Age-dependent biological characteristics of embryonal mass of maritime pine in relation to the embryogenic potential

Krstyna Klimaszewska, Carlos Noceda, Gervais Pelletier, Philippe Label, Roberto Rodriguez, Marie-Anne Lelu-Walter

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Introduction

Somatic embryogenesis (SE) in conifer is at the heart of many biotechnology protocols that potentially might be developed. SE is a powerful tool for micropropagation that has opened avenues for employment of nuclear and stromal regenerative potential in forest plants (Platen et al. 2005). Although the SE process can be managed for high efficiency and high frequency, there are several parameters that require further manipulation of some factors. One such factor is the use of various substances in SE, which may have a direct or indirect impact on the efficiency of SE. For example, sodium azide is one of the substances that is commonly used in SE and may alter the efficiency of SE (Klimaszewska et al. 2005, Lata et al. 2005). Such alterations in culture media may also influence the growth and developmental stages of SE-derived embryos.

The objectives of this study were to get an insight into differences between young, primary lines of embryonal masses of maritime pine that produced somatic embryos and the lines of significantly increased age that did not produce somatic embryos. We analyzed in both types of materials the following:

- transcription of their metabolites
- metabolites
- global DNA methylation levels and patterns
- effect of differentially acting agents on the embryonic tissue viability, embryogenic potential and DNA
- morphological levels and patterns.

Material and Methods

In this study we used embryogenic cultures of Pinus pinaster (primary line) from the MNR of different ages (Fig. 1) and different embryogenic potential (Table 1, Fig. 2).

Fig. 1: Examples of changes in the methylation pattern in culture treated with 10 and 20 µM 5-azaC. Related to the basic level of culture, the changes (a) and (b) were treated with different concentrations of the above agents for up to 14 days (Fig. 1A). Each of the changes (a) and (b) were determined in the in vitro culture at different stages after treatment.

Table 1: Summary of changes in the methylation pattern in culture treated with 10 and 20 µM 5-azaC. Related to the basic level of culture, the changes (a) and (b) were treated with different concentrations of the above agents for up to 14 days (Fig. 1A). Each of the changes (a) and (b) were determined in the in vitro culture at different stages after treatment.

Results

Hormones

Table 2: Quantification of hormones and their metabolites in embryogenic cultures of Pinus pinaster (primary line) from the MNR of different ages (Fig. 1) and different embryogenic potential (Table 1, Fig. 2).

The level of hormones and their metabolites varied in the three types of material (Table 2). In general, in old cultures (2) there was a higher level of free cytokinins and their metabolites, whereas in young cultures (1) the levels of free cytokinins were significantly higher in the MNR of old cultures. In young cultures (2) the levels of free cytokinins were significantly higher in the MNR of old cultures.

Polymethines (PMA)

Fig. 3: Profiles of the phytohormone content. The data are presented as mean ± SD (n=3). ABA, abscisic acid; ABA-epi, 9-epi-ABA; IAA, indole-3-acetic acid; IAA-epi, 1-epi-IAA; IAA-x, 1-IAA-x; MA, meyoabscisic acid; MH, methylhistidin.

Conclusions

Maritime pine cultures (from MNR) of different ages and embryogenic potential varied with respect to the hormonal and polyamines contents.

- The old cultures (2) that lost embryogenic potential were characterized by elevated levels of cytokinins and their metabolites compared with young embryogenic cultures (1). This possibly reflected the high proliferation of the transitory cells of differentiation. Similarly, in old cultures (2) the levels of free cytokinins were significantly higher in the MNR of young cultures (1). The ratio of 5-IAA to 5-IAA-x was highest in old cultures, which might coincide with the loss of embryogenic potential.

Global DNA methylation levels were similar in both types of material. However, the methylation pattern was different. Aging of the cultures led to the relative changes 100% (± SD) in the DNA methylation levels (Table 3, Fig. 4). The ratio of the ratios of 5'-CCGG to 5'-CCGC was highest in the young cultures and the lowest in the old cultures. Treatment with 5-azaC resulted in changes in the methylation patterns. This effect changes on somatic embryogenesis being studied.

References


