IMMUNOLOCALIZATION OF CYTOKININS IN NORWAY SPRUCE SOMATIC EMBRYOS

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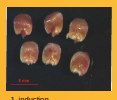
Aims:

1. the development of a method of immunohistochemical localization of selected cytokinins in spruce ESM and somatic embryos
2. the description of cytokinins localization in somatic embryos during their development

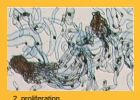
Material:

Embryogenic culture of Picea abies was used in all experiments. It was cultivated on the solidified GD medium (Gupta, Duryan 1986) as described elsewhere (Vágner et al. 1999). Proliferation medium contains 5µM 2,4-D, 2µM kinetin and 2µM BA. Maturation medium is supplemented by 20µM ABA.

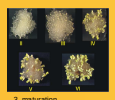
5 steps of conifers somatic embryogenesis:



1. induction
usually from immature zygotic embryos

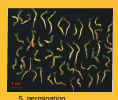


2. proliferation
maintenance of culture



3. maturation
4 - 6 weeks on medium with ABA

4. desiccation
3 weeks of desiccation in high relative humidity



5. germination
without hormones

Methods

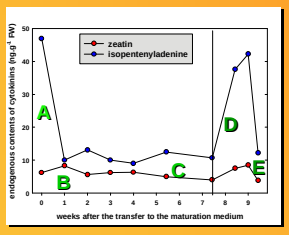
The material was fixed in formaldehyde, which is specific for free bases (Sossountzov et al 1988). The cryosections were treated with antibodies against zeatin riboside and against isopentenyladenosine. The antibodies were purified by protein A, visualised by biotinylated secondary antibody and avidin-biotin-alkaline phosphatase complex. For the control of specificity we used the inhibition of immunoreaction with a mixture of antibodies and corresponding free hormones.

Endogenous levels of cytokinins were characterized using HPLC separation followed by the ELISA quantification (Macháčková et al 1993).

Introduction

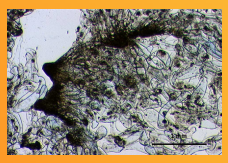
The process of conifers somatic embryogenesis consists of 5 steps /see Material on the left/. It is controlled by the exogenous treatments of plant growth regulators (auxins, cytokinins, ABA). Embryonic suspensor mass /ESM/ is treated by cytokinins and auxin during proliferation.The embryo maturation occurs on medium supplemented by ABA. Auxin and cytokinins treatments are not necessary in this step. We found high endogenous levels of ABA and marked peak of endogenous auxin in ESM and in somatic embryos during maturation. Endogenous level of cytokinins decreases after the transfer of ESM from proliferation medium to the cytokinins free maturation medium and remains low during further somatic embryo development. A transient increase of cytokinin levels was found in the mid of desiccation step (see the graf below).

Endogenous cytokinins in spruce somatic embryos during their development

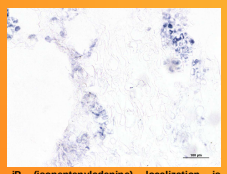


A - proliferation (early somatic embryos)
B - the start of maturation
C - the end of maturation
D - the start of desiccation
E - the end of desiccation

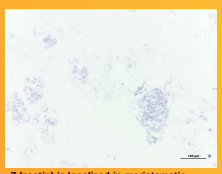
A. Proliferation



- embryonic suspensor mass consists of meristematic cells and suspensor cells
- early somatic embryos form polyembryonic complexes



-iP (isopentenyladenine) localization is predominantly detected in meristematic cells
-iP free suspensor cells

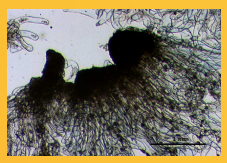


- Z (zeatin) is localized in meristematic cells
- suspensor cells are Z free

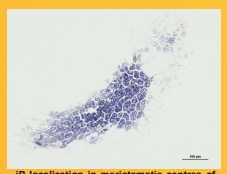
- no differences in iP and Z localization during proliferation
- endogenous level of these cytokinins depends on the ratio between meristematic and suspensor cells in embryonic suspensor mass

B. The start of maturation

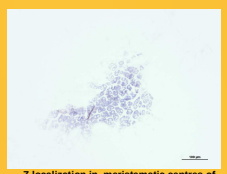
1 week of cultivation on maturation medium



- meristematic centres in polyembryonic complexes grow larger (single somatic embryos reach from polyembryonic complexes and start their development)
- each somatic embryo consists of meristematic centre and well organised suspensor tail



- iP localization in meristematic centres of polyembryonic complexes
- suspensor tails (and cells) are iP free

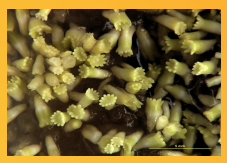


- Z localization in meristematic centres of polyembryonic complexes
- suspensor tails (and cells) are Z free

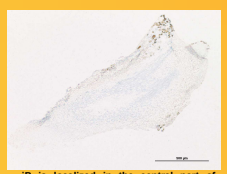
- no differences in iP and Z localization during the 1st week of maturation
- endogenous levels of these cytokinins depend on the number of developing somatic embryos (quantity of developed meristematic centres)

C. The end of maturation

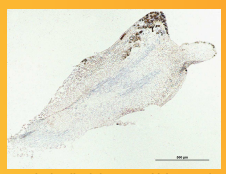
6 weeks of cultivation on maturation medium



- fully developed somatic embryos (cotyledons arround the apical meristem, root pole with root cap, columella and root meristem, fully developed procambium and cortex)



- iP is localized in the central part of somatic embryos (in prokambium) and near to the root cap



- Z is localized in procambial part of somatic embryos and near to the root cap

- the distribution of these cytokinins into the different parts of somatic embryos occurss during the step of embryo polarisation (during the 4th and 5th week of maturation)
- cytokinins localization is clearly defined in fully polarized and fully developed somatic embryos at the end of maturation
- no differences in iP and Z localization in matured somatic embryos
- endogenous levels of iP and Z depend on the number and quality of properly developed somatic embryos

Desiccation of fully developed somatic embryos

(macroscopical picture of desiccating embryos see above)

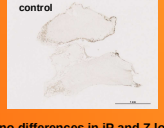
- matured somatic embryos
- no significant differences in anatomical structure of somatic embryos at the end of maturation and during desiccation

D. The start of desiccation

1 week of desiccation

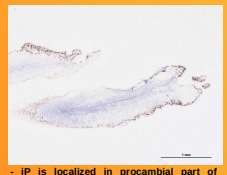
- iP is localized in procambial part of somatic embryos and mainly in the root cap

- Z is localized in procambial part of somatic embryos and in the root cap

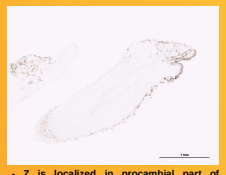


E.The end of desiccation

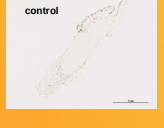
4 weeks of desiccation



- iP is localized in procambial part of somatic embryos and mainly in the root cap



- Z is localized in procambial part of somatic embryos and in the root cap



- no differences in iP and Z localization in desiccated somatic embryos
- endogenous level of these cytokinins depends on somatic embryos quality

Conclusions

-iP and Z are detected by immunohistochemical methods in ESM and somatic embryos of Picea abies; no marked differences were found in iP and Z localization

-both cytokinins are localized in meristematic centres of ESM during proliferation

-the localization of both cytokinins persists in meristematic centres during the beginning of maturation

- both cytokinins are unequally distributed in fully developed embryos and in embryos during desiccation, they are predominantly localized in the procambial part of somatic embryos and in the root cap

References

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Vágner M, Vondráková Z, Špačková J, Cvikrová M, Eder J, Lipavská H, Albrechtová J, Svobodová H, Macháčková I. (1999): Plant Biotechnology and In Vitro Biology in the 21th Century. Altman et al (eds.) 93 –96. Kluwer Academic Publishers, Netherlands.

The research was supported by the Ministry of Education, Youth and Sports (projects LN00A081 and COST 843.50).