Profiles of the volatile organic compounds emitted by the masses of *Abies nordmanniana* somatic embryos at maintenance and maturation stages

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Introduction

To face the foreign competition existing at the European level on the Christmas tree market, the Walloon producers are seeking for improving and differentiating their plant material with the addition of aroma to well-shaped trees as a priority.

Today, somatic embryogenesis is the most promising technology for breeding conifers with no the genetic re-combinations linked to classical hybridizations. Additionally, this is the only way to propagate clonally the improved genotypes of *Abies nordmanniana* afterwards, according to protocols already developed (Misson et al., 2006, Misson and Druart, 2008).

Screening precociously the cellular clusters that could express the targeted trait during plant regeneration process is challenging. It requires to prevent the cell cultures from disturbances while keeping their environment sterile. That is the reason why the composition of the headspace atmosphere has been considered. Volatiles organic compounds emitted by callus (Nieds et al., 1997), embryogenic cultures (Alonzo et al., 2001), have been reported. Subjected to changes during subcultures, the variation of these bioactive compounds offers a non-destructive approach for investigating the plant physiology under controlled stress conditions (Predieri and Rapparini, 2006, Spinelli et al., 2011).

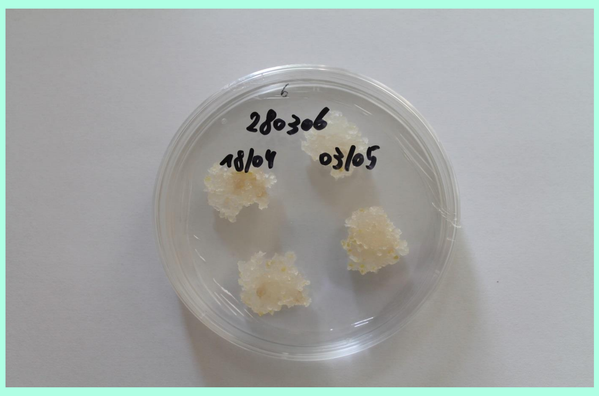
We report hereunder the method developed for the analysis of VOCs emissions in the headspace of Petri dishes where embryogenic masses of *A. nordmanniana* and *A. balsamea* are cultivated. The first results will concern 2 stages: the maintenance of immature embryo masses and the first step of their maturation.

Materials and method

Culture conditions and plant material for the experimentation

During the maintenance stage, the proliferating somatic embryos formed secondary embryos and it was carried on BP6PN culture medium (Picture1 , Misson and al. , 2006). The maturation of the embryos led to the formation of cotyledons (Picture 2) in a 3 successive step culture corresponding to different nutrient media (data not shown). Only the first step of maturation was performed on a Schenk & Hildebrandt (1972) medium (SH2) modified after Misson and al (2006). Cultures were carried out in " RITA " containers (configured for temporary immersion cultures) to allow atmosphere sampling under sterile conditions.

The plant material was composed of a strain of *A. balsamea* provided by Dr. YS Park (National Resources Canada) and of 2 strains of *A. nordmanianna* embryogenic masses initiated in the CRA-W laboratory (Misson and Druart, 2008) and caracterized by their maturation ability. This caracterisation showed that no maturation has occured for Nord 11308 and that Nord 280306 was able to maturate.

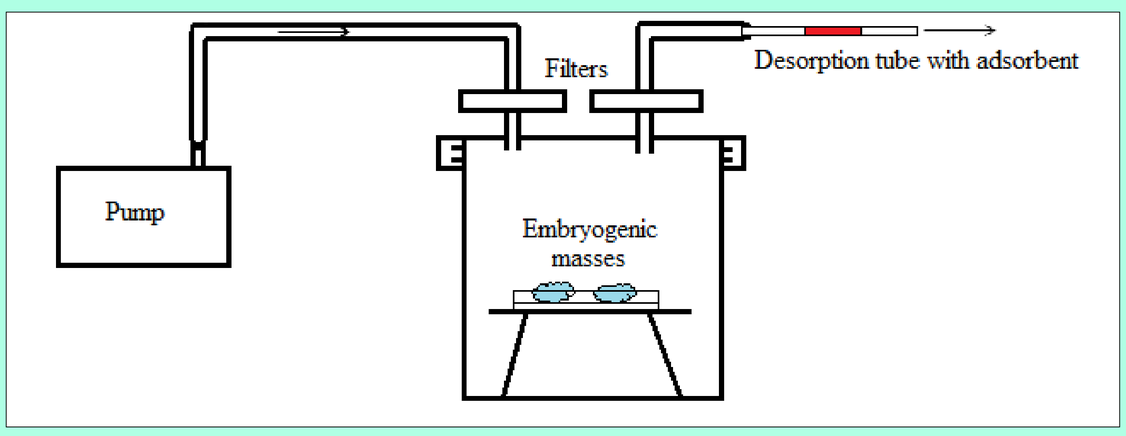


Picture 1. Embryogenic masses of *A. nordmanniana* at the maintenance stage

Picture 2. Somatic embryos of *A. nordmanniana* with cotyledons, at the end of the maturation process

GC-MS conditions

The volatile organic compounds (VOC) emitted by the embryogenic masses were sampled during 4 h, by using a desorption tube filled with tenax as adsorbent.



The optimal analytical conditions were:

Desorption temperature : 280 °C
Polar column VF-WAX ms 260 °C: 30 m x 250 µm x 0.25 µm

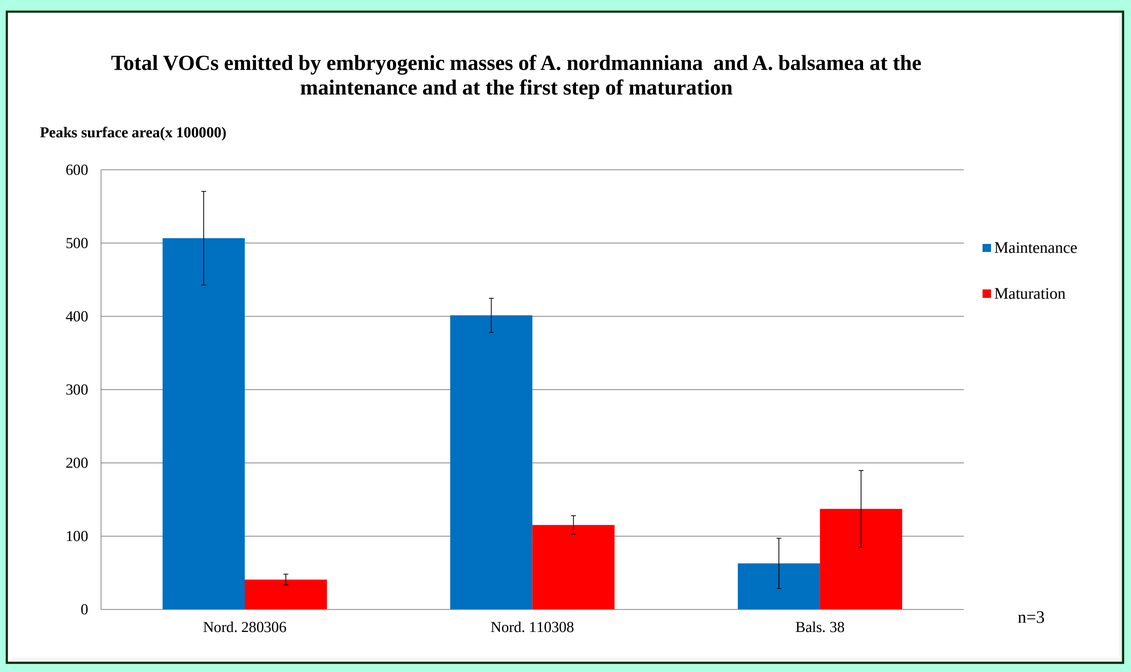
Oven temperature
35 °C for 2 min
then 3 °C/min to 50 °C for 0 min
then 5 °C/min to 115 °C for 0 min
then 10 °C/min to 250 °C for 2 min

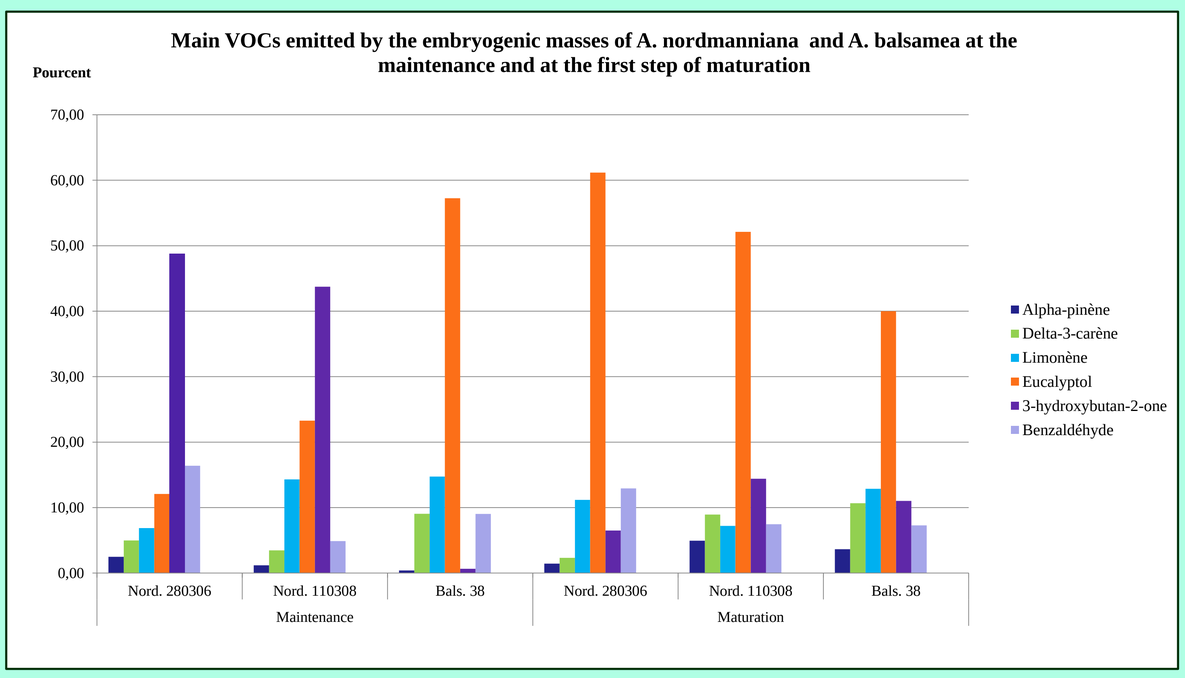
Results and discussion

Quantitatively (Figure 1), both lines of *A. nordmanniana* emit more volatile organic compounds than *A. balsamea* strain during the maintenance stage. Emissions then fall drastically for these two lines after the maturation (SH2 medium) while at the same time, the emission by *A balsamea* line seems to progress but not significantly.

The composition of VOCs profiles obtained from *A. nordmanniana* changes with the culture medium. During maintenance, the 3 - hydroxybutane -2-one is dominant compared to eucalyptol while in maturation, it is the opposite . Further the ratio between these two specific components are more related to the strang reduction of 3 - hydroxybutane -2-one (10-fold) compared to eucalyptol which decreases moderately (by a factor of 2.5). *A. balsamea* profile shows no reversion of these two components in relation with the cultivation stage. Stability is observed with the maintenance of eucalyptol relatively predominant on 3 - hydroxybutane-2-one, although it is attenuated at maturation.

The distinction between embryogenic masses of *A. nordmanianna* is not sharp even if the ratio 3 - hydroxybutan -2-one/ eucalyptol is higher for the line able to mature (4.04) than for not maturated one (1.87) .





Conclusions

The distinction between *Abies* species is obtained from the embryogenic masses at the maintenance stage: *A. nordmanniana* is characterized by 3-hydroxybutane-2-one while *A. balsamea* by eucalyptol.

The distinction between culture stages (more related to the physiological state of the masses) only occured with both *A. nordmanniana* strains that show a reverse relationship between 3-hydroxy-butane-2-one (predominant in the maintenance stage) and eucalyptol (predominant in the preliminary stage of maturation).

No conclusion about the distinction between strains of *A. nordmanniana* according to their respective of maturation can be obtained. It requires to be performed on a larger number of lines,

Acknowledgements

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