w klin i okulizacji. Podsumowując, badanie wykazało, że 4000 ppm IBA i szczepy PGPR (zwłaszcza M3 i OSU142) zwiększały powodzenie szczepienia czarnej morwy. Zastosowanie PGPR może być korzystne w szczepieniu odmian morwy, zwłaszcza w uprawach organicznych. Poza tym stwierdzono, że szczepienie w klin i okulizacja mogą być z powodzeniem stosowane w szczepieniu morwy czarnej.

Słowa kluczowe: IBA, PGPR, metody szczepień, rozmnażanie

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