

In vitro Propagation of *Betula schmidtii* from Germinated Seedlings

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I. Introduction

Betula schmidtii Regel. is a large deciduous tree and has very hard, heavy, closegrained and tenacious wood. It has been used for utensils, building materials, furnitures etc. (KURATA, 1968).

Recently it is attentioned as a material for seals in Japan. For its genetic improvement a technique for clonal propagation is needed for the propagation of selected plus-trees.

In vitro propagation of *B. schmidtii* was reported by LEE *et al.* (1986). They succeeded in the plant regeneration from shoot tips and/or axillary buds of 4-year-old seedling of this birch and also succeeded in mass propagation of clone by the successive subculture.

In tissue culture of Japanese white birch (*Betula platyphylla* var *japonica*), GA₃ (Gibberellic acid) concentration is effective for adventitious buds and multiple shoots induction (IDE, 1987, KAWAI, 1991, SAITO and IDE, 1985). In this experiment we intended to clarify the effects of GA₃ concentrations to the medium in the culture of germinated seedlings of *B. schmidtii*. And we established *in vitro* propagation method for mass propagation of this birch from germinated seedlings.

II. Materials and Methods

Seeds of *B. schmidtii* was obtained from the university forest in Chichibu, the university of Tokyo. Their provenance was at the altitude of 800 m at Oochigawa in Ootakimura, Saitama Prefecture, Japan. They were collected at August 1991 and stored in refrigerator at 5°C until using.

The seeds were surface sterilized by 70% ethanol for 1 min, by antiformine solution containing about 5% effective chloride and 1% triton-x for 10 min and 3% hydrogyperoxide for 10 min successively. They were laid on the germination medium. The medium was 1/2MS medium where the concentration of macro inorganic salts of MS medium (MURASHIGE and SKOOG, 1962) were reduced to half and containing 1% of sucrose.

After ten days on germination medium, explants were prepared by removing radicles from germinated seedlings. They were inoculated onto 1/2MS medium containing 0.8 mg/l of BAP (6-benzylaminopurine), 0.02 mg/l of NAA (α -naphthylacetic acid), 10 mg/l of GA₃ and 2% of sucrose.

After two weeks of culture, explants were cut into three segments i. e. cotyledonal node, hypocotyl segment and epicotyl segment (Fig. 1). They were inoculated onto fresh 1/2MS medium containing 0.8 mg/l BAP, 0.02 mg/l NAA and 0 or 10 mg/l of GA₃.

After one month culture of divided tissues, shoots which derived from explants and elongated over 0.5 cm were harvested and transplanted onto 1/2MS medium containing 0.5 mg/l of IBA (β -indolebutyric acid) and 0.02 mg/l of NAA for rooting.

Shoots were harvested again after two weeks of previous harvesting and transplanted

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onto rooting media. Hormone free 1/2MS medium was tested besides former rooting medium to confirm the effect of auxine concentration on rooting.

After shoot harvesting, basal tissues of cotyledonal nodes cultured on GA_3 containing medium and epicotyl segments cultured on GA_3 free medium were cut into small pieces about 5 mm in diameter and transplanted onto two types of fresh media for subculture. One medium contained 0.8 mg/l of BAP, 0.02 mg/l of NAA and 10 mg/l of GA_3 and the other medium contained 0.8 mg/l of BAP and 0.02 mg/l of NAA. The cultures were divided into equivalent number of tubes for each medium.

All culture medium used in this experiment were solidified by 0.8% of agar and pHs were adjusted to 5.8 before autoclaving.

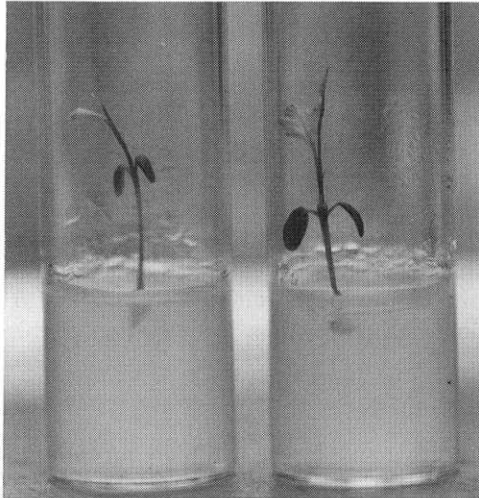


Fig. 1. Hypocotyl and epicotyl elongation of seedlings which radicles had been removed and cultured on GA_3 containing 1/2MS medium. After 45 days of culture.

Cultures were kept at 25°C and under fluorescent illumination for 16 h photoperiod.

Rooted plantlets were transplanted to soil mixture (vermiculite and peat moss; 1 : 1 (v : v)) after removing agar medium and acclimatized in a growth chamber. In the chamber, relative humidity was kept at 90% for 1 weeks and lowered to 70%.

III. Results and Discussion

Primary culture

Hypocotyls of seedlings of which radicles had been removed were 5–7 mm in length at the beginning of culture. During 10 days of culture, hypocotyls elongated to 15–20 mm. Gibberellin has an effect of inducing elongation of juvenile embryonic tissues (MURAKAMI, 1980). The rapid hypocotyl extension appeared to be induced by GA_3 .

Some explants were kept in the medium for continuous observation. Epicotyls of

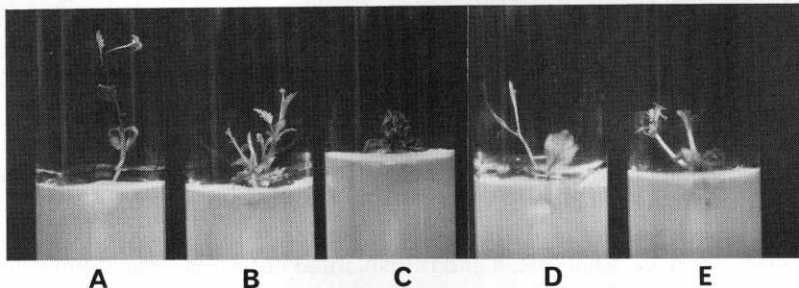


Fig. 2. Shoot elongation from divided tissues after one month of culture.
 A: Seedling which did not divided and cultured on GA_3 containing medium.
 B: Cotyledonal node cultured on GA_3 free medium.
 C: Cotyledonal node cultured on GA_3 containing medium.
 D: Epicotyl segment cultured on GA_3 free medium.
 E: Epicotyl segment cultured on GA_3 containing medium.

Table 1. Number of shoots derived from divided seedling tissues.

Tissue	GA ₃ concentration (mg/l)					
	0			10		
	1st	2nd	Total	1st	2nd	Total
Cotyledonal node	0.3	0.7	1.0 (3)	4.0	6.5	10.5 (4)
Epicotyl segment	2.3	1.7	4.0 (3)	1.0	0.0	1.0 (4)
Hypocotyl segment	0.0	0.0	0.0 (3)	0.0	0.0	0.0 (4)

1st: 1st harvest of shoots after 1 month culture of divided tissue.

2nd: 2nd harvest of shoots after 2 weeks of 1st harvest.

Total: Total harvest during culturing period.

Numbers of shoots are shown as mean for a explant.

Numbers in parentheses are numbers of cultured explant.

Table 2. Number and length of shoots derived from 55 days of subculture.

Original tissue	GA ₃ concentration (mg/l)			
	0		10	
	Number	Length (mm)	Number	Length (mm)
Cotyledonal node (4)	7.8	17.7 ± 1.7	9.8	22.4 ± 2.3
Epicotyl segment (3)	2.7	17.6 ± 3.9	1.0	13.0 ± 7.5

Cotyledonal nodes were cultured on GA₃ containing medium and epicotyl segments were cultured on GA₃ free medium in the primary culture respectively.

Numbers in parentheses are the number of original seedlings.

Tissues derived one original seedling were divided into equivalent number of tubes.

these explants grew longer and calluses were formed at the cut end of hypocotyl after 40 days of culture (Fig. 1). But adventitious shoot or axillary shoot was never elongated (Fig. 2A).

Another explants were cut into three parts and used for successive culture. After two weeks culture of divided tissues, multi shoots were elongated from a cotyledonal node which cultured on the medium containing GA₃. After one month of culture, they were elongated to 5–20 mm (Fig. 2B). On the other hand, few shoots were formed from a cotyledonal node which was cultured on the medium without GA₃ (Fig. 2C). Epicotyl segments elongated plural shoots on the medium without GA₃ (Fig. 2D). No shoot was formed from epicotyl segments cultured on the medium without GA₃ (Fig. 2E).

No shoot formation was observed on hypocotyl segment. Calluses were formed on the hypocotyl segments in both medium. Callus formation was rather active on the medium containing GA₃ than on the medium without GA₃.

Elongated shoots were harvested and transplanted to rooting medium. After tow weeks of 1st harvest of shoots newly elongated shoots were harvested again.

Numbers of shoots obtained from two times of harvesting are shown in Table 1. The cotyledonal nodes cultured on the medium with GA₃ produced 10.5 shoots from a explant. Epicotyl segment cultured on GA₃ free medium produced four shoots from a explant. And other combinations of media and explants produced only one shoot from a explant.

These results suggested that the culture of cotyledonal nodes on the medium containing 10 mg/l of GA₃ is appropriate for multiple shoot proliferation.

The multiple shoot formation also was induced from epicotyl segments when they

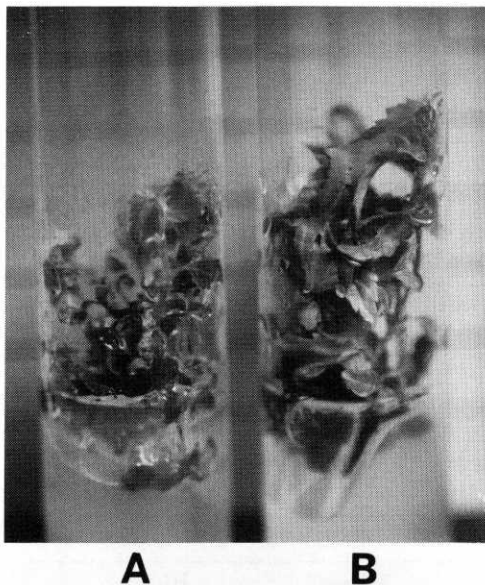


Fig. 3. Multiple shoot formations by subculture of cotyledonal node and epicotyl segment.

After 55 days of subculture.

A: Tissue derived from cotyledonal node which cultured on GA₃ containing medium and subcultured on the same medium.

B: Tissue derived from epicotyl segment which cultured on GA₃ free medium and subcultured on GA₃ free medium.

were cultured on GA₃ free medium. While the concentration of GA₃ has a specific effect on shoot multiplication at cotyledonal nodes, on the contrary it suppress shoot multiplication at epicotyl segments. It is supposed that GA₃ stimulates shoot elongation when shoot apices exist and it stimulates adventitious shoot formation when shoot apices are absent.

Efficiency of GA₃ application to birch tissue culture was reported on *Betula platyphylla* var *japonica* (SAITO and IDE, 1985b; IDE, 1987; KAWAI, 1991). GA₃ was used for adventitious bud induction and it was not necessary for shoot elongation in these cases. This phenomenon agrees well with the results of this experiment.

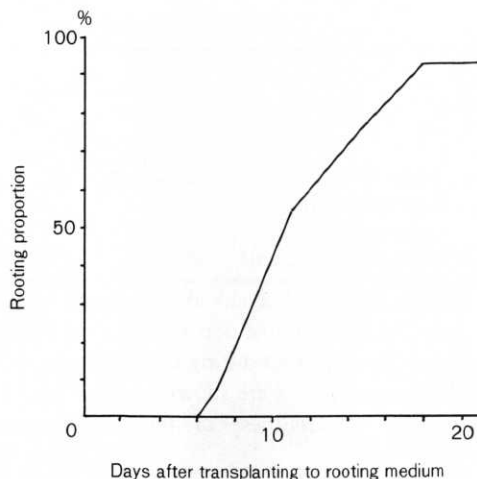


Fig. 4. Change in the proportion of rooted shoots which harvested primarily from divided seedlings.

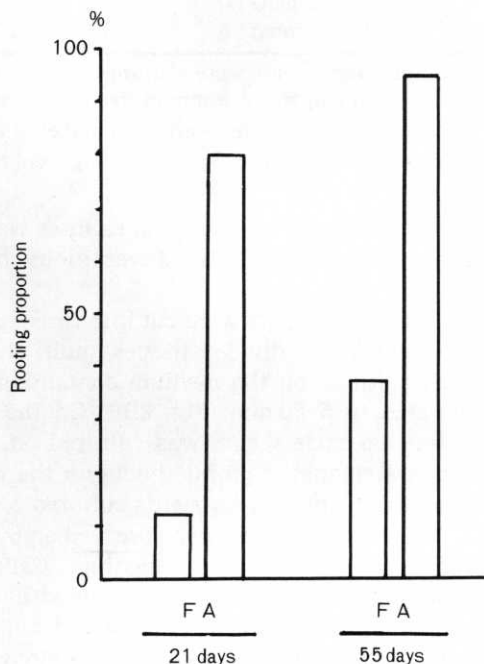


Fig. 5. Rooting proportion of shoots which harvested secondarily from divided tissue on different rooting media. F: Hormone free medium. A: Medium contained 0.02 mg/l NAA, 0.5 mg/l IBA.

Subculture

The numbers and length of newly elongated shoots were counted and measured after 55 days of subculture as shown in Table 2. The tissues derived from cotyledonal segments cultured on GA₃ contained medium in the primary culture developed more shoots in the secondary culture than the tissues derived from epicotyl segments cultured on GA₃ free medium (Fig. 3). There was no significant of GA₃ concentration to the medium is not necessary for subculturing shoots.

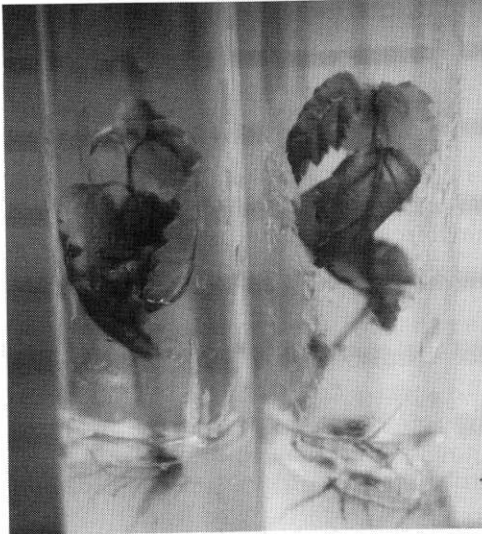


Fig. 6. Regenerated plantlets on rooting medium.
After 45 days of culture on rooting medium.

Rooting of shoots

The shoots harvested first time from separated tissues were transplanted to rooting medium. One weeks after the inoculation to rooting medium, rooted individuals were first observed. During 18 days on the rooting medium 92% of shoots were rooted (Fig. 4). Rooting occurred regardless of the media on which shoots were derived.

The shoots harvested second time were cultured on two types of rooting medium. Rooting was observed after one weeks of inoculation on the medium containing IBA and NAA. Rooting proportion achieved 80% after two weeks of culture. And after 55 days of culture it increased to 95%. On the hormone free medium, first rooting individual was observed after 10 days of culture. After three weeks of culture, rooting proportion was only 12.5% and it was 37.5% after 55 days of culture (Fig. 5). Regenerated



Fig. 7. Plantlets successfully acclimatized and grown in room condition.
One month after acclimatization.

plantlets after 45 days culture on rooting medium were shown in Fig. 6.

We concluded that the rooting medium with 0.5 mg/l of IBA and 0.02 mg/l of NAA is appropriate for rapid clonal propagation of this birch. While LEE *et al.* (1986) achieved 100% of rooting in the culture of 4-year-old *B. schmidtii* on the medium containing 0.5 mg/l of IBA. This combination of IBA and NAA concentrations to rooting medium also was effective on rooting of another birch species such as *B. platyphylla* var. *japonica* (IDE, 1987), *Betula maximowicziana* (IDE and YAMAMOTO, 1991) and *Betula ermanii* (IDE and YAMAMOTO, 1991) too. The combination of hormones used in the present experiments probably have wide adaptability for rooting in birch tissue cultures.

Acclimatization

Two weeks of acclimatization procedure in growth chamber allowed that 80% of plantlets were successfully grown in out door condition (Fig. 7).

By this experiment, we demonstrated the effect of GA₃ concentration to the media on shoot multiplication from cotyledonal node and epicotyl segment of *B. schmidtii*. And also established mass propagation procedure of this birch from germinated seedlings.

Acknowledgement

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Summary

Plantlets regeneration was achieved by the culture of germinated seedlings of *Betula schmidtii*. When cotyledonal nodes of seedlings were cultured on 1/2MS medium containing 0.8 mg/l of NAA and 10 mg/l of GA₃, multiple shoots were formed and when they were cultured on GA₃ free medium, they did not produce multiple shoot. On the other hand, epicotyl segment produced multiple shoot on GA₃ free medium, and they did not produce multiple shoot on GA₃ containing medium. Multiple shoots also were obtained by the successive subculture of these tissues. For the rooting medium, 1/2MS medium containing 0.02 mg/l of NAA and 0.5 mg/l of IBA was appropriate. The regenerated plantlets were successfully acclimatized on soil mixture.

Key words: *Betula schmidtii*, Germinated seedling, Tissue culture, Plant regeneration, Gibberellic acid

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オノオレカンバ芽生えの組織培養による植物体増殖

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要 旨

無菌的に発芽したオノオレカンバの芽生えの組織を培養することによって植物体を再生することができた。子葉節を 0.8 mg/l の BAP および 0.02 mg/l の NNA, 10 mg/l の GA₃ を含む 1/2 MS 培地上で培養するとマルチプルシュートが形成された。一方, GA₃ を含まない培地で子葉節を培養してもマルチプルシュートの形成はみられなかった。さらに, 上胚軸部分を同じ培地で培養した場合には, GA₃ を含まない培地でマルチプルシュートが形成され, 含む場合には形成されなかった。マルチプルシュートの形成がみられた組織の継代培養により継続的にシュートを収穫することができた。発根培地としては, 1/2MS 培地に 0.02 mg/l の NAA と 0.05 mg/l の IBA を添加したものが適当であった。発根再生した幼植物体は用土に鉢上げ後順化することができた。

キーワード: オノオレカンバ (*Betula schmidtii*), 芽生え, 組織培養, 植物体再生, ジベレリン