Short Note: Tissue Culture Studies on Chinese Poplar (Populus tomentosa)

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Summary

Chinese poplar clones (*Populus tomentosa* Carr.) were multiplied as callus cultures and some of them were regenerated to plants, which could be transferred to soil. During the process of regeneration it was found out that the regeneration success is mainly genotype dependent; only 1/3 of the clones could be induced to callus growth but also an environmental influence of the medium type could be pointed out: organogenesis and regeneration of complete plantlets was possible only on medium 2.

Key words: Tissue culture, regeneration, genotype, environment, *Populus tomentosa*.

Introduction

Chinese white poplar (*Populus tomentosa* Carr.) is a species in the Leuce section of *Populus*. It is a native tree species distributed mainly over 1,000·000 square kilometers of ten provinces in the valleys of the Yellow, Huai and Hai Rivers in the northern part of China (Zhu Zhi-Ti, 1988).

P. tomentosa is a suitable material for investigating growth and differentiation because it has a good wood quality and is resistant to pests and diseases. But the seed set is low and the stem cuttings are recalcitrant to rooting hampering vegetative propagation of this valuable tree. Grafting is expensive and cumbersome. Sometimes suckers are used for propagation: Roots are cut, placed in soil and these produced rarely new shoots. Clonal multiplication and genetic manipulation of poplar and aspen clones have now become a major subject of investigation through tissue culture, because investigators are getting interested in improving the biomass production of available resources of especially fast growing trees. The aim of this study is to describe the regeneration and vegetative propagation of *Populus tomentosa* with methods in vitro.

Material and Method

Cuttings of 63 different clones of *Populus tomentosa* were supplied by Zhu, Z. T., Beijing Forestry University. Internodal pieces from little branches of al 63 clones were sterilied by washing in 70% alcohol followed by 10 min. treatment with 10% sodium hypochlorite. The explants were finally washed with sterile water twice, cut into 5 mm segments up to 50 per clone and implanted on Murashige and Skoog's (MS, 1962) medium supplemented with the following substances (in mg/l): 30 adenine sulphate, 50 Asparagine, 50 Glutamine, 100 Lysine. 1 Naphtyl acetic acid (NAA), 1 Benzylaminopurine (BAP) and 100 caseinhydrolysate (CH). The medium was gelled with 0.475g agar/l and pH was adjusted to 5.8 before autoclaving. The cultures were maintained under continuous light (1000 lux). For regeneration experiments the callus obtained on the above medium was transferred either to M1 (MS + 0.5 NAA, 0.5 IAA, + 1 BAP + 1000 CH; all mg/l) or to M2 (MS + 0.1 NAA, + 0,1 IBA + 1.0 Z + 500 CH; all mg/l). The growth response of callus on these two media was determined after six weeks. Increase of fresh weight as well as microscopical observations of the morphology and texture of the calli was recorded.

Results

Initiation of cell proliferation in the majority of clones started after one week. The explants remained green throughout and the cut ends produced callus. The first sign of initiation of callusing was noticed after 12 days. By the end of six weeks enough subculturable callus was obtained. The explants were then divided into two pieces with one end having the callus. After another six weeks the entire explant was covered with callus which was then transferred to fresh media (nos 1 and 2).

Concerning the increase of callus fresh weight a *genotype effect* could be seen: A group of 20 genotypes out of the initial 63 cuttings brought from China and taken into in vitro culture, showed callus growth: 12 of them produced rather poor or medium callus growth. The resulting callus mass after 6 weeks was only two- or threefold, starting with 0.2 mg or 0.3 mg per callus. 5 genotypes resulted in a good amount of callus (callus mass was tenfold or more) on M1.

A *medium effect* was demonstrated, when callus pieces were transferred to M2: Three genotypes failed to produce callus on M1, but showed good callus growth and even regeneration capacity on M2 as well as most of the clones which produced medium callus growth on M1. Overall, 10 genotypes responded by regenerating either roots or shoots.

The best regeneration was achieved in 2 clones, which differentiated shoots within 3 weeks after transfer on medium 2 (see *Fig. 1*). The shoots attained a height of 4 cm within 4 weeks growing on M2. In fact, as many as 20 to 25 shoots could be harvested from each callus piece. These shoots were then separated and grown on a medium having lower concentration of BAP (0.2 mg/l) and no auxin (see *Fig. 2*).

There was a remarkable corresponding effect between callus morphology and texture and the regeneration ability of the clones: Genotypes producing losely-textured, light green callus mostly regenerated roots, whereas in compact dark green calli shoot differentiation or both, roots and shoots were induced.

Discussion

In vitro studies with *P. tomentosa* have been tried earlier by Zhu et al. (1980). They reported on the production of plantlets from anthers for this species. In both

Figure 1. — Micro shoots regenerated from callus of a *Populus tomentosa* clone on a modified MURASHIGE-SKOOG medium containing NAA, IBA and Zeatin.

Figure 2. — Regenerated plantlets of a *Populus tomentosa* clone on a modified MURASHIGE-SKOOG medium containing low concentration of BAP but no auxins.

these studies, however, no comparison was made in response between the clones. It is now getting establislied that genotypes do play an important role on in vitro response of different explants (see GLOCK and GREGORIUS, 1986).

In the present study also an interesting correlation between callus growth and regeneration and genotype was observed. Of the 63 clones tried, 20 produced some amount of callus growth, and 10 of these clones showed organogenesis. At least two proved quite encouraging in terms of regeneration of complete plantlets. On the other hand, there was a pronounced environmental effect (medium composition) on callus growth and regeneration: On M1 all differentiating clones only showed callus with varying degrees of chlorophyll and texture, whereas, on M2 several of the clones were enabled to regenerate organs and some of them even complete plantlets.

Our present study may prove helpful in inducing regenerants from selected clones because the outcome of our experiments demonstrates for the genus *Populus* the same sensitive interaction during differentiation processes as could be shown earlier for *Betula pendula* (GLOCK and GREGORIUS, 1984) and *Pseudotsuga menziesii* (GLOCK et al., 1988): the genotype-dependent response of the clones to the environmental conditions (medium type) and the medium effect upon the genotypes by the variable response of one clone to different media.

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