Beta-1,3-GLUCANASES AND CHITINASES IN SOMATIC EMBRYOS OF NORWAY SPRUCE (*Picea abies*)

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INTRODUCTION

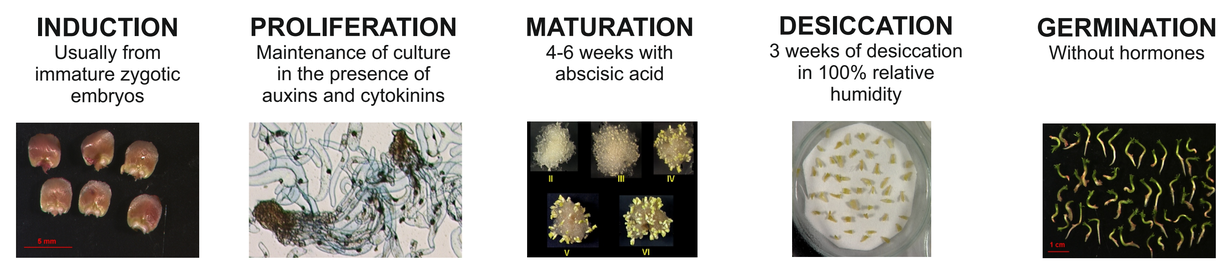
Somatic embryogenesis is a developmental process where a plant somatic cell can dedifferentiate to a totipotent embryonic stem cell that has the ability to give rise to an embryo under appropriate conditions. Desiccation is the final phase of normal embryonic development in most angiosperms and appears to be important in the transition from embryogeny to the ability to germinate and form normal seedlings. Different conditions during desiccation affect the biochemical changes, which occur in this phase.

Chitinases (EC 3.2.1.14) and β-1,3-glucanases (EC 3.2.1.39) are hydrolytic enzymes that often act together in plants. They have been widely studied in the context of plant pathogenesis (e.g. Ceasar and Ignacimuthu 2012). However, their role in the defense against abiotic stresses has been also proven. Chitinases and glucanases are induced by different abiotic stresses such as osmotic stress, cold stress, salt stress, drought stress and heavy metal stress (e.g. Dalen et al. 2015, Fossdal et al. 2009). Recent studies confirm that both of these enzymes are also implicated in a wide range of developmental processes in plant. Among others they play an important role in the development of gymnosperm and angiosperm somatic embryos (Dong and Dunstan 2000, Dyachok et al. 2002).

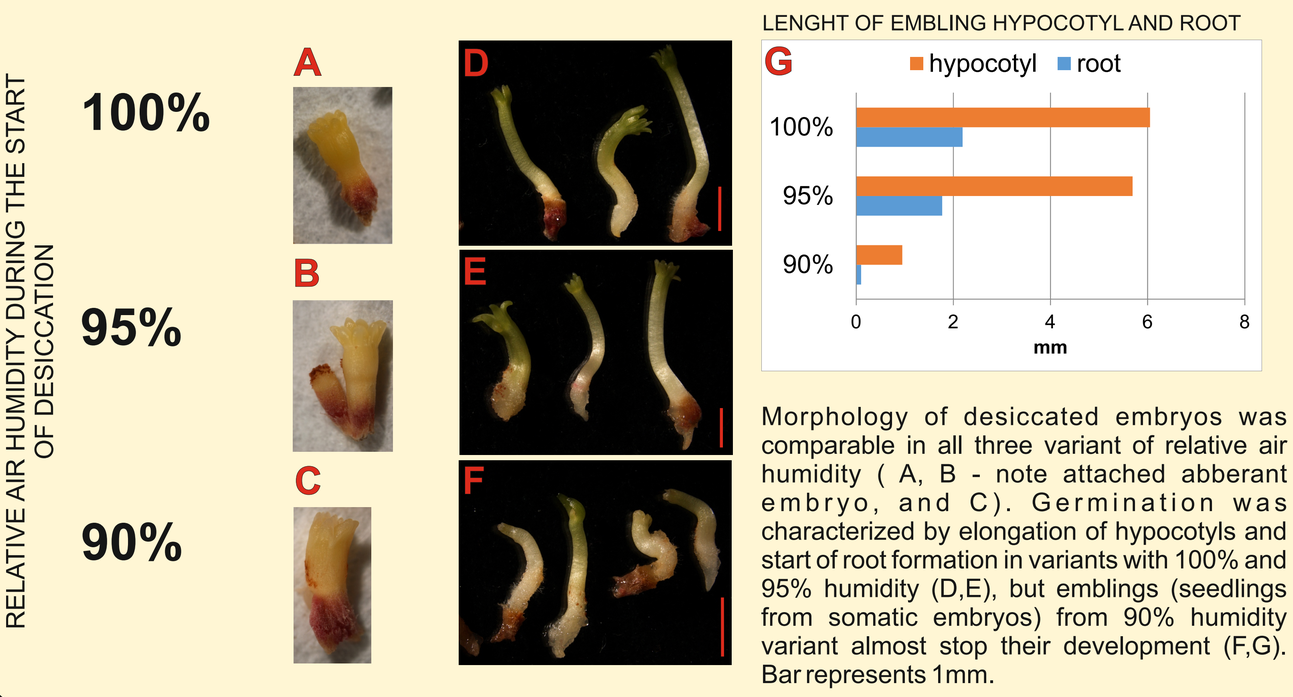
AIMS

- to describe the changes of the chitinase and β - 1,3 glucanase activities during Norway spruce somatic embryo development
- to define the effect of different air humidity in the desiccation phase on embryo development and chitinase and β - 1,3 glucanase activities

PHASES OF CONIFEROUS SOMATIC EMBRYOGENESIS

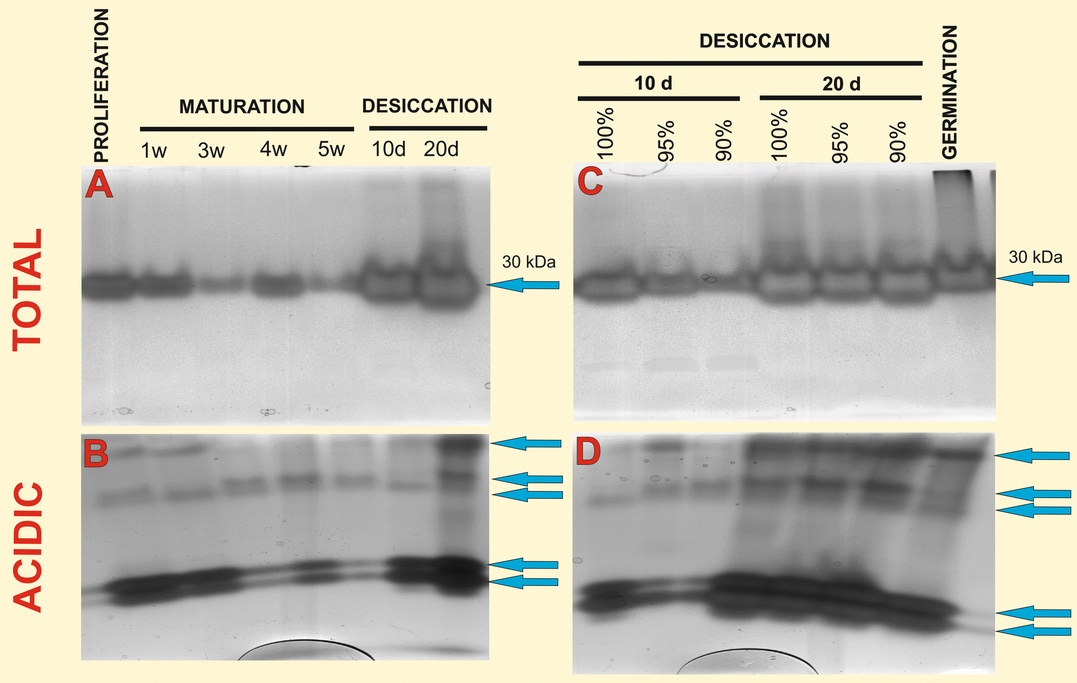


MORPHOLOGY OF EMBRYOS AFTER DESICCATION IN DIFFERENT RELATIVE AIR HUMIDITY



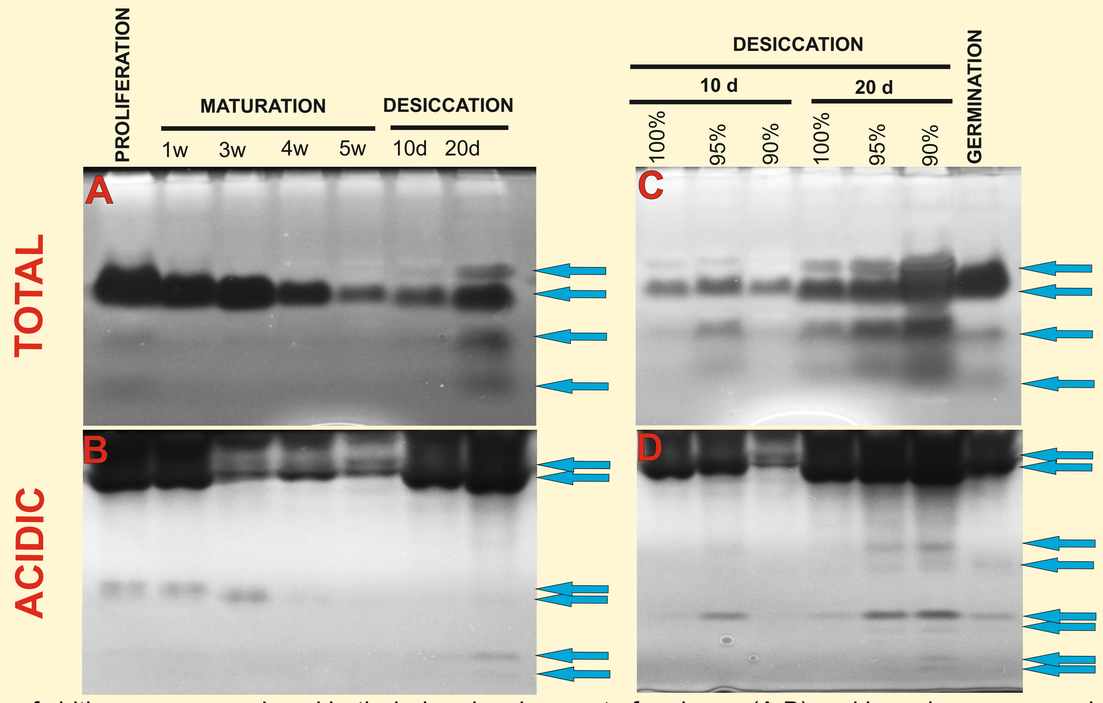
Morphology of desiccated embryos was comparable in all three variant of relative air humidity (A, B - note attached abberant embryo, and C). Germination was characterized by elongation of hypocotyls and start of root formation in variants with 100% and 95% humidity (D,E), but emblings (seedlings from somatic embryos) from 90% humidity variant almost stop their development (F,G). Bar represents 1mm.

β-1,3-GLUCANASES



Activity of β- 1,3 glucanases was analyzed both during development of embryos (A,B) and in embryos exposed to reduced humidity during desiccation (C,D).
After SDS-PAGE separation one majority isoform was detected (A and C, 30 kDa), which activity decreased during maturation and again increased in desiccation. Low humidity in desiccation reduced its activity (C,10d), which was restored after embryo rehydration at the end of desiccation (C, 20d) and persisted to germination.
Five isoforms with different molecular weihgts were detected after separation in acidic conditions (B,D), their levels of activity coresponded to most abundant isoform from total SDS-PAGE separation.

CHITINASES



Activity of chitinases was analyzed both during development of embryos (A,B) and in embryos exposed to reduced humidity during desiccation (C,D).
After SDS –PAGE separation one major isoform of chitinases was detected (A). Its activity decreased from proliferation to 10 d of desiccation (A) and after 10 d of desiccation was not significantly affected by different humidity. After embryo rehydratation (20 d of desiccation) the activity of major isoform increased and three new isoforms of chitinases appeared (A,C). These isoforms were highly detectable in embryos desiccated in 100 % and 95% air humidity. After desiccation in the lowest humidity their activities were reduced. Six isoforms with different molecular weihgts were detected after separation in acidic conditions (B,D), their levels of activity coresponded to most abundant isoform from total SDS-PAGE separation.

CONCLUSIONS

- individual phases of somatic embryogenesis are characterized by different activities of both β- 1,3 glucanases and chitinases
- abundance of distinct β- 1,3 glucanase and chitinase isoforms varies during embryo development
- reduced relative air humidity in desiccation decrease the activity of β- 1,3 glucanases and chitinases
- 90% relative air humidity in desiccation negativelly affects germination of embryos

MATERIAL AND METHODS

Cultivation:

Embryogenic cultures of Norway spruce (Picea abies L. [Karst.]) were cultivated on solidified (proliferation) resp. liquid (maturation) GD medium (Gupta and Durzan 1986) as described elsewhere (Vágner et al. 1998). The proliferation medium contained 5 mM 2,4-D, 2 mM BA and 2 mM kinetin, maturation medium was supplemented by 20 mM ABA and 3.75% (w/v) PEG 4000. The fully developed embryos were desiccated in three different levels of air humidity (90%, 95%, 100%) for 10 days, the rest of desiccation (another 10 days) took place in 100% air humidity. Germination medium without phytohormones was supplemented with active charcoal.

Morphology of embryos:

Light transmission microscop Jenaval (Karl Zeiss) with DS-5M Nikon camera was used to monitor the effect of drought stress in desiccatin phase of embryo development. Obtained images were processed by Lucia image analysis system.

Biochemical analysis:

Total proteins were extracted from embryos (Hurkman and Tanaka 1986). Aliquots (20 µg) were separated in 12.5 % polyacrylamide gels to detect total enzyme profiles, profiles of acidic/neutral and basic/neutral isoforms (respectively) under standard conditions. For detection of chitinases, the gels contained 0.01 % (w/v) glycolchitin as the enzyme substrate. The glucanase activities of protein fractions were detected in gels with 0.01 % (w/v) laminarin.

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