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Influence of inorganic salts on biomass production, biochemical composition, and bioethanol production of *Populus alba*

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Inorganic salts are very important for the biosynthesis of major components such as cellulose and lignin. In order to investigate biomass production, major components of the biosynthesis of plant cell wall and the bioethanol production of *Populus alba*, we examined the effect of inorganic salts on *in vitro* culture systems without specific mineral salts. The medium without $H_2PO_4^-$ was supportive for *Populus alba* shoot growth, while the absence of NH_4^+ resulted in poor shoot growth. The medium without $H_2PO_4^-$ and Fe^{3+} inhibited above-ground biomass production, whereas NH_4^+ and K^+ deprivation led to an enhancement of the same. The root/shoot ratio of *Populus alba* in the medium without $H_2PO_4^-$ was high compared with plants cultured in the control medium. $H_2PO_4^-$ is deeply involved in lignin biosynthesis, and its removal has been shown to reduce the biosynthesis of lignin. Plants grown on nitrate-free medium were found to be good for enzymatic saccharification and ethanol production. The plants grown in the medium without NO_3^- showed 72.0% enzyme digestibility, and the yield of ethanol showed 9.58% ethanol productivity after 12 hours. These results can be used as the basis for producing high-quality biomass for future bioethanol production.

Keywords: Enzymatic Hydrolysis, Fermentation, Inorganic Salts, *In vitro* Culture, *Populus alba*

Introduction

In vitro plant cultivation provides easy control of environmental factors. The *in vitro* testing of optimal plant requirements can provide accurate information on cultivation without planting in fields. The preliminary design of the cultivation environment of the plant to be cultivated through *in vitro* culture methods can be called "*in vitro* monitoring". Nutrient media for plant tissue cultures are designed to maintain the plant tissue in a fully artificial environment. *In vitro* culturing allows the monitoring of plant responses under a variety of biochemical and physiological conditions (Shibli et al. 2000).

The short rotation coppice (SRC) is known as an effective way to enhance biomass massive production and reduce atmospheric CO_2 accumulation (Amichev et al. 2012). SRC energy crops such as poplar (*Populus* spp.) are grown commercially for heat and power generation as a consequence of their rapid growth rate and favorable energy ratio (Rowe et al. 2009). *Populus* species play significant roles in global ecosystems and provide raw materials for the forest industry and fuels for rural areas in developing countries (IPCC 2008). *Populus alba* (*P. alba*) is the fastest growing among the *Populus* species and is easily propagated, and thus it serves as a model for tree biotechnology (Bradshaw et al. 2000).

Crops grown for their high production require nutrients in the soil to be carefully considered. SRC cultivation should also take into account soil nutrients and fertilization (Dimitriou & Rutz 2015). Several studies of SRC, such as poplar and willow, have studied these nutrients, but they focused on the optimal cultivation environment. There have been no studies on the effect of inorganic salt components on plant cell wall composition.

Nutrient stress can cause a change in the content of lignin and other secondary metabolites in trees (Chapin 1991). Tree growth is generally associated with the ability to obtain mineral nutrients from the soil in a balanced amount. The factors that can alter mineral nutrition in plants may also affect the lignification process. Yet, the role of the mineral nutrients in lignification is still unclear. The regulation of nutrient supply in plants may be one of the most easily practicable ways of directing the biosynthesis of lignin and other compounds in plants.

Lignocellulosic biomass can be converted to bioethanol after pretreatment procedures, hydrolysis and subsequent fermentation. The lignocellulosic biomass is mainly composed of cellulose, hemicelluloses and lignin, along with smaller amounts of pectin, protein and ash (Jørgensen et al. 2007). Pretreatment has been viewed as one of the most important and expensive steps in releasing sugars from hemicellu-

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