Wood formation of trees in relation to potassium and calcium nutrition

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Summary
Potassium and calcium are essential for tree metabolism and various physiological processes related to growth. In recent years, special interest was therefore accorded to the effect of both cations on cambial activity and xylem development. Various studies revealed a distinct correlation between potassium as well as calcium nutrition and wood formation. When poplar trees were grown under low K⁺ or Ca²⁺ regimes, the cambial activity as well as the seasonal rate of wood increment and the vessel size were significantly reduced. Molecular, biochemical and electrophysiological investigations indicate (i) a strong involvement of specific K⁺ channels in the regulation of xylem cell expansion and (ii) a significant influence of Ca²⁺ on the onset of cambial reactivation after winter dormancy as well as on wood structure and chemistry. These studies highlight the important role of potassium as well as calcium in xylelogenesis. Based on that knowledge, further research will be directed towards a better understanding of the mechanisms governing K⁺- as well as Ca²⁺-dependent wood formation.

Keywords: EDXFA, electrophysiology, immunolocalization, K⁺ channels, poplar, wood production.

Introduction
As essential macronutrients in higher plants, potassium and calcium play decisive physiological roles in plant development and function. Potassium is essential for osmoregulation, cell expansion, stomatal movements and enzyme activation in respiration and photosynthesis. Recently, it was shown in longan trees that application of KClO₃ at temperatures >20 °C induced floral induction (Potchanasin et al. 2009), indicating that potassium also may be involved in initiation of flowering. Concerning calcium, this mineral element appears to play an important role in the synthesis of cell walls (Eklund and Eliasson 1990), particularly in the middle lamella where pectin chains are linked together via calcium. It is also required during cell division and as a second messenger for numerous responses to environmental and hormonal signals (White and Broadley 2003). Additionally, intracellular calcium acts as a membrane stabilizer, having also a protective effect against passive ion influx. Both calcium and potassium are taken up passively by the fine roots and transported via the transpiration stream to the shoots and the leaves. By using multiple stable isotope labelling, Kuhn et al. (1995) followed the path of potassium and calcium during uptake into the root and long-distance transport into the shoot in spruce (Picea abies (L.) H. Karst) trees, showing that 60–70% of the Ca²⁺ and 30% of the K⁺ content in xylem cell walls originate from labelling solutions. With regard to calcium, Page et al. (2008) showed that calcium isotopes were fractionated along the transpiration streams of sugar maple (Acer saccharum Marshall) and American beech (Fagus grandifolia Ehrh.) because roots had the lowest ⁴⁴Ca/⁴⁰Ca ratios and leaf litter the greatest. After leaving the xylem, potassium can be transported via the phloem to different sinks such as developing wood, leaves and roots as well as young fruits (Eschrich et al. 1988, Deeken et al. 2000, Ache et al. 2001). In Sitka spruce (Picea sitchensis (Bong.) Carr.), the internal cycling of potassium has been shown to be independent of current nutrient supply (Weatherall et al. 2006). At the molecular level, the uptake and transport mechanisms of potassium have been investigated within different cell types of the root, shoot and leaf. Various uptake channels and carriers are responsible for K⁺ uptake from the soil (Gassman et al. 1996, Hirsch et al. 1998, Ivashikina et al. 2001). For example, a K⁺ efflux channel of the Shaker type within the Arabidopsis thaliana (L.) genome, the so-called SKOR (Gaymard et al. 1998) plays an important role in K⁺ release into the xylem sap and is expressed by potassium. Within the phloem, K⁺ channels were identified in several species such as Arabidopsis (Deeken et al. 2002) and Vicia faba (Ache et al. 2001), where they affect phloem loading and unloading, respectively.

In contrast to potassium, calcium is immobile in the phloem and is mainly deposited in the cell walls after leaving the xylem cells. During xylem differentiation, it has a strengthening effect on cell walls (Brett and Waldron 1996), and after it is bound in the cell wall layers, it is no longer available for metabolic processes in the symplast. Excess calcium ions can be accumulated as calcium oxalate crys-
tals in the obliterated phloem or in vacuoles, e.g., in leaf cells of *Carya ovata* (Borchert 1990) or in thick-walled cells of oak (*Quercus robur* L.) and poplar (*Populus tremula* L.) bark (Trockenbrodt 1995). In contrast to its strong appearance in the apoplast, calcium occurs only in very low concentrations (0.1–0.2 µM) in the symplast (Hirschi 2004), acting as an effective signal transducer via small variations in its concentration upon abiotic and biotic stimuli (Sanders et al. 1999, Knight 2000).

The present review mainly focuses on the interaction of potassium as well as of calcium supply and wood formation. Microscopic, molecular and biophysical techniques reveal that both mineral nutrients, potassium and calcium, play important roles in xylogenesis. While potassium is mainly required for cell expansion in the developing xylem, calcium seems to be essential for the onset of cambial reactivation and cell division in spring.

The effect of potassium on tree and wood growth

A number of fertilization studies have shown that potassium has a significant impact on tree growth. In eucalyptus (*Eucalyptus grandis* (W. Hill ex Maiden)) trees, after K⁺ fertilization, the above-ground net primary production increased almost up to 100% over the first 36 months after planting, mainly through the enhancement in leaf area index (Laclau et al. 2009). In *Pinus radiata*, a doubling of stem growth response to K⁺ fertilization was associated with a 270% increase in foliar K⁺ (Smethurst et al. 2007), and in *Picea abies*, stands fertilized with K⁺, Ca²⁺ and Mg²⁺ show 30% more biomass and a significant increase in periclinal cell divisions in the cambium (Dünisch and Bauch 1994). When poplar (*Populus tremula* L. × *P. tremuloides* Michx.) plants were grown under potassium deficiency, the first observable symptoms were mottled or marginal chlorosis at the leaves after 5–7 weeks, which then develops into necrosis (Arend et al. 2004, Wind et al. 2004). Occasionally, the leaves were also found to crinkle or curl. Since potassium is mobile within plant organs and tissues, the internal available K⁺ can be transported to the younger leaves and the chlorotic symptoms occur first on leaves of advanced maturation. Moreover, poplars grown under potassium starvation were distinctively smaller and did not show upright growth during the whole growth period of 5 months.

With regard to wood formation, a seasonal variation with a high K⁺ content in spring and summer and a strong reduction in autumn and winter was observed in the poplar cambium (Wind et al. 2004). These variations in K⁺ levels strongly correlated with the radial width and the osmotic potential of the cambial zone, indicating that potassium also plays an important osmotic role during cambial cell expansion. By using a laser ablation system coupled to a high resolution inductively coupled plasma mass spectrometer, Barrelet et al. (2006) found potassium especially accumulated in the latewood of Norway spruce, pointing also to a K⁺ function in latewood formation. Moreover, using energy-dispersive microanalysis (EDXA), it was shown in poplar that the K⁺ concentration in the cambial zone and developing xylem was much higher than in the mature xylem and the phloem (Figure 1). In addition, the K⁺ concentration was distinctly higher in the vessels in comparison with the fibres (Langer et al. 2002). The difference between cell types was most pronounced in poplars grown under non-limiting K⁺ supply (10 mM). Plants grown at low K⁺ levels (0.05 mM) revealed no significant differences in K⁺ concentration between young fibres, vessels and cambial cells. When plants were grown under non-limiting K⁺ supply also the developing xylem zone was threefold larger and the cambial K⁺ level was significantly higher than in plants grown under K⁺ depletion. In plants grown under low K⁺ regimes, secondary cell walls were initiated earlier than in trees well supplied with K⁺. The distribution of K⁺ in the cam-
bium and developing xylem appeared in good agreement with the size of the newly formed vessels and fibres. With rising K⁺ supply in the nutrient solution, the vessel size clearly increased in poplar. In contrast, treatment with tetrathylammonium (TEA), a K⁺ channel blocker, significantly reduced the size of the newly formed vessels (Langer et al. 2002). Since the size of newly formed fibres was neither affected by K⁺ supply nor by TEA treatment, the osmotic role for K⁺ seems to be restricted to vessel and cambial cell expansion. From an ultrastructural perspective, the strong vacuolization of active cambial cells and differentiating vessels is assumed to be a prerequisite for effective cell expansion (Arend and Fromm 2003).

Molecular analysis of K⁺-dependent wood formation

So far, K⁺ has been studied in two tree species. First, from Eucalyptus canadensis, two cDNAs, EcHKT1 and EcHKT2, have been isolated by Fairbairn et al. (2000). The cDNAs encode potassium transporter polypeptides with homology to the wheat K⁺–Na⁺ symporter HKT1 and are expressed in leaves, stems and roots. Both complemented the K⁺-limited growth of an Escherichia coli K⁺-uptake-deficient triple mutant and mediated Na⁺ and K⁺ uptake when expressed in Xenopus laevis oocytes. A comparison of the EcHKT1 and EcHKT2 sequences and their transport properties indicated that these cDNAs represent two K⁺ transporters with distinct functional characteristics, suggesting that they play an important physiological role (Fairbairn et al. 2000). Second, to date 10 K⁺ channels have been found from the poplar genome (Ache et al. 2010). From the cambium of Populus tremula × tremuloides, the EST database (Sterky et al. 1998) was checked for sequence homologies to known K⁺ transporters, and several DNA fragments with homologies to channels and carriers of Arabidopsis were identified (Langer et al. 2002). Corresponding full-length cDNAs were cloned and the homologues were named PTORK (P. tremula outward rectifying K⁺ channel), in agreement with SKOR, the Arabidopsis gene for the outward rectifier expressed in endodermis and xylem parenchyma cells (Gaymard et al. 1998). PTK2 (P. tremula K⁺ channel 2) is another homologue that corresponds to the phloem channel AKT2/3 (Deeken et al. 2000) while KPT1 (P. tremula K⁺ uptake transporter) corresponds to the guard cell channel of the KAT1 type (Anderson et al. 1992). Evidence was given that PTORK and PTK2 are involved in xylem and phloem transport of poplar twigs (Langer et al. 2002), while KPT1 is associated with K⁺ uptake during stomatal opening and bud development (Langer et al. 2004). After isolation of mRNA from various tissues for quantitative RT–PCR analyses, the highest amounts of PTORK and PTK2 were detected in the petioles and the phloem. In order to connect the various K⁺ transporters to seasonal changes in xylogenesis, the expression profiles were compared throughout the year. PTORK and PTK expression was low during cambial dormancy in winter and was induced at temperatures >10–15 °C during cambial reactivation in spring. This pattern appears to be in close correlation with the increase in K⁺ concentration in the cambial zone.

In addition, electrophysiological measurements were performed to study the biophysics of poplar K⁺ channels. First, gene products of PTORK and PTK2 cRNAs were analysed by the double-electrode voltage-clamp technique after injection into Xenopus oocytes. Measurements showed that membrane depolarization elicited an outward rectifying current with a slow sigmoidal activation kinetic and that PTORK is under control of the membrane potential and external K⁺ concentration, enabling K⁺ release in a voltage- and K⁺-dependent manner. In opposition to PTORK, PTK2, like its counterpart AKT2/3 in Arabidopsis, mediates both uptake and release of K⁺ in response to changes in membrane potential, calcium and pH (Langer et al. 2002). Second, in vivo patch-clamp studies were performed on isolated protoplasts from PTORK and PTK2 expression suspension cultures derived from meristematic tissues of poplar branches. With regard to PTORK, results show that the properties of this channel are similar to the PTORK measurements in oocytes as well as to other plant depolarization-activated K⁺ release channels (Gaymard et al. 1998, Ache et al. 2000). Concerning PTK2, it shows inward rectification and, therefore, its voltage dependency in protoplasts differed from that measured in PTK2-expressing oocytes where inward rectification was weak. These opposite features of PTK2 might be explained by the fact that functional Shaker K⁺ channels are formed by four alpha subunits (MacKinnon 1991) and that members of different subfamilies are able to form hetero-tetramers (Daram et al. 1997, Dreyer et al. 1997, Ehnhardt et al. 1997). Therefore, it is assumed that poplar suspension cells could express an additional K⁺ channel alpha subunit that transforms PTK2 into an inward rectifier.

To follow ion channel gene activity at the cellular level, antibodies were raised against a unique N-terminal region of PTORK, and their specificity was checked by western blot analysis of Xenopus oocytes expressing PTORK. By using fluorescence microscopy PTORK labelling was found in the plasma membrane of differentiating fibres and vessel-associated cells (VACs) of the ray parenchyma as well as in sieve elements on tissue sections of poplar branches (Arend et al. 2005). Due to reduced potassium transport during cambial dormancy in winter, PTORK activity is restricted to the period of wood formation in spring and summer, indicating essential functions in xylem development. Since PTORK was absent in vessels, the hypothesis was raised that PTORK might limit the radial expansion of differentiating fibres by mediating K⁺ efflux (Arend et al. 2005). Potassium release from fibres can also contribute to K⁺ accumulation of differentiating vessels, which require high K⁺ levels for their expansion. In contrast, the occurrence of PTORK in VACs of the rays in mature wood points to a release of K⁺ into vessels from where it can be recycled within the branches. Addition-
ally, K\(^+\) release from VACs into vessels might be necessary for charge balance during uptake of metabolites from the vessels [Van Bel 1990]. It is well known that the rays provide a translocation pathway for nutrients from the phloem via the cambium to the xylem (Fromm 1997). After arriving in the VACs, the sugars can be translocated via contact pits into the vessels, while K\(^+\) can be shifted via PTORK into the vessels to be remobilized within the shoot.

In addition to PTORK, the plasma membrane H\(^+\)-ATPase could be localized in the cambial zone as well as in developing xylem cells and in VACs of the mature xylem (Arend et al. 2002, 2004). In coincidence with the localization pattern of PTORK, the activity of the H\(^+\)-ATPase was restricted to the active period of wood formation. Interestingly, when auxin was applied to dormant plants, the formation of the proton pump was induced (Arend et al. 2002), indicating that auxin stimulates H\(^+\) excretion. Since endogenous auxin generally occurs in high concentrations in the active cambium (Sundberg et al. 2000), an upregulation of H\(^+\)-ATPase by auxin seems to be an important process during cambial reactivation in spring. The pump generates the required proton-motive force for the uptake of potassium and nutrients into developing wood cells and VACs of the rays. In the latter, an increased abundance of H\(^+\)-ATPase as well as an enhanced efflux of H\(^+\), measured via H\(^+\)-selective microelectrodes, occurred under conditions of low K\(^+\) supply (Arend et al. 2004). These results indicate an upregulation of the plasma membrane H\(^+\)-ATPase in VACs under K\(^+\) deficiency and point to its essential role in the uptake of K\(^+\) from the xylem stream.

**Figure 2.** Effect of Ca\(^{2+}\) deficiency on xylem structure and cambial width. Light microscopy of stem cross-section of poplar (*Populus tremula × Populus tremuloides*) clones grown under reduced Ca\(^{2+}\) supply (0.1 mM, A) in comparison with full-strength Ca\(^{2+}\) supply (5 mM, B). Under Ca\(^{2+}\)-limiting conditions, both vessel (V) size and wood increment decreased. TEM analysis revealed the cambial cells (C) to be filled with a dense cytoplasm and the cambial width decreased under Ca\(^{2+}\) deficiency (C). In contrast, under full-strength Ca\(^{2+}\) supply, the cambial zone appears to be much wider and the cambial cells show large vacuoles (D). P, phloem. Courtesy of Dr Silke Lautner. This figure appears in color in the online version of *Tree Physiology*.
Yet the levels of glucose, fructose and sucrose increased in leaves but a reduction in the bark (Lautner et al. 2007). HPLC analysis showed a rise in sugar concentrations in poplar (Arend and Fromm 1993). In correlation with these findings, TEM analysis revealed a decrease in calcium content in poplar shoots (Figure 1). An increase in Ca$^{2+}$ accumulation in the bark was also found in Norway spruce seedlings when they were treated with elevated calcium concentrations in nutrient solutions (Österas and Greger 2006). Moreover, when the cambium resumes cell division and expansion in spring, calcium bridges of acidic pectins in the middle lamella have to be degraded (Funada and Catesson 1991) and calcium appears to be available in its elemental state. In the course of subsequent lignification in the developing xylem, Wimmer and Lucas (1997) confirmed this suggestion by showing that low calcium content led to lower lignin proportion in spruce wood, leading to changes in wood hardness and elasticity. Thus, a close relationship between calcium content, lignin concentration and mechanical properties of wood appears to be obvious (Wimmer et al. 1997). Important key enzymes during lignification are apoplastic peroxidases, converting the hydrophilic gel of the primary wall into lignin. Since peroxidases from horseradish and zucchini are known to bind pectin in their calcium-in-duced structure (Penel and Greppin 1996), these enzymes could also play an essential role in the lignification process of the developing wood. Direct evidence for the involvement of calcium in lignification was provided by FTIR spectroscopy on poplar wood. Plants grown under calcium starvation showed a reduction in carbonyl as well as methoxyl groups from S-lignin (Lautner et al. 2007), indicating a general drop in lignin concentration.

On the level of a whole forest, McNulty et al. (1991) observed reduced lignin in needles in red spruce to be significantly related to lower calcium and magnesium concentrations in the foliage and on the forest floor under conditions of increased nitrogen deposition. The calcium-de-
K⁺-and Ca²⁺-Dependent XYLOGENESIS

1145

To summarize current knowledge on the role of potassium in wood formation, it was shown in various tree species that K⁺ represents one mandatory factor. Figure 3 (left) shows the sequence of events leading to wood formation in trees under non-limiting K⁺ nutrition. The latter caused a high cambial K⁺ level as well as a high cambial osmotic potential. As a consequence, cell expansion, cambial width and vessel size in the developing xylem increased. Moreover, K⁺ channels and transporters relevant for K⁺ transport and homeostasis have been identified in recent years, and their activity was found to correlate with the wood formation process. Special emphasis was given to VACs of the rays which play an important role in K⁺ recycling within the stem. In these cells, the K⁺ channel PTORK facilitates K⁺ efflux into the vessels while a plasma membrane H⁺-ATPase provides the driving force for K⁺ uptake from the vessels.

Apart from K⁺, various studies have demonstrated that calcium has a significant effect on wood production of trees. Non-limiting Ca²⁺ nutrition caused a strong increase of the cambial calcium level during cambial reactivation in spring. This increase promotes processes such as cell division in the cambial zone (Figure 3, right). Also, cell wall chemistry is affected by calcium because cross-linking of carboxyl groups within the pectin layer and lignification depend on calcium supply and indicate an impact of calcium on both wood structure and chemistry. To obtain a deeper understanding of the functions of potassium and calcium in the tree stem, future studies will focus on

- calcium-mediated enzymes and molecular as well as electrophysiological analysis of calcium channels in the developing xylem. Since knowledge regarding the molecular involvement of calcium in wood formation is lacking so far, this goal will be our first priority in future;
- the coordination between radial and elongation growth with respect to K⁺ and Ca²⁺ transport processes;
- the study of poplar mutants with overexpressed and repressed K⁺/Ca²⁺ channels;
- the impact of K⁺ and Ca²⁺ concentration on water transport in vessels;
- the development of new methods such as microarray analysis of single wood cells.

Conclusions and perspectives

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