Tree Physiology 30, 1140–1147
doi:10.1093/treephys/tpq024

INVITED REVIEW: PART OF AN INVITED ISSUE ON TREE NUTRITION

Wood formation of trees in relation to potassium and calcium nutrition

JÖRG FROMM
Institute for Wood Biology, University of Hamburg, Hamburg, Germany
Corresponding author (j.fromm@holz.uni-hamburg.de)

Received January 1, 2010; accepted February 26, 2010; published online May 2, 2010

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 xylogenesis. 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: EDXA, 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 significantly reduced cell expansion, stomatal movements and enzyme activation in respiration 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⁺ fertilizer 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-

![Figure 1. Distribution of potassium and calcium in actively growing poplar shoots under optimal nutrient supply. Representative EDXA line-scans of relative K⁺ as well as Ca²⁺ concentration reveal the highest K⁺ concentrations in the developing xylem while the highest Ca²⁺ concentrations were found in the phloem (cps, counts per second; F, fibre; V, vessel). Courtesy of Dr Silke Lautner.](https://treephys.oxfordjournals.org/content/30/9/1140)
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 tetraethylammonium (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 kinetics 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, Ehhrhardt 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.
Figure 3. Summarizing scheme of the effects of K⁺ and Ca²⁺ nutrition on physiological and structural processes in the cambium and the developing xylem. This figure appears in color in the online version of Tree Physiology.

Calcium-dependent wood formation

Similarly to potassium, calcium is also essential for basic processes of wood formation. When poplars (P. tremula L. × P. tremuloides Mix.) were grown hydroponically under calcium deficiency, a reduction in wood increment, vessel size and fibre length was observed by microscopic analysis (Lautner et al. 2007). Figure 2 shows characteristic differences in vessel size between plants grown under calcium deficiency (A) and plants grown under non-limiting Ca²⁺ supply (B). In coincidence with these structural changes, EDX analysis revealed a decrease in calcium content in the phloem, the cambium and the developing xylem. By using TEM, a narrow cambial zone of only three to five cells in radial direction and a dense cambial cytoplasm with numerous small vacuoles were also observed in calcium-deficient trees (Lautner et al. 2007, Figure 2C), similar to the ultrastructure of dormant cambial cells (Arend and Fromm 2003). Based on the fact that cell expansion in the developing xylem depends on large vacuoles and high osmotic potentials (Wind et al. 2003), the smaller vessel size might be caused by decreased vacuolation. With respect to phloem physiology, a lower phloem loading rate in the leaves and a reduction in phloem unloading in the stem were measured in poplars grown under calcium deficiency (Schulte-Baukloh and Fromm 1993). In correlation with these findings, HPLC analysis showed a rise in sugar concentrations in leaves but a reduction in the bark (Lautner et al. 2007). Yet the levels of glucose, fructose and sucrose increased in the developing xylem under calcium deficiency, indicating that the reduction of carbon in the bark cannot be the cause of a reduced wood increment. Thus, the results point to a direct effect of calcium on xylogenesis.

With the rhythm of the seasons, cambial cells undergo structural and functional changes in temperate areas. In early spring, these cells resume their metabolic activity (Sibon et al. 1993) and a dramatic increase in amino acid concentration was measured in the xylem sap of willow (Fagus sylvatica (L.)) (Sauter 1981) and in the calcium level of beech (Follet-Gueye et al. 1998) as well as poplar (Arend and Fromm 2000) at the same period. Calcium was also observed to gradually rise in the apical meristem during swelling and bud break in spring, indicating that it may be important in boosting cell division (Lautner and Fromm 2010). The temporary increase in calcium in the cambium and apical meristem might be involved in the enzymatic hydrolysis of starch and proteins because calcium is known to activate enzymes such as ATPases, amylases and lipases (Bangerth 1979). In this context, the question concerning the origin of the cambial calcium comes up. Most likely, it could have been mobilized from oxalate crystals of the bark (Trockenbrodt 1995) or the phloem, because EDX analysis has shown that phloem cell walls serve as a calcium reservoir in poplar shoots (Figure 1). An increase in Ca²⁺ 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, Westermark (1982) suggested that calcium is involved in lignin polymerization within the cell wall. 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-induced 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-
pended lignin decrease is more likely to appear in the crown because of increased distances from the roots and higher competition for calcium within the transpiration stream. Also, Wessmann et al. (1989) found foliar lignin content reduced in the upper canopies of oak–maple and pine forests in the USA. Recently, Smith et al. (2009) compared the effects of Ca\(^{2+}\) fertilization treatment on calcium concentration in wood and calcium oxalate concentration in foliage of red spruce at two locations with different initial Ca\(^{2+}\) levels in the soil. These authors found greater amounts of calcium in wood from the high calcium location than from the low calcium location. These results provide evidence for interrelated processes between soil and canopy chemistry and confirmed that calcium cycling plays a key role in the health and productivity of red spruce forests in the northeastern USA. In addition, calcium fertilization also had a significant effect on net photosynthesis rate in secondary forest vegetation regrowing on abandoned pastures in central Amazonia, Brazil (Moura da Silva et al. 2008). In contrast to phosphorus alone, fertilized trees growing on phosphorus- and calcium-fertilized plots increased photosynthesis, indicating that calcium is an important limiting nutrient in post-pasture secondary succession.

Conclusions and perspectives

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 Ca\(^{2+}\) 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 priority in future;
- the coordination between radial and elongation growth with respect to K\(^{+}\) and Ca\(^{2+}\) transport processes;
- the study of poplar mutants with overexpressed and repressed K\(^{+}\)/Ca\(^{2+}\) channels;
- the impact of K\(^{+}\) and Ca\(^{2+}\) concentration on water transport in vessels;
- the development of new methods such as microarray analysis of single wood cells.

References


