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Soluble carbohydrates and acid invertases involved in the rapid growth of developing culms in *Sasa palmata* (Bean) Camus

E. Magel*, S. Kruse, G. Lütje and W. Liese

Abteilung Holzbiologie, Zentrum Holzwirtschaft, Universität Hamburg, Leuschnerstr. 91,
21031 Hamburg, Germany

Developing bamboo culms reach their final height of several meters (5-20) within a very short period of two to four months. This rapid extension growth of bamboo is not well understood and no information about the physiological and molecular processes underlying this phenomenon exist. Extension growth is generally turgor-dependent and in many cases is regulated by invertases, either alone or in combination with sugars and plant hormones. Therefore, we investigated the pool sizes of the non-structural carbohydrates (glucose, fructose, sucrose, and starch) and the catalytic activities of acid invertases (soluble and cell wall bound) in the growing culm, in mature culms of different age-classes, and in the rhizome of bamboo, *Sasa palmata*. Our data show, that cell wall invertases (AI_{cw}) dominate in the growing culm, where high catalytic activities of AI_{cw} create a strong sink for sucrose. In the rapidly growing culm, high activities of cell wall and acid soluble invertases, together with the resulting high hexose (glucose, fructose) contents correlate with cell elongation and expansion, possibly by modulating osmotic pressure and cell turgor. The high availability of hexoses could also be the basis for maintaining a high mitotic activity. The findings thus give evidence that invertases are involved in the provision of organic solutes, necessary for elongation growth in bamboo culms.

The growth of a bamboo culm is still a mystery and not well understood. Only a few investigations have focused on limited aspects of this growth phenomenon. The culm grows by expanding its internodes, whereby cellular reorganization has been preformed in the buds (McClure 1997). The rate of daily expansion varies among species with an average of 5-20 cm (up to 60-80 cm), until a final length of several meters (up to 20-25 m) has been reached. Some of the bigger bamboos, such as *Guadua angustifolia*, produce about 500 cm³ wall substance per day (Liese 2004), amounting to a total of about 0.1 m³ biomass for the entire culm. This high quantity of biomass is produced within the growing season of about 3-5 months. There is no other plant with a comparable biomass production. Therefore, bamboos are

considered highly suitable for biomass sequestration plantations, and are discussed with regard to the Kyoto protocol.

It has to be noted that the growing culm hardly contributes by itself to the biomass accumulation: as photosynthetically active leaves do not develop before full culm expansion (Nath et al. 2004). During expansion, the culm itself is covered by a dense layer of culm sheaths with hardly any net rate of assimilation. In the late stages of growth this cover is shed, enabling positive autotrophic carbon gain by the culm (Judziewicz et al. 1999). Consequently, the biomass production of an expanding culm has to be facilitated by internal supplies of carbon, which are reallocated from storage tissues of the rhizome and of older, previous years' culms.

* Corresponding author:
Phone: ++49 40 73962403
Fax.: ++49 40 428912835
Email: Elisabeth.magel@uni-hamburg.de

Nonstructural carbohydrates are not only prominent in carbon storage (starch), but also provide transport of carbon (sucrose), or nutrient carbon substances in plants. Carbohydrates also function as metabolic signals and regulatory molecules, and thus affect the expression of different classes of genes (Koch 1996, Roitsch and Gonzalez 2004). Moreover, it is not just the presence of the carbohydrates that is of importance, but also the capacity of the tissues to use them in metabolic processes or facilitate their import and export that is just as significant. Sucrose, and the products of its hydrolysis, glucose and fructose are in this context of central importance. Hydrolysis of sucrose is mediated by invertases. In plants, three types of invertases are present. They are located in the apoplast, the vacuole and the cytoplasm. Besides their subcellular localization, they are characterized by their solubility, pH-optima and isoelectric points. Alone or in combination with sugars and plant hormones, invertases regulate many aspects of growth and development of plants (Strum and Tang 1999, Roitsch and Gonzalez 2004).

In order to gather information about the regulation of growth in new bamboo shoots, we investigated the seasonal changes in pool sizes of these sugars, and of the levels of activity of soluble (vacuolar) and cell wall acid invertases in the rhizome, one-, and two-years-old, as well as the developing culms of *Sasa palmata*. *Sasa palmata* (Bean) Camus was chosen for this investigation as it is small and therefore easy to handle and study. The results, however, can be extended to the large bamboos.

MATERIALS AND METHODS

Plant material

Sasa palmata plants were investigated between March 2004 until February 2005. Specimens of the rhizome, of one-year-old culms, of two-years-old culms, and current year culms were harvested in the field near Hamburg, Germany. In order to follow the development of the emerging culm in detail, harvest took place in a weekly course from March 2004 until May 2004 (March 18th,

March 25th, April 2nd, April 13th, April 20th, April 27th, May 2nd, May 10th). During this time, the diameter of the harvested developing culms were about 10 mm, whereas the length extended from 25 cm (April 13th), 70 cm (April 20th), 120 cm (April 27th), 170 cm (May 2nd), and 220 cm (May 10th), respectively. Additionally, specimen were collected in summer (June 15th), fall (October 18th), and winter (February 3rd, 2005) from fully developed, current-year culms. For analysis of mature culms (e.g. one-year-old and two-years-old culms), material of the 6th internode was used. Immediately after harvest, the specimen were quickly frozen. After freeze-drying, the material was homogenized to a fine powder and kept under vacuum at -30°C until use.

Determination of starch, glucose, fructose, and sucrose

Identification of the dominating soluble carbohydrates was done by thinlayer chromatography (Magel and Höll 1993). As it turned out that glucose, fructose, and sucrose dominated by far, their amounts, as well as the amounts of starch, were quantified enzymatically as described in Magel et al. (2001). After denaturing endogenous hydrolytic catalytic activities by heat treatment, non-structural carbohydrates were extracted from 6 mg plant material in 750 µl of doubly distilled water. For quantification, micro plate reader assays in 96-well micro plates (300 µl cavity volume; Greiner, Nürtingen, Germany) in a Spectra Thermo micro plate reader (Tecan, Crailsheim, Germany) were used.

Preparations of crude extracts for enzyme assays

Fifteen mg of lyophilized plant material was mixed with 20 mg of insoluble polyvinyl-pyrrolidone (MW 360000, Polyclar AT, Serva). Soluble invertases (AIsol) were extracted by adding 750 µl of ice-cold Tris/borate/2-mercaptoethanol buffer (100/300/2 mM, pH 7.2; extraction medium I), under occasional vortexing for 15 min on ice. After centrifugation (10000g, 10 min, 4°C), aliquots of the supernatants were taken for ammonium sulphate mediated protein-precipitation (Li et al. 2003). The precipitated protein was collected by

centrifugation, redissolved in extraction medium I, and desalted on a Sephadex G-25 PD-10 column (Amersham Pharmacia biotech, Sweden; extract I). Ten- μ l-aliquots of the cooled filtrate were used for determination of the enzyme activities.

Extraction of ionically cell wall bound invertases (Alicw) was done by re-extraction of the pellet with buffer of high ionic strength (extraction buffer supplemented with 2 M NaCl; extract II). For the measurements of covalently cell wall bound (insoluble) invertases (Alccw), the pellet was resuspended with extraction medium I and the homogenate was used in the assays.

Assays of enzyme activities

The assays of the enzyme activities were based on the published micro plate reader assays for pine (Uggla *et al.* 2001) and walnut tissue (Magel *et al.* 2001), and were adapted to the specific requirements of bamboo tissues.

Activities of AIsol and Alicw were assayed by quantifying the amounts of glucose and fructose formed from sucrose in the specific step (total volume of 70 μ l, pH 7.0, 150 mM Hepes-buffer, containing 3 mM MgSO₄, 1.55 mM NADP, 4.07 mM ATP, phosphoglucose-isomerase [4.3 U ml⁻¹], glc6P-dehydrogenase [2.2 U ml⁻¹], hexokinase [9.2 U ml⁻¹]). For the assay of AIsol and Alicw, 10 μ l of extract I or II respectively were incubated in a total volume of 55 μ l at pH 4.0 (68 mM citrate/86 mM phosphate) containing 220 mM sucrose. For the measurement of the activities of Alccw the specific step was performed with the homogenate, at pH 4.0 and 200 mM sucrose. Termination of the reaction was achieved by adjusting the pH to 7.5 and centrifugation, and then amounts of glucose and fructose formed were quantified in the supernatant (see above).

Blanks were run either with inactivated enzyme extracts (10min, 98°C), double-distilled water and with sample-free extraction buffer instead of the specific substrate (sucrose).

Protein determination

The protein content of the extracts (I and II) was determined in a micro plate assay using the BIO-Rad (BioRad, Munich) reagents according to the manufacturer's protocol.

RESULTS AND DISCUSSION

In the bamboo *Sasa palmata*, like other photoautotrophic and heterotrophic plant tissues, glucose, fructose and sucrose constitute the major soluble carbohydrate fraction. Together with starch these sugars represent the non-structural carbohydrates of this bamboo species. In mature above-ground organs such as one-year-old and two-years-old culms, the monosaccharides glucose and fructose dominate the soluble carbohydrate fraction during the cold season (February, March), and reach values of up to 120 nmol/mg dw. The high contents of monosaccharides and of sucrose (up to 100 nmol/mg dw; Figs: 1 b, c) together with low amounts of starch (down to less than 10 nmol starch-bound hexoses/mg dw; Figs: 1 b, c) during this period can be taken as an indication of cryoprotection. This has also been reported for other perennial plant organs, such as branches or stems of trees (Magel *et al.* 1994, Sauter and Marquardt 1966, Sauter and Wellenkamp 1998, Sauter *et al.* 1996). Starting with higher temperatures towards the end of March, sucrose becomes the dominating sugar fraction and starch accumulates in the parenchyma cells. This is most probably due to a surplus of photoassimilated products, resulting in highest amounts of starch in early summer (for example in conifers see Egger *et al.* 1996). In samples of one-year-old and two-years-old culms collected on April 20th, this steady starch increase is retarded. During autumn and winter, starch pools are low in mature culms (Figs. 1b, c).

In the bamboo under-ground organ, the rhizome, cryoprotection of the tissue during wintertime is also characterized by higher contents of soluble carbohydrates and decreased pools of starch (Fig. 1a). Contrasting to overwintering, one-year-old and two-years-old bamboo culms contain sucrose as the preponderant component of the soluble carbohydrate fraction throughout the year. Starch contents in the rhizome peak in early spring (end of March; Fig. 1a) and then later on during early summer (May, June). Thus, the rapid spring growth of the new shoots, leads to reduced total amounts of non-structural

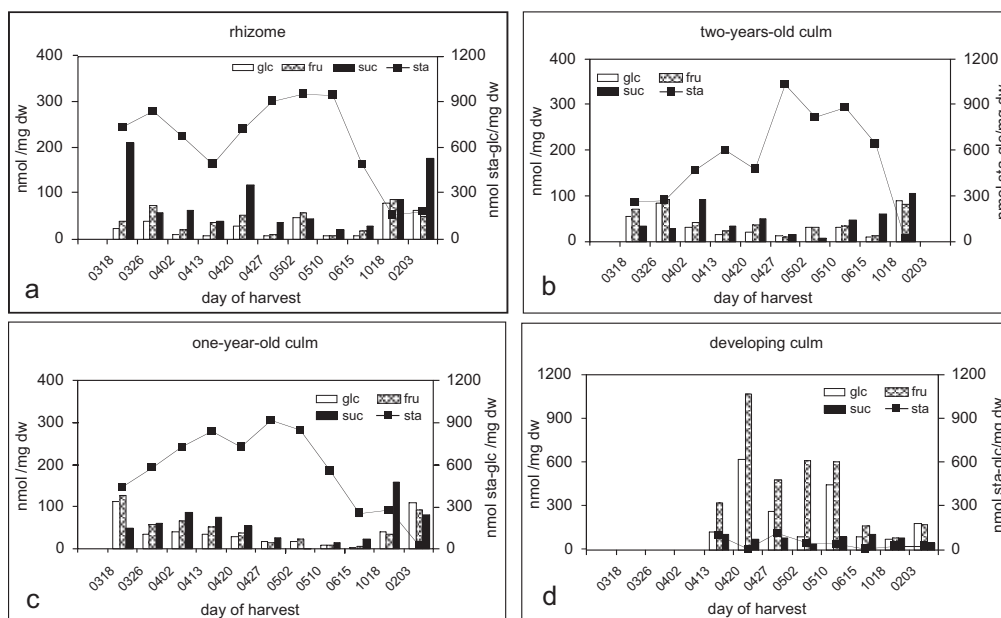


Figure 1. Seasonal course of contents of glucose (glc, open bars; nmol/mg dw), fructose (fru, grey bars; nmol/mg dw), sucrose (suc, closed bars; nmol/mg dw), and starch (sta, closed squares; nmol sta-glc/mg dw) in the rhizome (a), one-year-old (b), two-years-old (c), and the developing (d) culm of *Sasa palmata* from March 18th, 2004 (0318), until February 3rd, 2005 (0203). The first two digits identify the month, the second two the day. Values given are means of three replicates. As standard deviations were lower 5% no SD is given.

carbohydrates in the rhizome (Li et al. 1998). It is noteworthy, that both the rhizome and the mature culms exhibit starch levels of up to 1 μmol starch-bound hexoses/mg dw, and thus starch constitutes more than 20 % of the dry weight.

Between April 2nd and April 13th, the current year's culms emerged (Fig. 1d). On April 13th, this new shoot was about 25 cm in length. A constant daily growth rate of about 7 cm was maintained until the final height of about 220 cm was reached on May 10th. During this growth period, starch contents were negligible and soluble carbohydrates reached amounts up to 1.8 μmol hexoses/mg dw (Fig. 1d); thus sugars constituted more than 30 % of the dry weight (for comparison sugar content of the phloem sap and of sugar storing tissues of sugar cane and sugar beet is about 15%). Moreover, hexose-contents found in the elongating tissue of bamboo culms are 5 fold higher than those present in the zone of enlarging tracheids within the cambial differentiation zone of pine trees

(Uggla et al. 2001) and up to 10 fold higher than in the apical meristem of developing conifer seedlings (Einig et al. 1999). In vigorous growing culms, fructose contents exceed by far the contents of glucose and/or sucrose (Fig. 1d). This implies, that in the developing culm like in the dividing and expanding tissues of the cambial region of pine trees, glucose is faster consumed in metabolic and/or biosynthetic pathways (Uggla et al. 2001).

Based on these findings, we concluded that highest concentrations of osmotically active components in the vigorous growing culm, such as the monomeric and dimeric carbohydrates, glucose, fructose, and sucrose, enable the elongation of the bamboo tissue, which appears to be driven, at least partly, by changes in the solute potential (Cosgrove 1986, Hoffmann-Benning et al. 1997).

In rapidly elongating tissues, such as internodes, fibrous roots, early stages of fruit expansion, young sink leaves or expanding cambial cells increased amounts of monomeric

sugars are associated with high catalytic activities of soluble acid invertase (Morris and Arthur 1985, Quick and Schaffer 1986, Uggla *et al.* 2001). Soluble acid invertases are located in the vacuole. They control sugar composition in fruits or storage organs, respond to environmental stresses or wounding, and are involved in osmoregulation and cell enlargement (Roitsch and Gonzalez 2004). This pivotal role of acid invertase for cell elongation was also shown in transgenic tobacco plants, expressing a yeast-derived invertase in the vacuole (Hoffmann-Benning *et al.* 1997). In elongating bamboo culms, like in tissues which undergo rapid cell expansion (Tymowska-Lalanne and Kreis 1998), highest contents of monomeric sugars coincide in time and tissue distribution with the highest catalytic activities of acid soluble invertases, both when calculated on a dry weight or protein basis (Fig. 2). This can be taken as further proof that expansion and elongation of the cellular organization of the culm, which has been preformed within the buds, is at least partly controlled by osmotic pressure and cell turgor.

During these developmental processes, the heterotrophically growing culm depends on carbon supply from other source tissues. Sharp decreases in starch pools in the rhizome during times of the early growing period of the new culm (April 13th), as well as lower starch accumulation in the mature culms (April 20th), indicate a reallocation of stored carbon from

these tissues towards the developing culm. In addition, current photoassimilates of the older culms appear to be translocated to the rapidly growing new culm (Koyama and Ogawa 1993).

In most plant species, carbon is transported from source to sink tissues in the form of sucrose. At the sink area, sucrose is exported or leaked from the translocation path (e.g. sieve elements) into the apoplast. Here, cell wall located invertases hydrolyse sucrose into glucose and fructose. The hexoses are then taken up into the sink cells by hexose transporters (Roitsch and Gonzalez 2004). In the rapidly elongating culm (until May 2nd), high catalytic activities of cell wall invertases could facilitate such an import of sucrose into this sink tissue, and thus indicate apoplastic unloading in the expanding bamboo culm (Fig. 3). Ionically bound cell wall invertases dominate this developmental stage.

After cessation of the growth in height (June 15th) of the new culm, pools of soluble carbohydrates, and catalytic activities of the vacuolar (AI_{sol}) and cell wall (AI_{icw} , AI_{ccw}) invertases were similar to those of mature culms, whereas starch contents remained low.

In summary we conclude, that cell wall invertases play an important role in sucrose partitioning towards the growing culm, and hydrolysis of sucrose by cell wall invertases (AI_{cw}) contribute to establishing sink strength (Sturm and Tang 1999). The correlation between high activities of AI_{cw} and AI_{sol} , and

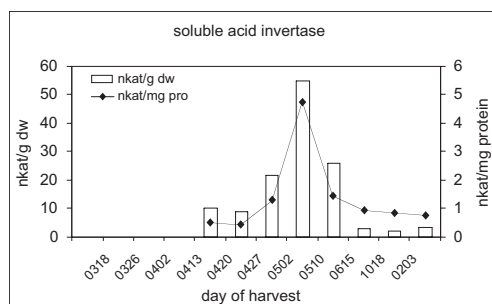


Figure 2. Catalytic activities of soluble, vacuolar acid invertase calculated on a dry weight (open bars; nkat/g dw) and protein (closed rectangles; nkat/mg pro) basis in the developing culm of *Sasa palmata*. Values given are means of three replicates. As standard deviations were lower 5% no SD is given.

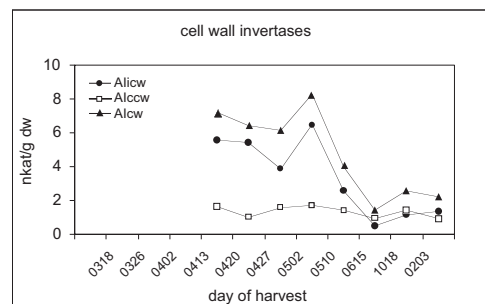


Figure 3. Catalytic activities of ionically (AI_{icw} ; closed circles; nkat/g dw), covalently (AI_{ccw} ; open squares; nkat/g dw), and total (sum of AI_{icw} plus AI_{ccw} ; closed triangles; nkat/g dw) cell wall invertases in the developing culm of *Sasa palmata*. Values given are means of three replicates. As standard deviations were lower 5% no SD is given.

the high resulting hexose contents, control cell elongation and expansion by modulating osmotic pressure and cell turgor. Most probably, these sugar supplies are also important for maintaining high mitotic activity in the growing culm (Roitsch and Gonzales 2004). Our findings add to the evidence that invertases in combination with sugars regulate growth and elongation of developing bamboo culms. The biochemical and genetic regulation of the phenomenon of rapid growth and elongation of developing bamboo culms will be the focus of future work.

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LITERATURE CITED

- Cosgrove, D. 1986. Biophysical control of plant cell growth. *Annual Review of Plant Physiology* 37: 377-405.
- Egger, B., W. Einig, A. Schlereth, T. Wallenda, E. Magel, A. Loewe and R. Hampp. 1996. Carbohydrate metabolism in 1- and 2-year-old spruce needles and stem carbohydrates from three months before until three months after bud break. *Physiologia Plantarum* 96: 91-100.
- Einig, W., A. Mertz and R. Hampp. 1999. Growth rate, photosynthetic activity, and leaf development of Brazil pine seedlings (*Araucaria angustifolia* [Bert.] O. Ktze.) *Plant Ecology* 143: 23-28.
- Hoffmann-Benning, S., L. Willmitzer and J. Fisahn. 1997. Analysis of growth, composition and thickness of the cell walls of transgenic tobacco plants expressing a yeast-derived invertase. *Protoplasma* 200: 146-153.
- Judziewicz, E.J., L.G. Clark, X. Londono and M.J. Stern. 1999. *American Bamboos*. Smithsonian Institution Press, Washington London.
- Koch, K.E. 1996. Carbohydrate-modulated gene expression in plants. *Annual Review of Plant Physiology and Molecular Biology* 47: 509-540.
- Koyama, N. and Y. Ogawa. 1993. Growth characteristics of Nezasa dwarf bamboo (*Pleioblastus variegatus* Makino): 1. Photosynthesis and utilization of stored nitrogen. *Journal of Japanese Society of Grassland Science* 39: 28-35.
- Li, R., M.J.A., Werger, H.J. During and Z.C. Zhong. 1998. Carbon and nutrient dynamics in relation to growth rhythm in the giant bamboo *Phyllostachys pubescens*. *Plant and Soil* 210:113-123.
- Li, X., M. Pfiz, M. Küppers, W. Einig, H. Rennenberg and R. Hampp. 2003. Sucrose phosphate synthase in leaves of mistletoe: its regulation in relation to host (*Abies alba*) and season. *Trees* 17: 221-227.
- Liese, W. 2004. *Guadua* in Kolumbien. *Bambus Journal* 16: 4-6.
- Magel, E.A., C. Jay-Allemand and H. Ziegler. 1994. Formation of heartwood substances in the stemwood of *R. pseudoacacia* L. II. Distribution of non-structural carbohydrates and wood extractives across the trunk. *Trees* 8: 165-171.
- Magel, E.A. and W. Höll. 1993. Storage carbohydrates and adenine nucleotides in trunks of *Fagus sylvatica* in relation to discolored wood. *Holzforschung* 47: 19-24
- Magel E.A., A. Abdel-Latif and R. Hampp. 2001. Non-structural carbohydrates and catalytic activities of sucrose metabolizing enzymes in trunks of two *Juglans* species and their role in heartwood formation. *Holzforschung* 55: 135-145.
- McClure, F.A. 1997. *The bamboos*. Smithsonian Institution Press, Washington London.
- Morris, D.A. and E.D. Arthur. 1985. Invertase activity, carbohydrate metabolism and cell expansion in the stem of *Phaseolus vulgaris* L. *Journal of Experimental Botany* 36: 623-633.

- Nath, A.J., G. Das and A.K. Das. 2004. Phenology and culm growth of *Bambusa cacharensis* R.Majumdar in Barak Valley, Assam, North-East India. *Bamboo Science and Culture* 18: 19-23.
- Quick, P. and A.A. Schaffer. 1986. Sucrose metabolism in sources and sinks. In: Zamski, E. and A.A. Schaffer (eds.). *Photoassimilate distribution in plants and crops. Source-sink-relationships*. Marcel Dekker, New York: 115-156.
- Roitsch, T. and M.C. Gonzalez. 2004. Function and regulation of plant invertases: sweet sensations. *Trends in Plant Science* 9: 605-613.
- Sauter, J.J. and H. Marquardt. 1966. Untersuchungen zur Physiologie der Pappelholzstrahlen. *Zeitschrift für Pflanzenphysiologie* 55: 246-258.
- Sauter, J.J. and S. Wellenkamp. 1998. Seasonal changes in content of starch, protein and sugars in the twig wood of *Salix caprea* L. *Holzforschung* 52: 255-262.
- Sauter, J.J., M. Wisniewski and W. Witt. 1996. Interrelationships between ultrastructure, sugar levels, and frost hardiness of ray parenchyma cells during frost acclimation and deacclimation in poplar (*Populus x canadensis* Moench 'robusta' wood. *Journal of Plant Physiology* 149: 451-461.
- Sturm, A. and G.Q. Tang. 1999. The sucrose-cleaving enzymes of plants are crucial for development, growth and carbon partitioning. *Trends in Plant Science* 4: 401-407.
- Tymowska-Lalanne, Z. and M. Kreis. 1998. The Plant Invertases: Physiology, biochemistry and molecular biology. *Advances in Botanical Research* 28:71-117.
- Uggla, C., E. Magel, T. Moritz and B. Sundberg. 2001. Function and dynamics of auxin and carbohydrates during earlywood/latewood transition in scots pine. *Plant Physiology* 125: 2029-2039.