ABS ACTA BIOLOGICA SLOVENICA
LJUBLJANA 2003 Vol. 46, Št. 2 : 55 - 60

Effect of sucrose nutrition on the histological structure of carob shoot cultures

Branka VINTERHALTER[1], Dragan VINTERHALTER[2]

[1]Institute for Biological Research, 29. novembra 142, Belgrade, Yugoslavia
[2]Faculty of Science, Zmaja od Bosne 33–35, Sarajevo, Bosnia and Herzegovina

Abstract. Analysis of carob (*Ceratonia siliqua* L.) shoot cultures grown on 2 and 8 % sucrose in light and darkness (etiolation) showed marked differences in histological structure. Etiolated, dark grown shoot cultures were hypolignified on both sucrose concentrations. They had highly reduced sclerenchyma and xylem production, endodermis with Casparian bands and absence of lenticel formation. In light grown cultures shoots in transection showed nearly concentric ring of sclerenchyma (outer) and xylem (inner) ring and the absence of Casparian bands in endodermal cells. Increased sucrose nutrition promoted lignification and efficiently prevented lenticel hypertrophy characteristic for low sucrose concentration.

Key words: carob, *in vitro*, sucrose, light, etiolation, lenticel hypertrophy, lignification, hypolignification, Casparian bands

Introduction

Effect of sucrose on the growth of plant *in vitro* cultures has been subject of numerous studies. In most species cultured *in vitro* optimal concentration of sucrose is 2–3 %. Apart from nutritive role sucrose has been recently attributed a regulatory role since changes in sucrose concentration can serve in gene activation and signal sensing (Koch & al., 1992, Mitra & al. 1995, Smeekens & Rook 1997). Increased sucrose nutrition can induce morphogenetic effects as for instance axillary bud formation in the rooting stage of *Dracaena fragrans* (Vinterhalter & Vinterhalter 1997). In carob sucrose together with light regulates leaf size (Vinterhalter & Vinterhalter 2001). It is well documented that sucrose stimulates xylogenesis in callus of angiosperms (Wetmore & Rier 1963) and in internodes of *Coleus* (Rier & Beslow 1967). Warren Wilson & al. (1994) confirmed the stimulatory effect of exogenous sucrose on xylogenesis. However, the optimal sucrose concentration was species dependant. In letuce pith explants it was at 0.2 % and in tobacco pith explants at 3.0 % sucrose.

Carob shoot cultures grown on low sucrose concentrations (2 % sucrose or less) exhibit lenticel hypertrophy a rare physiological disorder of *in vitro* cultures described also in *Populus euphratica* (Lledo & al. 1995). Histological investigation showed that lenticels appear only on hypolignified internodes (Vinterhalter & al. 1997). Increased sucrose nutrition promotes the growth of carob shoot cultures (Vinterhalter 1998) and at the same time decreases the frequency of lenticel hypertrophy. Another treatment in which lenticel hypertrophy in carob was found to be absen was

cultivation in the darkness which results in formation of etiolated shoot cultures (VINTERHALTER & VINTERHALTER 2002).

In the present study we investigated the histological structure of carob shoot cultures grown in conditions of high and low sucrose nutrition both in the light and darkness. We were particulary interested in a possible connection between lignification and lenticel hypertrophy.

Material and methods

Carob shoot cultures were established and maintained on MURASHIGE & SKOOG (1962) medium with 2.25 μM BA and 0.5 μM IBA as previously described (VINTERHALTER & VINTERHALTER 2001). Treatments consisted of media supplemented with 2 % (low) and 8 % (high) sucrose in the light and darkness. The light 46.5 μmol $m^{-2}s^{-1}$ was provided by cool white fluorescent lamps. All treatments lasted for 35 days. The material for histological study was prepared and stained according to JOHANSEN (1940). Shoots were fixed in 1 : 1 : 18 formalin-acetic acid - ethanol (FAA) embedded in the paraffine and cut on a rotary microtome in the 8–10 μm thick sections. Sections were double stained with safranine-light green or safranine-wasser blau. Presence of lignine was demonstrated in fresh material using hand sections stained either with an acidified phloroglucinol or aniline sulphate. Hand sections of etiolated tissues were stained with Lugol-s reagent (JJK) in combination with acidified phloroglucinol or safranine.

Results

Experiments conducted in the light: At the end of subculture, shoot explants, initially consisting of an apical bud and 2–3 internodes develop 5–8 new internodes. Internodes of the initial explant become basal internodes whilst apical and central internodes develop in the current subculture. Basal internodes submersed into the medium produce a callus which appears in the endodermis and replaces all cortical and surface tissues developing in a form of pseudo-cortex.

Apical internodes in transection have characteristic 5 lobed structure and maturation of vascular tissues in not yet complete. Central internodes are circular in transection and have typical mature structure with well differentiated vascular tissues and two massive circumferential rings of lignified cells, outer sclerenchyma and inner xylem ring. Difference in color (sclerenchyma is more orange) indicates possible difference in lignine composition. Epidermal and subepidermal cells are red from accumulation of anthocyanin and resins.

Basal internodes of cultures grown on 2 % sucrose, typically undergo lenticel hypertrophy with massive surface proliferation of phelloderm cells. Inner tissues of such internodes show severe hypolignification, meaning that the two massive rings of lignified cells (sclerenchyma and xylem) are reduced or missing. In conditions of low sucrose concentration lenticel hypertrophy is therefore always associated with hypolignification. The analyses of hand sectioned fresh material showed that in the earliest stages of lenticel hypertrophy sclerenchyma ring is ruptured or missing (Fig. 1).

Frequency of lenticel hypertrophy significantly decreases if cultures are grown on the media with increased sucrose concentration (Fig. 5). Accumulation of anthocyanins and resins in epidermal and subepidermal cells is stimulated same as abundant formation of amyloplasts in all parenchymatous cells. In central and basal internodes sclerenchyma ring is well developed and there is a massive formation of both phloem and xylem (Figs. 3, 4).

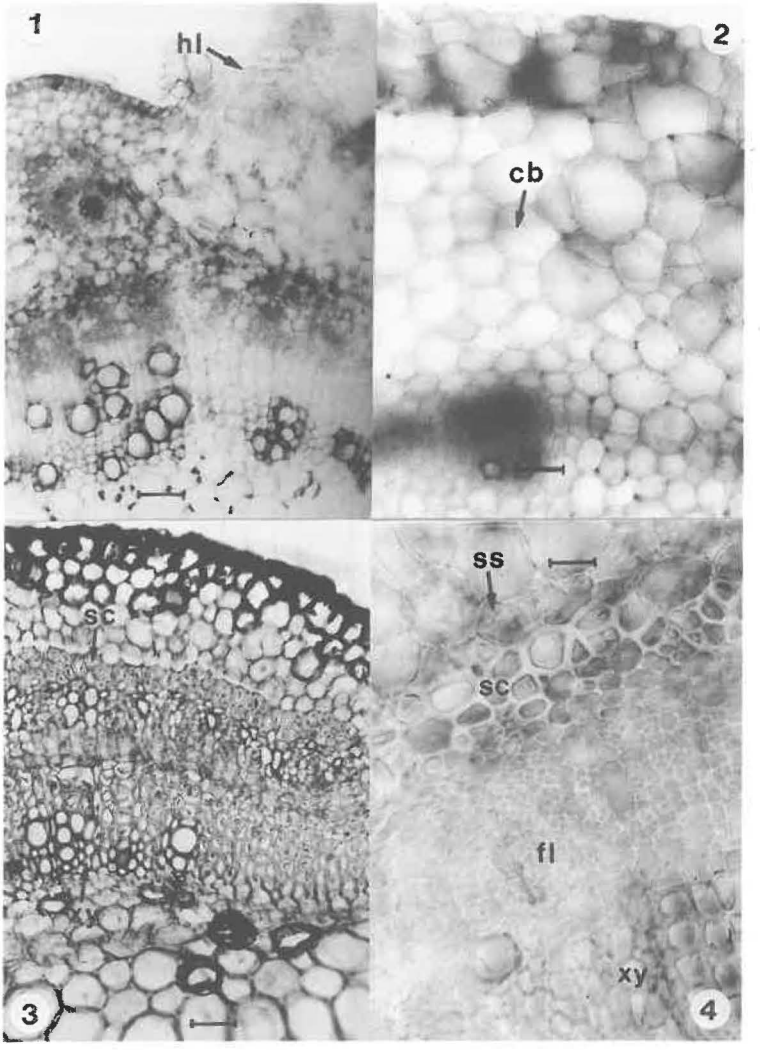


Figure 1: Early stage of lenticel development on 2 % sucrose in light, basal internodes, bar = 60 μm. Hand section of fresh material stained with Lugol and safranine. Sclerenchyma ring absent, xylem organised in smal groups, prominet lenticel (hl).

Figure 2: Transection of a dark grown etiolated shoot, 2 % sucrose, bar = 22 μm. Hand section of fresh material stained with Lugol and safranine. Endodermal cells with Casparian bands (cb), sclerenchyma ring absent and replaced with large, thin-walled parenchymatous cells.

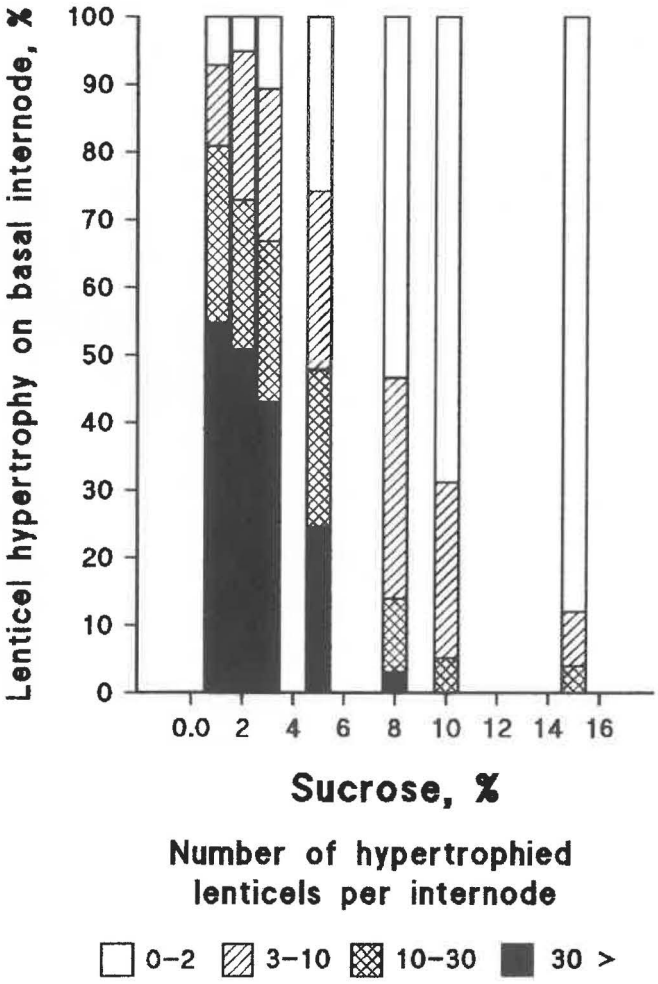
Figure 3: Transection of a basal internode on 8 % sucrose in light, bar = 60 μm. Paraffine section stained with safranine- light green. Typical heavy lignified structure, lenticels absen. Epidermis and subpeidermis rich with anthocyanins an resins, circumferential sclerenchyma ring (sc), phloem, cambal layer and a massive layer of lignified xylem (xy).

Figure 4: Transection of a basal internode on 8 % sucrose in light, bar = 22 μm. Hand section of fresh material stained with Lugol and safranine. Endodermis with amyloplast (starch sheet, ss), well deveoped sclerenchyma (sc), phloem (fl) and xylem (xy).

Experiments conducted in the darkness: After a short transition period cultures became etiolated. Paraffine sections of etiolated cultures which were soft and spongy were successfully replaced with hand sectioned fresh material.

Etiolation induced drastic changes in the shoot structure. Etiolated cultures are in general highly hypolignified. In the darkness no lignine is produced and rings of lignified sclerenchyma and xylem elements (characteristic for light conditions) are never formed. Xylem elements are rare and sclerenchyma layer is completely absent, replaced by large parenchymatic cells laying inner to the endodermis. Characteristic feature of this endodermal parenchyma are cells with Casparian bands which form a continuous or nearly continuous ring around the central cylinder (Fig. 2).

Hypertrophied lenticels were never observed to appear in etiolated shoot cultures irrespectively of the concentration of sucrose in the medium. However, etiolated cultures transferred to light quickly became de-etiolated and in favorable conditions (low sucrose in the medium) lenticel hypertrophy re-appeared.



Fig

Figure 5: Effect of sucrose concentration on the number of hypertrophied lenticels formed on the first internode.

Discussion

Hand sectioning of fresh material proved to be an exellent method for rapid investigation of lignified tissues in carob shoot cultures grown both in light and darkness. In etiolated shoot cultures it enabled us to observe Casparian bands.

Arzee & al. (1977) found that in carob seedlings phellogen formation starts at the end of the first year. In carob shoot cultures tissue differentiation is very fast and certain conditions may induce premature formation and hypertrophy of lenticels which we consider as a physiological disorder. What are the factors which induce this phenomenon?

Our first studies showed that lenticel hypertrophy is connected to cytokinin concentration (Vinterhalter & al. 1992) and restricted ventilation (Vinterhalter & Vinterhalter 1992). Absence of ventilation triggered lenticel formation but only if cytokinins were present in the medium, otherwise leaves were sheadeed and cultures perished.

In some species of trees lenticel hypertrophy is a physiological adaptation to anoxia induced by flooding and mediated by ethylene (Tang & Kozlowski 1984). Process includes synthesis of ethylene precursor (ACC) in submersed tissues, its translocation through the vascular tissues and conversion into ethylene in aerated tissues above the line of submersion.

Although lenticel hypertrophy *in vitro* has been described only in two species, *Ceratonia siliqua* (Vinterhalter & al. 1992) and *Populus euphratica* (Lledo & al. 1995) there are notions that it is a more widespread phenomenon. Is it possible that *in vitro* conditions mimic flooding in some plants species? In carob lenticel hypertrophy always appears on the basal internode, first internode above the surface of the medium and than it spreads acropetally.

Our studies showed that lenticel hypertrophy is affected by sucrose nutrition and light. High sucrose nutrition decreases frequency of lenticel hypertrophy whilst growth in darkness (etiolated cultures) completelly prevents it (Vinterhalter 1998, Vinterhalter & Vinterhalter 2002). In both cases there are changes in the histological structure which affects the abundance of cells with lignified walls as reported here. In the first case lenticel hypertrophy does not occur whilst sclerenchyma ring is present. Sclerenchyma ring is a barrier which efficiently prevents lateral movement of water in stems. It seems that undeveloped sclerenchyma ring characteristic for low sucrose concentrations is the factor which stimulates lenticel hypertophy and which the high sucrose nutrition prevents. In the second case lenticel hypertrophy is absent although etiolated shoots contain no lignified cells. Here sclerenchyma ring although absent is functionally replaced with another barrier, ring of cells with Casparian bands which are known to restrict lateral translocation of water (Kolda 1937). We thus suppose that lenticel hypertrophy in carob may be prevented by conditions which limit lateral translocation of water and solutes in shoots.

Literature

Arzee T., E. Arbel & L. Cohen 1977: Ontogeny of periderm and phellogen activity in *Ceratonia siliqua* L. Bot. Gaz. **138** (3): 329–333.

Johansen D.A. 1940: Plant microtechnique. McGraw-Hill. New York & London, 523 pp.

Koch K. E., K. D. Nolte, E.R. Duke, D. R. McCarthy & W. T. Avigne 1992: Sugar levels modulate differential expression of maize sucrose synthase genes. Plant Cell **4**: 59–69.

Kolda A. 1937: Zur Anatomie etiolierter und periodisch belichteter Pflanzen und über die Wirkung nachträglicher Kultur am Lichte. Bieh. Bot. Centralb. Abt. A., **57** (3): 319–380.

Lledo M.D., M.B.Crespo & J.B. Amo-Marco 1995: The role of cytokinins and ethylene inhibitors on lenticel hypertrophy generation and ethylene production in *in vitro* cultures of *Populus euphratica* Olivier. Israel Journal of Plant Sciences **43**: 339–345.

MITRA S., K. SUZUKI-FUJII & K. NAKAMURA 1995: Sugar-inducible expression of a gene for ±-amylase in *Arabidopsis thaliana*. Plant Physiol. **107**: 895–904.

MURASHIGE T. & F. SKOOG 1962: A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. **15**: 473–497.

RIER J.P. & D.T. BESLOW 1967: Sucrose concentration and differentiation of xylem in callus. Bot. Gaz. **128**: 73–77.

SMEEKENS S. & F. ROOK 1997: Sugar sensing and sugar mediated signal transduction in plants. Plant Physiol. **113**: 293–304.

TANG Z.C. & T.T. KOZLOWSKI 1984: Ethylene production and morphological adaptation of woody plants to flooding. Can. J. Bot. **62**: 1659–1664.

VINTERHALTER B. 1998: Uticaj svetlosti i saharoze na rastenje i građu izdanaka rogač (*Ceratonia siliqua* L.) u kulturi *in vitro*. Ph.D. thesis. Univ. Belgrade, 134 pp.

VINTERHALTER D., D. GRUBIŠIĆ, D. BOJOVIĆ-CVETIĆ & S. BUDIMIR 1992: Lenticel hypertrophy in shoot cultures of *Ceratonia siliqua* L. Plant Cell Tiss.Org.Culture **31**: 111–114.

VINTERHALTER D. & B. VINTERHALTER 1992: Factors affecting *in vitro* propagation of carob (*Ceratonia siliqua* L.). Arch. Biol.Sci. **44**: 177–186.

VINTERHALTER D. & B. VINTERHALTER 1997: Micropropagation of *Dracaena* Species. Biotechnology in Agriculture and Forestry Vol. **40**, High-Tech and Micropropagation VI, editor Y.P.S. Bajaj, Springer, 131–146.

VINTERHALTER B., D. VINTERHALTER & M. NEŠKOVIĆ 1997: Effect of sucrose nutrition and light on the histological structure of carob (*Ceratonia siliqua* L.) shoot cultures. Procc. 3rd ICFWST Conference, Belgrade-Goč, 37–42.

VINTERHALTER B. & D. VINTERHALTER 2001: Effect of irradiance, sugars and nitrogen on leaf size of *in vitro* grown *Ceratonia siliqua* L. Biologia Plantarum **44**:185–188.

VINTERHALTER D. & B. VINTERHALTER 2002: Etiolated, dark grown shoot cultures and light emitting diodes - New approaches in plant biotechnology. In " Plant Physiology in the New Millenium (eds):. Quarrie S., Krstić B, Janjić V. Yugoslav Soc. Plant Physiol. Belgrade, 95–99. (ISBN 86-7384-011-2).

WARREN WILSON J.W., L.V. ROBERTS, P.M.W. WARREN WILSON & P.M..GRESSHOFF 1994: Stymulatory and inhibitory effects of sucrose concentration on xylogenesis in lettuce pith explants; possible mediation by ethylen biosynthesis. Ann. Bot. **73**: 65–73.

WETMORE R.H. & J.P. RIER 1963: Experimental induction of vascular tissues in callus of angiosperms. Amer.J.Bot. **50**: 418–430.