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Fast growing aspens in the development of a plant micropropagation system based on plant-produced ethylene action

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ARTICLE INFO

Article history:
Received 15 June 2012
Received in revised form
20 December 2012
Accepted 9 January 2013
Available online 13 February 2013

Keywords:
Aspen
Hormone
In vitro
Shoot proliferation
Short rotation forestry

ABSTRACT

Representatives of the genus *Populus* (poplars), such as *Populus tremula* L. (European aspen) and its fast-growing hybrids, are recognized as being among the most suitable tree species for short rotation coppicing in Northern Europe. Several technologies have been developed for fast propagation of selected aspen genotypes, including laboratory (*in vitro*) micropropagation, which is usually based on the action of exogenous plant hormones. Seeking to minimize the use of the latter, the present study was designed to test if the conditions suitable for increased accumulation of plant-produced gas, including the gaseous plant hormone ethylene, inside a culture vessel could contribute to commercially desirable changes in aspen development. Shoot cultures of several European and hybrid (*Populus tremuloides* Michx. × *P. tremula*) aspen genotypes were studied using two different types of culture vessels: tightly sealed Petri dishes (15 × 54 mm) designed to provide restricted gas exchange (RGE) conditions, and capped (but not sealed) test tubes (150 × 18 mm) providing control conditions. Under RGE conditions, not only the positive impact of the ethylene precursors 1-aminocyclopropane-1-carboxylic-acid (ACC) and ethephon on shoot proliferation was demonstrated but also a several-fold increase, compared to the control conditions, in the mean shoot number per explant was recorded even on the hormone-free nutrient medium. Moreover, the shoots developed under RGE conditions were distinguished by superior rooting ability in the subsequent culture. These results suggest that a plant micropropagation system based on the action of plant-produced ethylene rather than of exogenous hormones is possible.

1. Introduction

Fast-growing poplars (*Populus*) are recognized as a suitable model for biomass-related scientific studies [1]. In relation to short rotation forestry, a special interest in Northern Europe, including Scandinavian and Baltic countries, is paid to native aspen (*Populus tremula* L.) and its hybrids (particularly with North American *Populus tremuloides* Michx.) distinguished

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic-acid; PGR, plant growth regulator(s); RGE, restricted gas exchange.

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http://dx.doi.org/10.1016/j.biombioe.2013.01.005

by their fast growth [2–4]. Several technologies have been developed for the fast propagation of selected aspen genotypes, varying from root cuttings [5] to laboratory (*in vitro*) micropropagation [6]. *In vitro* culture provides the opportunity to obtain the largest number of new shoots during a short period of time; however, it is usually based on extensive use of exogenous plant hormones, particularly auxins and cytokinins [7]. This results not only in increased costs but also in some degree of uncertainty about the nature and persistence of possible side effects, since exogenously applied hormones can variously interact with plant-produced hormones. Therefore, an approach should be developed for more efficient exploitation of the latter, instead of using the former.

In plant tissues, auxins and cytokinins, as well as a variety of stressful environmental factors, are known to increase the synthesis of ethylene gas, which is also counted among plant hormones [8,9]. Many authors suggest that ethylene accumulation or signaling should be restricted in order to achieve better shoot regeneration and growth [10–12], or enhanced rooting [13,14]; however, some claim a positive role for ethylene in shoot and root development [15,16]. Thus, the present study was designed to see if the establishment of *in vitro* conditions suitable for the increased accumulation of plant-produced gas inside a culture vessel could induce commercially desirable changes in plant development and, if so, how these changes are related to the action of ethylene.

2. Materials and methods

2.1. Plant material and growth conditions

The present study involved three *Populus* genotypes (Table 1) cloned under laboratory conditions.

At the start of the cloning process, proliferating shoot cultures were established from 2 to 3 cm long segments (carrying at least one vegetative bud) of young aspen twigs that were collected from the middle part of the crown in early spring, just before the bud-break. These shoot cultures were maintained *in vitro* for several years through successive passages which were usually done every two months. During initial phase, the cultures were grown on a solidified (with 8.5 g L^{-1} phytoagar) Woody Plant Medium (WPM [17]) containing 25 g L^{-1} sucrose and 0.5 mg L^{-1} 6-benzylaminopurine (BAP; Duchefa Biochemie, Haarlem, The Netherlands). After several passages, BAP was excluded from the medium composition and, for a period of at least one year, the cultures were grown on a WPM free of plant growth regulators (PGR). In the subculture previous to the experiments, cultures were grown on such a PGR-free medium for a period of approximately two months (if not stated otherwise). Apical stem segments carrying two to three buds (including an apical bud) were used for the experiments. In some of the experiments, nodal stem segments (without apical bud) were also involved.

The basal WPM without any additional compounds (PGR-free) was used as control medium in all experiments. In certain experiments aimed at the study of ethylene's influence, the nutrient medium was supplemented with 2-chloroethyl phosphonic acid (ethephon, an ethylene-releasing compound [18]) or 1-aminocyclopropane-1-carboxylic-acid (ACC, a natural precursor of ethylene whose conversion to ethylene is catalyzed by the enzyme ACC oxidase [19]). These chemicals were obtained from Sigma–Aldrich Laborchemikalien GmbH (Seelze, Germany) and Sigma–Aldrich Chemie GmbH (Steinheim, Germany), respectively. Also, some of the experiments involved ethylene signal inhibitor [20] silver nitrate ($AgNO_3$; Duchefa Biochemie, Haarlem, The Netherlands). ACC was first dissolved in 0.5 mL of 1 μmol L^{-1} NaOH and then diluted with distilled water to a 50 mL volume, while ethephon and $AgNO_3$ were dissolved in 50 mL distilled water (pH value for ethephon solution was set well below 4.0). All PGR solutions were filtered using a 0.22 μm syringe-driven filter prior to adding them (at the appropriate volume) to the autoclaved nutrient medium, while the pH value of the medium was adjusted to 4.8 before autoclaving for 30 min at 121 °C.

Glass test tubes and polystyrene Petri dishes were used for culturing explants. More detailed characteristics of the conditions related to these different culture vessels are given in Table 2. Here, test tubes are considered to provide control conditions since they were routinely used while subculturing *Populus* explants prior to this study.

In each case, a single explant was provided with 5 mL of nutrient medium. All cultures were maintained in controlled environmental conditions under a 16 h photoperiod (white-light; irradiance 30 μmol m^{-2} s^{-1}) and a temperature regime of 25 °C/18 °C during day and night conditions.

2.2. Experiments

For testing the impact of ethylene on aspen shoot development, the basal nutrient medium for aspen DPL038 explants was enriched with ethylene precursors ACC and ethephon (at the concentrations of 1 μmol L^{-1}, 3 μmol L^{-1}, and 5 μmol L^{-1}). DPL038 responses to the aforesaid ethylene precursors were tested both under control and under RGE conditions.

Table 1 – Data on *Populus* genotypes and their respective donor trees involved in the study.

Tree code in the Lithuanian forest seed base catalog; species	Location of the donor tree	Tree parameters at the moment of collection of primary explants		
		Age, years	Height, m	Stem diameter (at the height of 1.3 m), m
DPL038; *P. tremula* L.	55°15′ N; 23°20′ E	70	33	0.64
DPL037; *P. tremula* L.	55°22′ N; 22°14′ E	70	33	0.66
DF1001; *P. tremuloides* Michx. × *P. tremula* L.	54°52′ N; 24°07′ E	25	24	0.33