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Shortening of Breeding Cycle by Forced Flowering in Forest trees Ⅱ. Enhancement of Flowering Induction by Treatment of Growth Regulators in *Betula pendula* Roth and *Betula platyphylla* var. *japonica* Hara[1*]

Min Sup Chung[2], Jinki Jo[2] and Sang Jin Park[2]

林木에 있어서 開花誘導에 의한 育種싸이클의 短縮 Ⅱ. 生長調節物質 處理에 의한 자작나무와 은자작나무의 開花誘導 促進[1*]

鄭珉燮[2] · 曺晋基[2] · 朴相珍[2]

ABSTRACT

Betula pendula and B. platyphylla var. *japonica* seedlings and/or grafts growing inside and outside of a plastic-greenhouse were treated with growth regulators to induce flowering at early stages of seedling and graft developments. The seedlings began to develop female catkins visibly in eight to nine months after seeding and in five months after the first treatment of growth regulators. Thirty three percents of the seedlings grown under controlled environment in the plastic-greenhouse with sufficient nutrient supply developed female catkins both in control plants and in the plants treated with IAA, GA_3 and kinetin, while none on the control plants grown in the field showed any sign of the development of floral organs. Sixty seven percents of the seedlings treated with ABA and SADH grown in the plastic-greenhouse developed female catkins. All the seedlings treated with 6.24 mM of SADH developed female catkins. SADH treatment to 2-5 year old seedlings and grafts of birch had a tendency of positive effect on inducing and increasing the flowering of the two birch species.

Key words : Betula spp. *induction of flowering, shortening of breeding cycle*

要 約

비닐하우스 및 야외에 식재된 자작나무와 은자작나무 實生苗와 接木苗에 대하여 幼苗 發育初期에 開花誘導를 목적으로 生長調節物質을 처리하였다. 자작나무와 은자작나무의 實生苗들은 播種 後 8-9 個月사이인, 生長調節物質 첫번째 處理後 5個月 부터 암꽃을 可視的으로 形成하기 시작하였다. 충분한 養分을 供給하면서 비닐하우스 內에서 環境調節 下에 養苗한 實生苗들 中 IAA, GA_3, kinetin을 처리한 것과 生長調節物質을 전연 처리하지 않은 것(비닐하우스 內의 比較木)의 33%가 암꽃을 형성하였으나 야외에 植栽된 比較木에서는 암꽃이 전연 形成되지 않았다. 비닐하우스 內에서 ABA와 SADH를 處理한 種苗의 67%가 암꽃을 형성하였으며 특히 6.24 mM의 SADH를 처리한 實生苗들은

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[2] College of Agriculture, Kyungpook National University, 702-701, Taegu, Republic of Korea
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본 논문은 1993-1995년도 교육부 학술진흥재단의 연구비 지원에 의해 수행되었음

모두가 암꽃을 形成하였다. 2~5년생 實生苗와 接木苗에 대한 SADH 처리는 開花誘導 및 開花量의 增加에 效果가 있는 것으로 나타났다.

INTRODUCTION

Seedlings of most woody perennials have a protracted juvenile phase during which they are incapable of flowering. Long juvenile periods and slow turnover of generations are major constraints to tree breeding and in modern silvi-culture. Strategies are required for induction of precocious flowering in seedlings and for speeding the generations.

The two birch species generally have relatively long juvenile phase of about ten to fifteen years in *B. platyphylla* var. *japonica* and *B. pendula* with some exceptions in isolates and/or in those on dry sites. Thus one breeding cycle of *B. platyphylla* var. *japonica* and *B. pendula* may well be over sixteen to twenty one years.

In early 1970's, research workers at Foundation for Forest Tree Breeding in Finland successfully induced flowering in *B. pendula* seedlings in a plastic-greenhouse with controlled environment-temperature control, nutrient supply and carbon dioxide enrichment in the ambient air etc(Lepisto 1973). The author at present work attempted to induce flowering at early stages of seedling development of *Betula pendula* and *B. platyphylla* var. *japonica* by a similar method as mentioned above. The result was satisfactory for inducing male flowering in the first and the following years after one cycle(about nine months) treatment for floral induction(Ryu *et al* 1995). Only a few female catkins developed with eleven percents of the treated seedlings in the second year. Most of the treated seedlings developed both male and female catkins from the third year of the treatment.

The aim of this study was to find out an effective method for flowering induction in *Betula pendula* and *B. platyphylla* var. *japonica* to shorten the breeding cycle and to produce large quantity of genetically improved seeds in short period of time.

MATERIALS AND METHODS

Seedlings of *Betula pendula*(German and Finnish seed sources) and B. *platyphylla* var. *japonica* (Japanese seed source) were grown in a plastic-greenhouse installed inside a laboratory for about three months until they were treated with growth regulators. The seedlings were grown under 19-hour day(about 12 to 14-hour natural incident light through windows and addi-tional light supplementation mainly with high pressure sodium lamps - 80% in electrical wattage - , and mercury lamps - 20% in electri-cal wattage - during night. Total radiant flux by the supplemental light was approximately 196~250J/m^2/sec. (maximum 385J/m^2/sec. at the top of tree crown) at tree crown level. Seedlings were transplanted into plastic pots(33cm both in diameter and height) when they were about 10cm in height, and regularly watered with nutrient solution with the following ingredients : 5mM KNO_3, 1mM KH_2PO_4, 4mM $Ca(NO3)_2$, 3mM $MgSO_4$, 0.046mM H_3BO_3, 0.008mM $ZnSO_4$, 0.003mM $CuSO_4$, 0.0003mM FeEDTA and 0.0001mM $(NH4)_2MoO_{24}$. Temperature was controlled to 24℃ for night and 30℃ for day.

The growing conditions of the seedlings were slightly modified from the fourth month as follows when the height reached proper developmental stages for floral induction - the day length ; 16 hour-day and 8-hour night, and the temperature ; 18℃ for night and 28℃ for day. The same nutrient solution but with reduced KNO_3 (3mM) and increased KH_2PO_4(3mM) was supplied every other day until the first reproductive organs develop visibly. Also ambient air inside the plastic-greenhouse was enriched with carbon dioxide up to 800 - 1200ppm to enhance the seedling growth whole through the induction period from seeding.

The seedlings in the plastic-greenhouse were treated three times with the following growth regulators : 0.05mM, 0.10mM of indoleacetic acid

(IAA), kinetin, gibberellic acid(GA_3) and abscisic acid(ABA), and 6.24 and 12.48 mM of succinate 2,2 dihydroxymethylhydrazide(SADH) when they were three, four, and five months old. Two to five year old seedlings and grafts in the field were also treated three times with 6.24 and 12.48mM of SADH in July and August for floral induction under natural conditions.

Table 1. Percentages of floral induction by the treatment of growth regulators in plastic greenhouse.

Growth regulators and concentrations		Replication	Catkins developed	Percentage of floral induction	
IAA	0.05mM	1 2 3	0 8 0	33%	33%
	0.10mM	1 2 3	0 0 43	33%	
Kinetin	0.05mM	1 2 3	1 0 0	33%	33%
	0.10mM	1 2 3	0 2 0	33%	
GA	0.05mM	1 2 3	0 1 0	33%	33%
	0.10mM	1 2 3	0 1 0	33%	
ABA	0.05mM	1 2 3	0 53 5	67%	67%
	0.10mM	1 2 3	1 37 0	67%	
SADH	6.24mM	1 2 3	2 3 38	100%	67%
	12.48mM	1 2 3	21 0 0	33%	
Controls	Inside plastic greenhouse	1 2 3	0 0 43	33%	33%
	Field	1 2 3	0 0 0	0%	0%

replication 1 and 2 : *B. platyphylla* var. *japonica*
replication 3 : *B. pendula*

RESULTS

The treatment of growth regulators did not affect the seedling growth significantly for one month after the first treatment. However, the treatment with 12.48mM of SADH retarded the seedling growth significantly(covariance analysis of the slopes of regression lines : $F=12.67$, $df=1,4$, $P<0.05$) compared with that of the 6.24 mM treatment.

The seedlings began to develop female catkins (determined by the position and the shape of initial developmental stages) in eight to nine months after seeding and in five to six months after the first treatment of growth regulators. The seedlings treated with 6.24mM of SADH developed catkins first. Some of the seedlings began to develop catkins in 11 months. The developing catkins did not show the normal shape. Most of the catkins had grown continuously and elongated shape just like the developing male catkins of the previous year of flowering and finally dried out without flowering during late July and August. Some of the catkins were erect and developed abnormal flowers with anthers between the scales of female flower.

Generally, birch species develop erect female catkins axially while drooping male catkins terminally on branches during flowering. But in this experiment all the seedlings that have developed floral organs had catkins axially on the female position except one seedling. In the previous work(Ryu *et al* 1995) only male catkins were developed during the first induction period when the seedlings and grafts were grown under controlled environmental conditions with 20-hour day length without treatment of growth regulators. Newly developing male catkins are visible from early May through late June in the previous year of flowering, and grow until fall before they enter dormancy under natural conditions in Taegu(35° 53' N, 128° 37' E, 57.8 m a.s.l.), Korea. Male floral primordia are initiated and developed mainly under long day conditions with increasing day length. Female floral primordia are thought to be initiated and developed to some extent inside the developing new buds from the late summer to fall of the previous year of flowering when the condition of day length is relatively short and decreased. Comparing the results of the previous and present works it is deduced that day length longer than twenty hours enhances the initiation of male catkins and shorter than sixteen hours enhances the female catkins.

Induction of flowering by the treatment of growth retardant ABA and SADH inside the plastic-greenhouse was effective(Table 1). Thirty three percents of the seedlings including controls grown under controlled environment in the plastic-greenhouse with sufficient nutrient supply developed female catkins without regard to the treatment of IAA, kinetin and GA_3 while none of the control plants grown in the field showed any sign of the development of floral organs. All the seedlings treated with 6.24mM of SADH developed female catkins. In this case the rate of flowering induction was significantly different ($X^2=6.00$ **, $P<0.025$) from the control in the field.

Induction of male flowering by SADH treatment to 2~5 year-old seedlings and grafts grown in the field and having no flowering record was very effective for male catkins both in *B. pendula* and *B. platyphylla* var. *japonica* ($X^2=4.36$ * and 4.71 * respectively). Induction of female flowering in *B. platyphylla* var. *japonica* was effective($X^2=4.71$ *), too(Table 2).

SADH treatment to two to five-year old seedlings and grafts grown in the field and having flowering record gave positive effects in increasing the quantity of flowering and/or in maintaining the persistency of flowering(Table 3). Most of the seedlings and grafts of *B. pendula* treated with SADH developed male catkins in the following year and the percentage of male flowering plants was significantly higher($X^2=4.16$ *) than that of the control.

DISCUSSION

Various methods have been reported for induction of precocious flowering and/or for the pro-

Table 2. Induction of flowering by SADH treatment to two to five year old and previously non flowering trees growing in field.

SADH concentr.	*B. pendula* Tree no	*B. pendula* Female	*B. pendula* Male	*B. platyphylla* Tree no	*B. platyphylla* Female	*B. platyphylla* Male
6.24mM	1		+	1	++	+
	2		++	2	+	+
	3	+	+	3	+	
	4	+	++	4		+
	5			5		+
	6			6		+
	7			7		+
	8			8	+	
				9	++	
				10		
				11	+	
				12	+	+
				13		
				14	++	++
% induction		25.0%	50.0%		57.1%	57.1%
12.48mM	1		+	1		
	2			2		
% induction		0.0%	50.0%		0.0%	0.0%
Total % induction		20.0%	50.0%		50.0%	50.0%
Control	6 trees	0.0%	0.0%	6 trees	0.0%	0.0%

+ : number of catkins less than 30, ++ : number of catkins more than 30

motion of flowering at early developmental stages of seedlings, grafts and cuttings in forest trees(Greenwood *et al* 1991, Harrison and Slee 1991, Ho 1988) and horticultural plants(Greene and Lord 1978, McLaughlin and Greene 1984, 1991a, 1991b, Wilkinson and Richards 1991). The most effective and promising methods for flowering induction in perennial woody species at present are the treatment of growth regulators and the ameliorated environmental treatment that enhances both vegetative and reproductive growth of seedlings.

Naphthalene acetic acid and GA_3 promote male flowering for four to seven year old *Picea engelmannii* grafts(Ross 1990) and for 7 year old *Pinus tabulaeformis* seedlings(Sheng and Wang 1990) respectively. GA_4 promotes female flowering both in 15 year old grafts of *Picea mariana* (Ho 1988) and *Pinus tabulaeformis*(Sheng and Wang 1990). Young birch seedlings treated with GA_3 and IAA at present work did not give similar effect on sex expression. Both of the treatment of GA_3 and IAA induced female catkins. Only one of the three seedlings treated with 0.05mM of ABA in the plastic greenhouse developed male catkins including female ones.

Vegetative growth of birch seedlings can be enhanced by long day treatment with high light intensities(Krizek and Zimmerman 1973) or by growing the seedlings under a CO_2 enriched atmosphere in controlled environment(Krizek *et al* 1969). Continuous growth of seedlings with sympodial growth behavior which is shown in birch species under the favorable environmental conditions with sufficient nutrient supply may lead to phase change from juvenile to mature ones within a short period of time at seedling stage as reported by Lepisto(1973).

The authors commenced the present work expecting the combined effect of environmental

Table 3. Promotion of flowering by the treatment of SADH to two to five year old trees grown in the field and flowered seldom and/or very poorly in the previous years.

SADH concentr.	*B. pendula*			*B. platyphylla*		
	Tree no	Female	Male	Tree no	Female	Male
6.24mM	1		+	1	++	++
	2	++	++	2	++	++
	3		++	3	+	+
	4		++	4	++	+
	5		+	5	+	+
	6	++	++	6	++	++
	7		+	7	+	+
	8			8	+	+
	9	+	+			
% flowering		33.3%	88.9%		100.0%	100.0%
12.48mM	1		+	1	+	++
	2	+	++	2	++	++
	3		+	3	+	+
	4		+	4	+	+
	5	+	++	5	+	++
	6	+				
	7	+				
	8	+				
	9	++	++			
	10		++			
	11	+	++			
% flowering		36.4%	100.0%		100.0%	100.0%
Total % of flowering		35.0%	95.0%		100.0%	100.0%
Control	1			1	+	++
	2			2	+	+
	3	+	+	3	+	+
	4		+	4	+	+
	5		++	5	+	+
	6	+	+			
% flowering		33.3%	66.7%		100.0%	100.0%

+ : number of catkins less than 30, ++ : number of catkins more than 30

control for ideal tree growth and the treatment of growth regulators for flowering induction at early stages of seedling development. The result was successful with the treatment of growth retardants, SADH and ABA(Table 1), for the enhancement of the efficiency of flowering induction at early seedling stages in birch species.

CONCLUSION

Juvenile phase of some forest tree species can be reduced dramatically, for instance, from ten or fifteen years to one or two years by treatment of growth regulators under controlled environ ment. From the result of present work it is possible to shorten one breeding cycle of *B. pendula* and *B. platyphylla* var. *japonica* to seven to eight years (two years for seed pro duction and five to six years for progeny test). This will give tremend ous returns in many ways to tree breeders. By shortening the breeding cycle with flowering induction we can save time, efforts and expendi tures for the management during the long breed ing cycle in forest trees.

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