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Effect of polystimulin growth regulators and scion clones on graft success and subsequent growth in Atlantic cedar (*Cedrus atlantica* Manetti)

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Abstracts: *Atlantic* cedar *(Cedrus atlantica Manetti) was grown, grafting onto the rootstocks of 2 years old Lebanon* cedar *(Cedrus libani L.). The mixture of polystimulin (PS) growth regulators was used to determine the effects on graft success and subsequent growth during three growing seasons. Scion clones had no effect on grafts success. PS increased the graft success by 20% in comparison to controls. PS-treated grafts burst their buds 18-20 days earlier than control grafts and increased shoot elongation. The PS-treated grafts had 4-5 cm longer shoots than controls at the end of three growing seasons. Thus, this research indicates the significance of PS-application on graft success and subsequent shoot growth on Atlantic* cedar. *It suggested that use of PS-treated grafts was more profitable than controls. Polystimulins which were used in small doses contributed significantly to the metabolism of Atlantic* cedar *seedlings after grafting.*

Key words: *Cedrus atlantica, Plant growth regulator, Grafting, Growth, Rootstock, Scion*

Introduction

The plant growth regulators have been used successfully in plant breeding and plant growing. Some plant growth regulators affect many physiological processes while, some affect specific functions in plant (Allahverdiev *et al*., 1999). Plant growth regulators increases not only the plant growth but also their resistance to environmental stresses. Polystimulins (PS-A6 like auxin and PS-K like cytokinin) are the polymer compounds which show high biological activity and have direct effects on development and growth of plant (Allahverdiev, 1988; Tsatsakis *et al*., 1993; Allahverdiev *et al*., 1998a). Polystimulins effect growth of plants and increase plant resistance to salt stress (Allahverdiev *et al*., 1998b). Kirdar and Ertekin (2001) pointed out that PS-A6 and PS-K hormones had positive effects on the seedling growth of *Magnolia grandiflora.* Kirdar and Allahverdiev (2003) also pointed out that polystimulins provoked the seedling growth in *Fagus orientalis*. Recently, there have been numerous reports on how plant growth regulators affect the seed germination and seedling growth and development (Makhmet and Katsalan, 1979; Keithly *et al*., 1990; Seaby and Selby, 1990; Misiha and El Ashrey, 1991; Shafi *et al*., 1991; Barnes and Kelley, 1992; Ashokan *et al*., 1995; Pigott, 1996; Aphola *et al.,* 1997). According to many researchers, cytokinins made the cell division start and auxins stimulates cell division (Schneider and Wightman, 1974; Cohen and Bandurski, 1982; Elliot *et al*., 1988).

The main purpose of this research is to determine the effect of polystimulin (PS) growth regulator on graft success and subsequent growth of Atlantic cedar. This necessitates the use of greenhouses or heated polythene tunnels to activate the rootstocks which takes polystimulin growth regulator inside their tissues . The warm environment also increases transpiration and water stress in the scion which must be reduced by maintaining a humid atmosphere around the plants. In order to avoid this type of problems, the graft unions made between dormant scions and rootstock was heated in *Corylus avellana* (Lagerstedt, 1981a, b, c) and *Picea sitchensis* (Barnet and Miller, 1993). To determine the graft success, grafts were observed for three years after grafting because of graft incompatibility. Graft incompatibility has long been recognized as a problem during first growing seasons after grafting (Haines and Nicles, 1987a, b). There are early and delayed forms of incompatibility (Haines and Dieters, 1990). Approximately 8% of clones are severely affected by early incompatibility, while a smaller proportion is affected by delayed incompatibility (Haines and Dieters, 1990). The use of seedling for rootstocks would seem to rule out genetic incompatibility as an explanation for high failure rate in certain scion clones (Barnett and Weatherhead, 1988). The success of grafting was very high at first but the incompatibility could be revealed in the following years (Fowler, 1967).

Materials and Methods

Two groups of experiments were carried out in this research. One was carried out to determine the effect of grafting time and grafting conditions on grafts success in January and April, the other one was carried out to determine the polystimulin (PS) effect and scion clone effects on grafts success and subsequent growth in April. For first experiment, a total of 240 rootstocks and scions were used, and for the second 360 were used. The optimum grafting conditions were prepared in the

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Table - 1: The mean monthly temperature and relative humidity recorded in the polythene tunnel and greenhouse

	January	February	March	April	May	January	July	August	September	October
Polythene tunnel										
Average temperature (°C)	21	21	25	26	28	28	30	32	29	26
Average humidity (%)	90	92	90	90	85	84	83	85	87	88
Greenhouse										
Average temperature (°C)	8	8	13	19	26	30.7	33	35.5	25	17
Average humidity (%)	70	83	70	70	68	65	60	55	70	80

polythene tunnel, which was heated and irrigated in order to avoid the problems of increased transpiration and water stress in the scions and rootstocks. On the other hand, the conditions of greenhouse were not suitable for January grafting, especially temperature and lack of actively growing rootstocks. The best growing rootstocks were chosen from Lebanon cedar (*Cedrus libani* L.) seedlings and scions were collected from four Atlantic cedar mature trees (20 years old). A total of 240 rootstocks were kept two months earlier in heated polythene tunnel and greenhouse for first experiment before the date of January and April grafts, at the campus of Bartin Forestry Faculty, Turkey. As soon as the growing of Lebanon cedar rootstocks start, the solution of Polystimulin-K + Polystimulin-A6 (prepared mixture of PS-K + PS-A6 with 200 mg/l) was applied with irrigation water to half of Lebanon cedar rootstock at 15 days interval at two times before grafting. The scions used in January and April were collected two days earlier before grafting time and treated with the mixture of PS-K + PS-A6 for 5 minutes just before grafting time. A total of 120 grafts were done on 20th of January and a total of 120 grafts were done on 3rd of April 2001. The mean air temperature in greenhouse and polythene tunnel were 8 °C and 21 °C, respectively in January and 19 °C and 26 °C, respectively in April. The mean relative humidity was 70±5% in January and 90±5% in April. First experiment was replicated three times in heated polythene tunnel and greenhouse in a randomized complete block design.

For the second experiment, a total of 360 Lebanon cedar rootstocks were kept three months earlier than grafting time in greenhouse. All scions were collected from 6 mature trees (20 years old) at the end of March when buds start swelling but not flushing. A total of 180 scions were treated with the mixture of PS-K + PS-A6 at 100 mg/l for 5 minutes just before grafting. Grafting experiments were carried out by using side vineer grafting method (Hallet *et al.*, 1981), on the first day of April, 2001. The second experimental design was completely random with three replicates with 30 grafts per clone (total 6 clones) with PS applied and non applied for a total of 360 grafts. The mean air temperature of greenhouse was 19 °C and the mean relative humidity was 70±5% in April.

The temperature and relative humidity were measured by thermohygrometer in greenhouse and polythene tunnel.

After grafting, the number of surviving grafts was determined during three growing seasons. The date of bud bursting was noted and the length of each surviving scion was measured monthly. Analyses of variance and Tukey's multiple range test were performed on PS effect.

Results and Discussion

Effects of grafting time and grafting conditions on graft success: The grafting conditions while grafting and during subsequent growth were summarized in Table 1. Conditions during grafting periods were relatively mild with the temperature (never falling below 23 °C) in heated polythene tunnel, but cooler inside the greenhouse. Relative humidity conditions were suitable for grafts in the polythene tunnel, especially after grafting period but not in the greenhouse.

According to results (Table 2), grafts in heated polythene tunnel were more successful than grafts in greenhouse. April grafts were more successful than January grafts. Differences between graft successes were observed during the growing season. These differences were supported statistically. On this occasion, according to data table and analysis of variance was done and the *F* test showed that different grafting conditions and grafting times had been very effective on graft success at the 0.999 confident level. Thus, there were significant differences between grafting conditions and times. To determine which treatment was best effective on graft success, Tukey multiple range tests were done (Table 3). According to the results of Tukey test, each treatment was separated in groups and those in the different group were named with different letter. That means with the different letter within a column was significantly different at the 0.95 confident level. The observations show that heated polythene tunnel which had best grafting conditions of temperature and humidity, leads to increase in graft success. In addition, the grafting time in April had a significant effect on the graft success. About 70% of the grafts done in January in heated polythene tunnel were flushed by 28 January, on the other hand, about half of January grafts kept in greenhouse were flushed by the end of May.

Thus, the impression at this stage was that heating of polythene tunnel led to greater successful rates and the successful grafts flushed earlier on January. Ninety percent of April grafts and PS-treated gray B flushed by the end of April. The time of bud open of April grafts was shorter than January grafts. The ratio of failed January grafts in greenhouse was very high than April grafts in heated polythene tunnel (Fig. 1).

Table - 2: Details of grafts prepared during the winter and spring of 2001/03

Protection type of grafts		January grafts						April grafts					
		Hormone application			Control			Hormone application			Control		
		Replication			Replication			Replication			Replication		
		1	2	3	1	2	3	1	2	3	1	2	3
Polythene tunnels	% success	70	70	60	50	50	40	90	90	80	70	60	50
	Failure rate in 2001 (%)	30	40	40	45	50	50	10	10	20	30	40	40
	Failure rate in 2002 (%)	10	10	-	-	10	15	-	-	10	10	10	-
	Failure rate in 2003 (%)	-	10	20	10	-	15	-	-	-	-	15	10
Greenhouse	% success	50	40	40	30	40	30	70	70	60	60	40	50
	Failure rate in 2001 (%)	50	60	60	70	60	70	30	30	40	40	60	50
	Failure rate in 2002 (%)	-	10	-	10	15	-	10	-	-	-	10	10
	Failure rate in 2003 (%)	20	-	30	-	15	20	-	-	10	20	-	10

Table - 3: Results of Tukey multiple range test for polystimulin, conditions and grafting time effects

	LS mean	Homogeneous groups		LS mean	Homogeneous groups		LS mean	Homogeneous groups
Control	47,5	a*	Greenhouse	48.33	c*	January	46.67	e*
PS	65,0	b	Polythene tunnel	64.17	d	April	65.83	f

* : Means with the different letter within a colum are significantly different ($p < 0.05$)

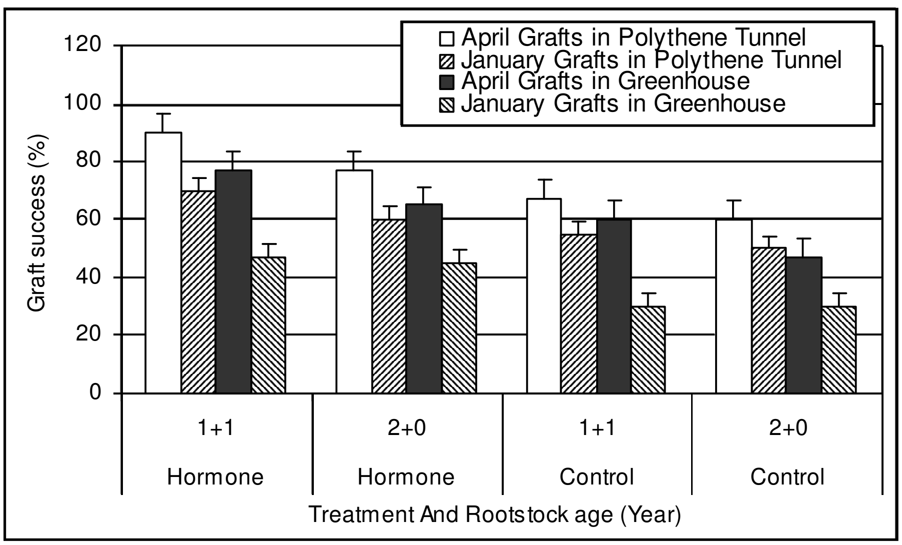

Fig. 1: The state of grafts kept in different condition and grafted and made in January and April

Effects of hormone treatment and scion clones on graft success: According to data, PS-treatment had a significant effect on grafts success at 0.999 confident level. The observations carried out during the growing seasons show that PS-treatment had a great contribution in graft success. In the first experiment, the success ratio of PS-treatment and control grafts was 66.7% and 43.3%, respectively in January and 86.7% and 60%, respectively in April in heated polythene tunnel. In the greenhouse, the success ratio of PS-treatment and control grafts was 43.3% and 33.3%, respectively in January and 66.7% and 50%, respectively in April (Fig. 1 and Table 4). In the second experiment, the success ratio of PS-treated grafts changed from 73.3% to 83.3% in greenhouse and the success ratio of control grafts changed from 43.3% to 56.7%. The application of PS to graft surface leads to significant increase in graft success as compared with controls in heated polythene tunnel. On the other hand, scion clones had no effect on grafts success. There were no significant differences among scion clones on graft success.

Differences between PS-treated and control grafts were continued during the next growing seasons and these differences

Table - 4: The results of scion clonal differences of April grafts

Treatment	Replication	Greenhouse					
		Scion clones					
		C1	C2	C3	C4	C5	C6
PS	1	70	80	80	70	70	90
	2	80	80	80	70	80	80
	3	70	90	70	80	70	70
Control	1	50	40	40	50	50	60
	2	40	50	50	50	60	50
	3	50	40	50	50	50	60

Table - 5: Results of Tukey multiple range test for PS (polystimulin) and clone effect

	LS mean	Homogeneous groups	LS mean	Homogeneous groups
Control	47,5	a*	Clone 1	c
PS	65,0	b	Clone 2	c
			Clone 3	c
			Clone 4	c
			Clone 5	c
			Clone 6	c

* : Means with the different letter within a colum are significantly different ($p < 0.05$)

were supported statistically. On this occasion, according to data table, analysis of variance was done and the *F* test showed that PS had been very effective on graft success at the 0.999 confident level. There were significant differences between PS-treated grafts and controls. To determine which treatment was the best effective one on graft success, Tukey's multiple range tests were done. According to results of Tukey test, each different treatment was separated in different groups and named with different letter. That means with the different letter within a column were significantly different at the 0.95 confident level (Table 5).

Bud bursting and subsequent shoot growth: About 70 % of PS treated grafts in January in heated polythene tunnel flushed by 5th of March, on the other hand, about half of control grafts flushed on 28th of March. Thus, the impression at this stage was that PS application in heated polythene tunnel led to greater successful rates and PS-treated grafts flushed their buds 23 days earlier than control grafts in January after grafting. While PS-treated April grafts flushed the buds on 09 of April, control grafts flushed on 27 of April in heated polythene tunnel. Bud bursting was seen on PS-treated April grafts 8 days later after grafting and seen on control grafts *i.e.* 27 days later in heated polythene tunnel. PS treatment made grafts buds burst 18 days earlier than control in April.

The time of bud bursting of April grafts was shorter than January grafts. Lots of April grafts, whether PS applied or not, showed bud bursting although no one of PS-treated and control grafts in January had swollen buds. Initial time for bud bursting from PS-treated January grafts was 5 of March in heated polythene tunnel, but was on the first day of June in greenhouse. Initial time for bud bursting from PS-treated April grafts was 9 of April in heated polythene tunnel, but 20 of April in greenhouse. Shortly, PS-treatment had a significant effect on bud bursting. It made bud bursting minimum 20 days earlier than controls in January and April grafts in heated polythene tunnel. Same results were obtained in greenhouse. PS-treated grafts burst their buds minimum 13 days earlier than control grafts buds.

The average extension of the scions (PS-treated and control grafts) for both grafting time were shown (Table 6 and Fig. 2 and 3). The PS-treated grafts in heated polythene tunnel and greenhouse flushed earlier than control grafts in January. Similar trend was also observed in April. PS-treated grafts showed a trend towards earlier growth than controls. The difference in growth between PS-treated grafts and controls for the late of growing season increased, being greatest for the April grafting. The time of bud bursting of PS applied grafts was 18-20 days earlier than control grafts in greenhouse and heated polythene

Table - 6: Summarizing the subsequent growth of the grafts after grafting

Condition	Treatment	Grafting time					
		15 January			01 April		
		Date of bud bursting and average shoot elongation (cm)			Date of bud bursting and average shoot elongation (cm)		
		2001	2002	2003	2001	2002	2003
Polythene tunnel	Control	28, March 4.5 cm	23, April1 4.0 cm	25, April 15.5 cm	27, April 9.0 cm	26, April 15.0 cm	23, April 15.5 cm
	PS	05, March 5.3 cm	01, April 16.3 cm	04, April 18.7 cm	09, April 17.0 cm	03, April 22.7 cm	02 April 23.5 cm
Greenhouse	Control	17, June 1.2 cm	28, April 11.4 cm	30, April I14.7cm	05, May 4.5 cm	18 April 12.3 cm	21 April 13.3 cm
	PS	01, June 1.3 cm	11, April 12.5 cm	14, April 16.6 cm	20, April 4.33 in	5, April 18.0 cm	7, April 19.6 cm

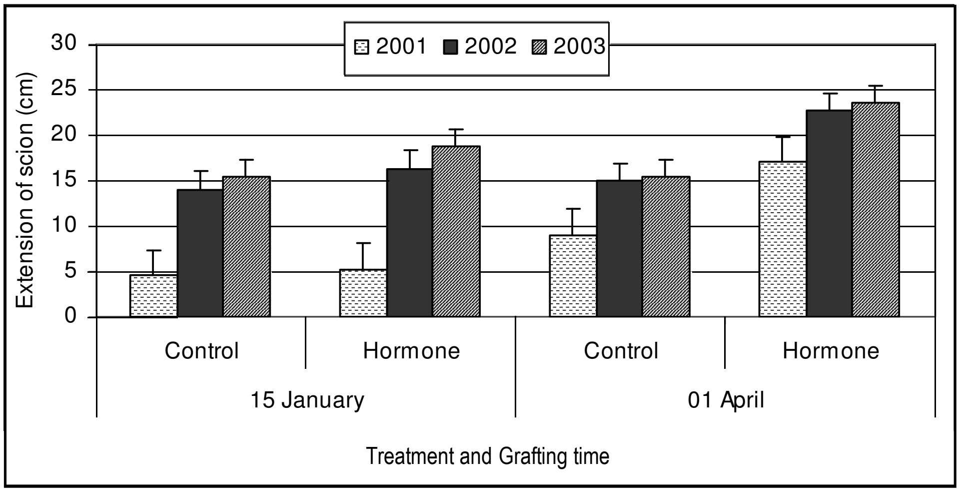

Fig. 2: Average shoot growth of grafts in polythene tunnel during three growing seasons after grafting

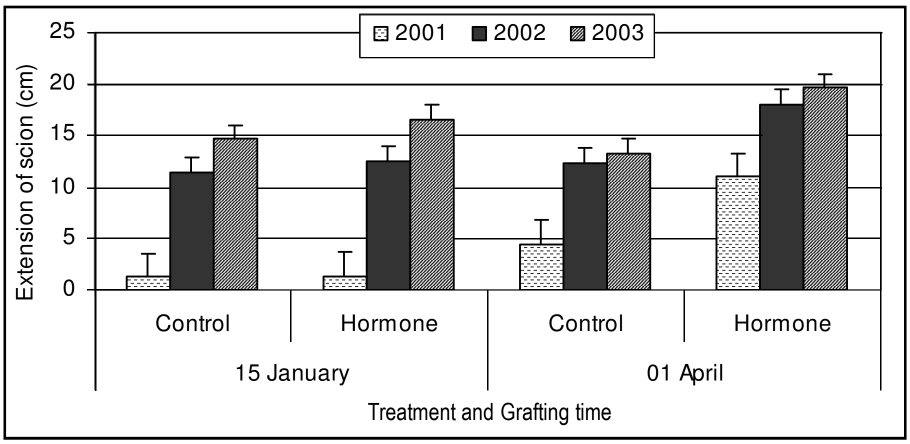

Fig. 3: Average shoot growth of grafts in greenhouse during three growing seasons after grafting

tunnel (Table 6). The average shoot elongation of PS-treated April grafts was 17.0 cm in heated polythene tunnel, but was only 4.5 cm of control grafts in first growing season. The average shoot elongation of PS-treated grafts was 11.0 cm in greenhouse in April. On the other hand, the average shoot elongation of PS-treated grafts and controls were 5.3 cm and 4.5 cm, respectively in polythene tunnel in January. As a result of these experiments, PS treatment affected the growth 4-5 times more than control grafts during first growing season.

In addition, best growing performance of PS-treated grafts continued by the end of three growing seasons. Generally, the average shoot elongation of PS-treated grafts was 4-5 cm longer than controls at the end of three growing seasons.

A noticeable improvement on grafts success and subsequent shoot growth was obtained for *Cedrus atlantica*. The results of the grafting experiments, demonstrate that the PS-treatment onto the graft surface and to rootstocks increases graft success rate. Indeed, the impression might be gained from observations carried out at the time of growing, that PS-treated grafts performed better than controls. The highest graft success was found at the treatment of PS. The highest grafts success was 86.7% in heated polythene tunnel in April comparing to 33.3% in control grafts kept in greenhouse in January. Applying PS to grafts resulted in earlier flushing of the scion bud. The present study also shows that PS-treatment increases shoot elongation of Atlantic cedar grafts. Fig. 2 and 3 show the final difference in length between the scions of PS-treated and control grafts. Thus, the difference in length achieved during the three growing seasons after grafting could be attributed to the earlier start obtained by PS applied grafts. Because scions were collected when their buds had swollen but not open, and rootstocks were in active. PS growth regulator was easily absorbed inside theirs' tissues. So, PS treatment made the best effect in April grafts. Shortly, PS-treatment to graft surface lead to significant increase on graft success and subsequent seedling growth as compared with controls. On the other hand, scion clones had no effect on grafts success.

Makhmet and Katsalan (1979) pointed out that treating the cut surfaces of the scion and rootstock with 0.1% dimethylsulphoxide improved the survival and growth of the grafts.

Allahverdiev (1988); Allahverdiev *et al.* (1998a, 1998b) and Tsatsakis *et al.* (1993) also described that polystimulins have many direct effects on the growth of plant. Some researchers pointed out that there was a strong dependence of the effects of phytohormones on seedling growth and phytohormones stimulate the plant growth (Keithly *et al.*, 1990). Kirdar and Ertekin (2001) found that PS-A6 and PS-K hormones had positive effects on the seedling growth of *Magnolia grandiflora*. Seaby and Selby (1990); Misiha and El Ashry (1991); Barnes and Kelley (1992); Ashokan *et al.* (1995) and Aphola *et al.* (1997) also pointed out that phytohormones increases seedling growth in their studies. Our observations confirm that PS-treatment was most effective if carried out when the buds start swelling, with both higher success rates and better subsequent growth.

Any successful grafts have suddenly been died three years later in this study. So, the ratio of graft success decreased in proportion to 20%. This indicated that incompatibility could be revealed following years after grafting for *Cedrus atlantica*. Haines and Nikles (1987a, b) have stated the deaths seen after grafting as a graft incompatibility. That was also stated by Fowler (1967); Haines and Dieters (1990) and Barnett and Weatherhead (1988).

The heating of polythene tunnel had a positive effect on graft success because it had increased the average temperature inside. So, PS had been taken easily by the tissues of rootstocks growing actively. The average temperature was never fallen below 23 °C. Heat application to activate the rootstocks had effect on the success rates in January grafts in polythene tunnel. Main reason of the success in grafting is to graft dormant scions onto actively growing rootstock during the winter and early spring. Lagerstedt (1981a, b, c) and Barnet and Miller (1993) pointed out that the heat application increases graft success.

This work has demonstrated the importance of PS-treatment in order to ensure success rates in growth of Atlantic cedar. From this point of view, it brings to our attention that using the only PS-treated grafts was more profitable than controls. To conclude, polystimulins which were used in small doses have contributed significantly to the metabolisms of Atlantic cedar seedlings after grafting to work rapidly and regularly.

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