Alterations in ultrastructure and subcellular localization of Ca\(^{2+}\) in poplar apical bud cells during the induction of dormancy

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Abstract

In poplar (Populus deltoides Bartr. ex Marsh), bud dormancy and freezing tolerance were concomitantly induced by short-day (SD) photoperiods. Ultrastructural changes and the alteration in subcellular localization of calcium in apical bud cells associated with dormancy development were investigated. During the development of dormancy, the thickness of cell walls increased significantly, the number of starch granules increased, and there was a significant accumulation of storage proteins in the vacuoles of the apical bud cells. The most striking change was the constriction and blockage of the plasmodesmata.

It was demonstrated that antimonate precipitation is a reliable technique for studying subcellular localization of calcium in poplar apical bud cells. Under the long day (LD) photoperiod, electron-dense calcium antimonate precipitates were mainly localized in vacuoles, intercellular spaces and plastids. Some antimonate precipitates were also found in the cell walls and at the entrance of the plasmodesmata. However, there were few Ca\(^{2+}\) deposits found in the cytosol and nucleus. After 20 d of SD exposure, when development of bud dormancy was initiated, calcium deposits in intercellular spaces were decreased, whereas some deposits were found in the cytosol and nuclei. From 28–49 d of SD exposure, while dormancy was developing, a large number of Ca\(^{2+}\) precipitates were found in the cytosol and nucleus. When deep dormancy was reached after 77 d of SD exposure, Ca\(^{2+}\) deposits became fewer in both cytosol and nuclei, whereas numerous deposits were again observed in the cell walls and in the intercellular spaces. These results suggest that under the influence of SD photoperiods, there are alterations in subcellular Ca\(^{2+}\) localization, and changes in ultrastructure of apical bud cells during the development of dormancy. The constriction and blockage of plasmodesmata may cause the cessation of symplastic transport, limit cellular communication and signal transduction between adjacent cells, which in turn may lead to events associated with growth cessation and dormancy development in buds.

Key words: Poplar, apical bud cells, Ca\(^{2+}\) subcellular localization, dormancy.

Introduction

The development of vegetative dormancy is an important adaptive strategy for the survival and growth of temperate perennial plants. It is generally believed that dormant plants are better able to withstand low, potentially damaging temperatures during winter. For most temperate perennial plants, the annual growth cycle involves an alternation between periods of active shoot growth and dormancy (Nooden and Weber, 1978). Cessation of shoot growth, development of cold hardiness and initiation of dormancy in most perennial plants are either accelerated or initiated by short days (SD) and inhibited or delayed by long days (LD) (Vince-Prue, 1975).

As in most perennial plants, vegetative dormancy in
poplar is initiated or accelerated by SD photoperiod (Nitsch, 1957; Pauley and Perry, 1954). Dormancy development in poplar also varies in an ecotypic manner. Generally speaking, cessation of growth and development of dormancy of plants from northern latitudes are initiated at longer day lengths than plants from southern latitudes (Pauley and Perry, 1954; Howe et al., 1995). There are distinct morphological and anatomical changes during the development of bud dormancy. The morphology and development of terminal and axillary buds subjected to a variety of photoperiod treatments were studied by Richards and Larson (1981) and Goffinet and Larson (1982). However, ultrastructural changes in buds in relation to dormancy induction were not examined.

Metabolic changes associated with the seasonal cycle of dormancy have been studied extensively in poplar. Changes in carbohydrates (Fege and Brown, 1984; Nelson and Dickson, 1981), amino acids (Cote et al., 1989; Sagisaka, 1974a), and enzyme activities (Sagisaka, 1974b) all demonstrated that metabolic changes are closely associated with the annual growth cycle of poplar. In addition, a 32 kDa poplar bark storage protein was accumulated during dormancy induction by short days (Coleman et al., 1991); and a full-length cDNA and a gene encoding for poplar 32 kDa bark storage protein have been isolated and sequenced (Coleman et al., 1992; Coleman and Chen, 1993). Although physiological changes that occur throughout the dormancy cycle have been well-characterized, little is known about the process of SD signal transduction and ultrastructural changes associated with the development of bud dormancy in poplar plants.

It is well-known that calcium plays an important role in plant growth and development. The alteration in cellular Ca\(^{2+}\) level regulates an astonishing variety of cellular processes from control of ion transport to gene expression (Hepler and Wayne, 1985; Bush, 1995). It is also known that environmental stimuli, such as temperature and light, etc., can alter cellular Ca\(^{2+}\) distribution (Hepler and Wayne, 1985; Bush, 1995). It was investigated whether there is a relationship between the alteration in cellular Ca\(^{2+}\) localization and ultrastructural changes and the development of dormancy in apical bud cells of poplar and the results suggest that SD-induced alteration in subcellular Ca\(^{2+}\) localization, and ultrastructural changes in plasmodesmata and cell walls are closely associated with the development of dormancy in poplar buds.

Materials and methods

- **Plant material**

Poplar plants (*Populus deltoides* Bartr. ex Marsh.) were established from greenwood stem cuttings that were rooted and grown in the greenhouse in individual 1.5 l pots containing a mix of 2 peat moss: 1 soil: 1 prolite (by vol.) under a photoperiod of 16 h light and 8 h darkness, and temperatures of 25/21 °C day/night. When the plants grew to about 40–50 cm tall, they were transferred into a SD growth chamber with photoperiod of 8 h light and 16 h darkness and temperatures of 25/21 °C day/night for up to 77 d. Plants which had been grown for different time periods in SD were used as experimental materials.

**Measurement of dormancy status**

At each sampling date (0, 10, 20, 28, 35, 49, and 77 d of SD exposure), one set of six poplar plants were transferred from SD growth chamber to LD greenhouse conditions at 25/21 °C day/night to determine dormancy status. Plants were manually defoliated and the shoot tips (about 2.5 cm) were removed. Dormancy status was expressed as the number of days to first bud break (the first visible shoot growth) in the decapitated and defoliated plants.

**Measurements of freezing tolerance**

The shoots were cut into short stem segments, each with two nodes. Five stem segments were placed in each of the test tubes, and there were three replicates (tubes) per sample. Tubes with samples were placed vertically in a low temperature liquid bath and then cooled down at a rate of 4–5 °C h\(^{-1}\). When the sample temperature reached −2 °C, ice pieces were added to the tubes to inoculate the stem segments to prevent supercooling. Samples were then lowered to each of −6, −8, −10, −12, −14, −16, and −18 °C for 3 h. The tubes were removed from the liquid bath at each temperature interval and the stem segments were removed from the tube and immediately placed on moist filter papers in Petri dishes which were held in an ice-filled box. After slowly thawing overnight in a cold room (2–4 °C), stem segments were incubated in a growth chamber at 25/21 °C day/night temperature and 14 h photoperiod. After 5 d, viability of the bark of the stems and buds was examined by visual observation of tissue browning. When bark and bud tissues became brown, death was recorded. Temperatures which caused 50% death of the total samples were referred to as killing temperature (LT\(_{50}\)).

**Sample preparation for cytochemical localization of Ca\(^{2+}\) and ultrastructural observations**

Cytocchemical localization of calcium was performed following the method of Slocum and Roux (1982) and adapted by Wang and Jian (1994). The samples were collected from the plants after either 10, 20, 28, 35, 49, and 77 d of SD exposure or LD plants (0 d in SD). To minimize the possible effects of circadian rhythm on intracellular Ca\(^{2+}\) fluctuation induced by environmental stimulus, such as photoperiod, all sample collections were conducted at 11.00 a.m., about 3 h after the beginning of light illumination. The apical buds were excised, cut into 0.5 x 0.5 x 0.5 mm slices, and immediately immersed in a fixative solution containing 4% glutaraldehyde and 2% potassium antimonate (K\(_2\)H\(_2\)Sb\(_2\)O\(_7\)). 0.4H\(_2\)O) in 0.1 M potassium phosphate buffer (pH 7.6) for 4 h at 4 °C. After fixation, the samples were washed three times, 20 min each, with 0.1 M potassium phosphate buffer (pH 7.6) containing 2% potassium antimonate, and then post-fixed in 1% osmium tetroxide (OsO\(_4\)) and 2% potassium antimonate in 0.1 M potassium phosphate buffer (pH 7.6) at 4 °C overnight. After post-fixation, the samples were washed twice in phosphate buffer containing 2% potassium antimonate, and followed by twice with pH 10 distilled water adjusted with KOH. Thereafter, the samples were dehydrated in an ethanol series, and embedded in EMBed 812 (EMS, New Jersey, USA). The embedded samples were then sectioned with...
glass knives and a RMC (Tucson, Arizona, USA) MT4000 ultramicrotome. The sections were stained with uranyl acetate, and then observed and photographed under a Philips (Mahwah, New Jersey, USA) CM12 TEM operated at 60 kV. At the same time, half of the samples were fixed in a fixative solution containing no antimonate. These tissue sections were used as the control for cytochemical localization of Ca$^{2+}$, as well as used for the observation of ultrastructural changes. In order to verify the location of calcium, additional treatment involving the chelation of calcium ion with EGTA was performed. Grids mounted with tissue sections previously examined by TEM were immersed in the solution of 100 mM EGTA (pH 8.0), and incubated at 60°C for 1 h. After treatment, the grids were rinsed briefly in distilled water and stained with uranyl acetate again, and then observed under EM.

**Results**

**Changes in dormancy status and freezing tolerance**

Under 8 h SD photoperiod and warm temperature, the growth rate of poplar plants decreased immediately upon transfer to SD from LD, and completely stopped by 20 d of SD treatment (data not shown). However, there was neither significant change in dormancy status, expressed as the days to bud break (Fig. 1), nor in freezing tolerance (Fig. 2) for plants exposed to SD for up to 20 d. There was a gradual increase in the degree of dormancy as evidenced by the delayed bud break which increased from 14–29 d after 28 d and 49 d of SD exposure, respectively. After 77 d of SD exposure, it took more than 3 months before any sign of regrowth became visible, indicating that a high degree of dormancy had been reached. The degree of dormancy of the terminal buds was similar to that of lateral buds of the same plants (Jian et al., unpublished results). During the SD treatment, there was also a gradual increase in freezing tolerance in both the stem and buds (Fig. 2). In plants that had been maintained under LD and warm conditions, the killing temperature was about −6°C and was constant throughout the experimental period of 77 d. Under SD, plants developed a higher degree of freezing tolerance when exposed to SD for more than 28 d. Plants given 77 d of SD exposure were able to tolerate about 12°C lower temperature than LD plants. Thus, the SD photoperiod clearly induced development of dormancy and cold hardiness simultaneously and in a similar manner to that observed for other temperate perennial plants (Weiser, 1970).

**Ultrastructural changes under SD conditions**

Plate 1A–C shows the ultrastructural features of the meristematic cells of poplar apical buds from plants which were grown under LD photoperiod. These cells contained relatively large nuclei which were localized in the centre of the cells, and which were characterized by their dense cytoplasmic and thin walls. Many mitochondria, plastids, endoplasmic reticuli, and ribosomes could be identified in the cytoplasm, and many plasmodesmata traversed the walls of neighbouring cells. After 77 d of SD exposure, many starch grains accumulated in the apical bud cells; the heterochromatin in the nuclei of these cells decreased or disappeared; the walls of these cells were markedly thickened; and the plasmodesmata in the cell walls were not visible (Plate 1D). This is in contrast to that observed in the samples collected from LD plants, in which many plasmodesmata were observed in the walls between two neighbouring cells (arrows, Plate 1A, B).

Plate 2A–D shows the high magnification view of plasmodesmata electron micrographs. Plate 2A is the cells of apical buds grown in LD; many plasmodesmata in the
Plate 1. Ultrastructural comparison of apical bud cells between LD and 77 d SD-grown plants. (A–C) The meristematic cells of apical buds in plants grown under LD condition. Note the relatively large-sized nucleus (N), dense cytoplasm, many mitochondria (M), plastids (P), endoplasmic reticulum (ER), small vacuoles (V) and thin cell wall (W); and many plasmodesmata (PD) traverse the cell walls of neighbouring cells (arrows) (A) ×4500; (B) ×7000; (C) ×18000. (D) After 77 d of SD exposure, the heterochromatin in nucleus decreased or disappeared; the cell walls were thickened markedly; the plasmodesmata are not visible in the cell walls; ×6000. S: starch granule. Bar: 1 μm.

walls of the neighbouring cells could be observed and their pores were generally larger in diameter. Plate 2B shows the cells from plants which had been exposed to SD photoperiod for 35 d. The walls of these cells were thickened and the pore diameter of plasmodesmata was reduced. After 77 d of SD exposure when the apical buds had reached a high degree of dormancy (Plate 2C, D), the plasmodesmata of these cells appeared to undergo a further alteration, i.e. the plasmodesmata were no longer distinguishable from the cell walls. Both ends of the plasmodesmata seemed to be blocked and the plasma membranes at the entrance of the plasmodesmata appeared to be fused with each other and formed a continuous membrane which was parallel with the cell wall. A number of electron-dense grains, which may be the remains of the original plasmodesmata, could still be identified at the plasmalemma (arrows, Plate 2C, D).

Plate 3A–D shows the ultrastructure of bud cortical cells from plants of 0, 28, 35, and 77 d of SD exposure, respectively, at the same magnification (×4500). It was clearly observed that during the induction of dormancy the cell walls were thickened; the precipitates in vacuoles increased; the central vacuoles in the cells from plants exposed to SD for 77 d were segmented to form many...
Plate 2. Plasmodesmata in apical bud cells of poplar plants which had been exposed to SD for various periods of time. (A) Cells of LD-grown plants. After 35 d (B) and 77 d of SD exposure (C, D). (A) × 9000; (B) × 5000; (C) × 24000; (D) × 24000. PM: plasmamembrane. Bar: 1 μm.

smaller vacuoles; and finally the number of starch granules in the cells was markedly increased. The plasmodesmata in the samples collected from plants that had been exposed to SD for 28 d were constricted (arrows, Plate 3B). In the cells of the samples collected from plants after 35 d of SD exposure, only a few small plasmodesmata could be observed in the walls of neighbouring cells (arrows, Plate 3C). After 77 d of SD exposure (Plate 3D), almost no distinct plasmodesmata could be found in these cell walls, the plasma membrane lost its intercellular connection, and thus the intercellular transport and communication of symplast might be discontinued.

These results indicate that during the development of dormancy induced by short days, there were a number of ultrastructural changes in both apical meristematic cells and differentiated cells directly under the apical meristematic cells. The changes could be summarized as follows: (1) the heterochromatin in nuclei decreased or disappeared; (2) starch granules increased; (3) a large number of precipitates, possibly storage proteins (Coleman et al., 1991; Wetzel et al., 1989), were accumulated in vacuoles; (4) the cell walls were thickened; and (5) the plasmodesmata were constricted, and their pores were blocked.

Localization of Ca$^{2+}$ in poplar apical bud cells grown in LD

For apical bud sections fixed with potassium antimonate, the calcium antimonate precipitates, which are indicative of calcium localization, appeared as electron-dense particles when observed under transmission electron microscope. The electron-dense calcium precipitates in the apical bud cells of LD plants were mainly localized in vacuoles (Plate 4A, B), implying that the vacuole is a main pool of Ca$^{2+}$ in cells of actively growing plants. There were many large electron-dense grains in intercellular spaces and plastids (Plate 4A, B). In addition, some antimonate precipitates were observed in the cell walls and at both ends of plasmodesmata (Plate 4A, B). On the other hand, few visible Ca$^{2+}$ deposits were observed in cytosol and nucleus (Plate 4A, B).

To demonstrate that the electron-dense grains were
indeed calcium deposits and not osmiophilic globules (lipid body), the tissue sections fixed in solutions with or without potassium antimonate were compared, and between sections which were fixed in solutions containing potassium antimonate but then treated with EGTA, a treatment that is known to remove calcium. The cells fixed without potassium antimonate did not show any electron-dense precipitates in plastids, intercellular spaces and vacuoles (Plate 4C). Furthermore, in the tissue sections that were treated with EGTA, there were small transparent holes in plastids, intercellular spaces and vacuoles, corresponding to where the electron-dense grains were localized before EGTA treatment (Plate 4D). Taken together, these results suggested that the location of electron-dense precipitates is a reliable indicator of cellular calcium localization.

**Plate 3.** Ultrastructural changes in the differentiated cortical cells of poplar apical buds during the induction of dormancy by short days. Ultrastructures of cells from LD (A), and from 28 d (B), 35 d (C), and 77 d (D) of SD-grown plants. Note the changes in plasmodesmata (arrows). ×4500. Bar: 1 μm.

**Changes of calcium distribution in poplar apical buds during dormancy induction by short day**

After 10 d of exposure to SD, the distribution of calcium in apical buds appeared to be similar to that of LD photoperiod, and no significant change was observed. Again, a large number of calcium antimonate precipitates were observed in intercellular spaces, vacuoles and plastids; and there were few visible Ca$^{2+}$ deposits in cytosol (Plate 5A).

After plants were subjected to SD photoperiod for 20 d, some marked changes occurred: the antimonate precipitates in intercellular spaces were decreased markedly, some calcium deposits were found to be localized in cell walls and on the external side of plasma membrane, and a few antimonate precipitates appeared in cytosol and nucleus (arrows, Plate 5B).
Plate 4. Subcellular localization of Ca\(^{2+}\) antimonate deposits in the apical bud cells of poplar grown under 16 h LD photoperiod. (A, B) The electron-dense calcium antimonate deposits (indicated by arrows) were mainly localized in vacuoles (V), intercellular spaces (IS) and plastids (P). Some were also observed at the entrances of the plasmodesmata (arrows) and in the cell wall (W). Few Ca\(^{2+}\) deposits were found in cytosol and nucleus. (A) \(\times 8500\); (B) \(\times 9000\). (C) Samples were fixed without potassium antimonate, \(\times 5000\). (D) Tissue sections were treated with EGTA. As indicated by the arrows, there were transparent holes in plastids, intercellular spaces and vacuoles, where the electron-dense grains were located before EGTA treatment, \(\times 5000\). Bar 1 \(\mu\)m.

After 28 d of exposure to SD, few Ca\(^{2+}\) deposits were observed in intercellular spaces. On the contrary, many antimonate precipitates distributed between the cell walls and the plasmalemma (arrows, Plate 5C), and many small-sized calcium deposits showed up in cytosol and nucleus. In addition, there were still some calcium antimonate precipitates observed in vacuoles and plastids (Plate 5C, D).

After plants were exposed to SD for 35 and 49 d, a large number of calcium deposits were localized between cell wall and plasmalemma. The number of Ca\(^{2+}\) deposits in cytosol and nucleus significantly increased. There were also many antimonate precipitates in plastids, mitochondria and small vacuoles (arrows, Plate 6A–D). In addition, Ca\(^{2+}\) deposits were detected in some intercellular spaces of the cells in plants which had been exposed to SD photoperiod for 49 d (Plate 6D).

After 77 d of SD exposure, the distribution of Ca\(^{2+}\) in apical buds changed again. Calcium deposits in cytosol and nucleus appeared to be decreased (Plate 7A, B). In some cells, although many Ca\(^{2+}\) deposits were still visible in cytosol, their distribution seemed to be concentrated in some regions but not uniformly distributed (arrows, Plate 7A, B). The calcium deposits distributed between the cell wall and the plasma membrane were also reduced. It is worth noting that a large number of Ca\(^{2+}\) deposits occurred again in intercellular spaces and in the cell walls (Plate 7A–D). The deposits were not observed in the
Plate 5. Subcellular localization of Ca$^{2+}$ antimonate deposits in apical bud cells of poplar under an 8 h SD photoperiod. (A) After 10 d of SD exposure, the pattern of Ca$^{2+}$ deposit distribution was similar to that of LD (see Plate 4A, B), $\times 11000$. (B) After 20 d of SD exposure, changes in Ca$^{2+}$ deposit localization occurred: deposits were markedly reduced in intercellular spaces; some calcium deposits were located on the outer face of plasma membrane (arrows); and some deposits were observed in cytosol and nuclei (arrows), $\times 13000$. (C, D) After 28 d of SD exposure, essentially no deposits were found in intercellular spaces, but a large number of antimonate precipitates distributed between the cell wall and the plasmalemma (arrowheads); many small calcium deposits were found in cytosol and nucleus; and there were still calcium antimonate precipitates in vacuoles and plastids. (C) $\times 11000$; (D) $\times 8500$. Bar: 1 $\mu$m.

Discussion

Changes in cellular calcium distribution during the induction of dormancy

There are several histo- and cytochemical methods that have been used to determine calcium localization in plant cells. Two of the most commonly used methods are X-ray microanalysis and the chlorotetracycline technique.
Plate 6. Subcellular localization of Ca$^{2+}$ antimonate deposits in the apical bud cells of poplar plants grown in SD condition for either 35 d (A, B) or 49 d (C, D). A large number of electron-dense calcium precipitates were widely distributed among cell wall, plasmalemma, plastids, cytosol and nucleus. Ca$^{2+}$ deposits were also found in some intercellular spaces of the cells exposed to SD photoperiod for 49 d (D). (A) x 4800; (B) x 10000; (C) x 8000; (D) x 7000. Bar: 1 $\mu$m.

Recently, fluorescent dyes (e.g. Quin 2, Fura-2, Fluo-3, Indo-1) have also been widely used to measure the changes in cytoplasmic Ca$^{2+}$ (Williams et al., 1990; Gilroy et al., 1991; McAinsh et al., 1992; Subbaiah et al., 1994). However, all of these methods have some limitations. For example, X-ray microanalysis can only show the average calcium concentration in a particular area, whereas the chlorotetracycline method can only reveal so-called membrane-associated Ca$^{2+}$ (Tretyn and Kopcewicz, 1988). When using fluorescent probes (dyes) to quantify the level of cytoplasmic free Ca$^{2+}$, it is not possible to determine the ultrastructural localization of the calcium ion, even by means of laser-scanning confocal microscopy. In the present study, the antimonate precipitation cytochemical technique was used. This procedure has been widely used for localization of Ca$^{2+}$ in animal cells (Weakley, 1979; Reith and Boyd, 1985). Wick and Hepler (1982), as well as Slocum and Roux (1982) have demonstrated the usefulness of this technique in localizing Ca$^{2+}$ in plant cells. Since then, many researchers have successfully applied this technique to determine the cellular localization of Ca$^{2+}$ in plant cells (Dauwalder et al., 1985; Lazzaro and Thomson, 1992; Tretyn et al., 1992; Wang and Jian, 1994; Hilaire et al., 1995). As mentioned above, the experimental results allowed the visualization of the dynamic changes
of Ca\textsuperscript{2+} localization in the cells of poplar apical buds during the development of dormancy induced by SD photoperiod.

Changes in subcellular Ca\textsuperscript{2+} distribution in bud cells seemed to be closely associated with the development of dormancy in buds under the growth chamber conditions of warm temperature and SD photoperiod. The dormancy status, expressed as days to bud break (Fig. 1), indicates that there was a low level of dormancy (<10 d to bud break) in buds from plants which had been exposed to SD for up to 20 d. After 28 d of SD exposure, buds started to develop a higher level of dormancy (>10 d to bud break). From 28–49 d of SD exposure, the degree of dormancy further increased. By 77 d of SD exposure, a high degree of dormancy was detected (>90 d to bud break). With the development of dormancy, there was also an increase in freezing tolerance in both buds and stem bark under SD photoperiod. The development of
freezing tolerance started after 28 d of exposure to SD (Fig. 2). By the end of 77 days of SD treatment, freezing tolerance in buds and stem bark was increased to $-18^\circ$C from $-6^\circ$C, a level of freezing tolerance observed in LD poplar.

Changes in calcium distribution in bud cells had already occurred prior to the initiation of dormancy, i.e. up to 20 d of SD exposure calcium deposits were decreased in intercellular spaces and increased in cytosol and nucleus, respectively. From 28–49 d of SD exposure, when the dormancy and freezing tolerance were developing, more calcium deposits accumulated in the cytosol and nuclei. When a high degree of dormancy was reached after 77 d of SD exposure, the levels of Ca$^{2+}$ in cytosol and nuclei were decreased, while accumulation of calcium in intercellular spaces and in cell walls was again observed. These results suggest that there is a dynamic change in Ca$^{2+}$ distribution in apical bud cells during the development of dormancy. It is not known whether the antimonate precipitates derived from free Ca$^{2+}$, bound Ca$^{2+}$ or both. Neither is it known what roles calcium may have in dormancy development, but the changes in subcellular calcium distribution, as detected by the antimonate cytochemical technique, do suggest the involvement of calcium in the initiation and development of bud dormancy induced by SD.

**Role of plasmodesmata in dormancy development**

In plant cells, functional plasmodesmata form a continuous network of connected protoplasm, termed symplast. The transport of organic materials and the transduction of signals between cells in the symplast depend upon the frequency and conductance of plasmodesmata. Therefore, plasmodesmata play an important role in plant growth and development, as well as in response and adaptation to environmental factors (Robards and Lucas, 1990; Lucas et al., 1993). The plasmodesmal channel is a dynamic structure, and its frequency can be variable (Botha, 1992; Lucas et al., 1993). When viral particles move through plasmodesmata or when plants are under anaerobic or osmotic stresses, the diameter of plasmodesmal pores become widened (Wolf and Lucas, 1994; Cleland et al., 1994; Schulz, 1995). Wille and Lucas (1984) reported that plasmodesmata existed in the cell walls between guard mother cell and neighbouring epidermal cells, as well as in the cell walls between developing young guard cells and neighbouring epidermal cells. During the development of the mature guard cell walls, however, the plasmodesmata were sealed. Thus, within the mature stomatal complex, there were no plasmodesmata connecting guard cells to sister guard cells or to adjacent epidermal cells. A similar case was also observed during the development of the embryo sac in angiosperms (Hawker et al., 1991). In overwintering wheat seedlings, the plasmodesmata between cells were blocked by glycoproteins or disconnected due to the contraction of protoplast (Jian and Sun, 1992). Such a change in plasmodesmata was considered to be an important mechanism for plant survival in a cold winter by preventing a rapid de-acclimation when the ambient temperature rose rapidly (Jian and Sun, 1992). In the present study, it was observed that the changes in plasmodesmata were closely related to the development of dormancy under SD photoperiod. During SD exposure and the development of dormancy, the frequency of plasmodesmata in the cell walls between neighbouring cells in apical buds decreased and the diameter of pores reduced. This was especially obvious in plants which had developed a high level of dormancy after 77 d of SD exposure. The number of plasmodesmata observed in the cell walls was reduced and those visible appeared to be disconnected, so that the symplastic connection between cells seemed to be discontinued. It is possible that these dramatic changes in plasmodesmata result in reduction or blockage of the intercellular transport of materials and of intercellular communication. Whether this has any connection to the lowered metabolic activity in the cells of dormant buds remains to be proven. Further studies are needed to explore the role of symplastic connections in the initiation of growth cessation and development of dormancy in poplar.

**Subcellular calcium localization may be involved in the regulation of the dynamic changes in plasmodesmata and cell walls**

The roles of Ca$^{2+}$ in the regulation of growth and development of plants have been studied extensively (Hepler and Wayne, 1985; Bush, 1995). Erwee and Goodwin (1983) proposed that cytoplasmic Ca$^{2+}$ concentration may directly regulate the permeability of plasmodesmata in the plant symplast. Micro-injection of buffered-carboxyfluorescein containing IP$_3$ or with Ca$^{2+}$-BAPTA into cells, revealed that elevated cytoplasmic Ca$^{2+}$ content inhibits cell-to-cell diffusion of carboxyfluorescent dye via plasmodesmata in staminal hairs of Setcresea purpurea, suggesting that calcium may regulate the opening and closing of plasmodesmal pores by modifying the conformation of the structural proteins surrounding the plasmodesmal pores (Tucker, 1988, 1990). Shepherd and Goodwin (1992) studied cell-to-cell communication in vegetative lateral branches of Chara corallina during winter and spring. Their experimental results suggested that in winter Chara cells undergo a period of vegetative dormancy characterized by relatively low plasmalemma potential differences, restricted cell-to-cell communication, inactive branch dactyls, and restricted growth. Spring branch internodes were characterized by high plasmalemma potential differences, extensive intercellular communication and active growing...
branch dactyl. Artificially increasing Ca\(^{2+}\) ion concentration in spring cells by application of ionophore A23187 or direct micro-injection of Ca\(^{2+}\) significantly restricted intercellular communication to a level similar to that found in winter cells. Eklund and Eliasson (1990) and Eklund (1991) reported that Ca\(^{2+}\) ion concentration influenced the synthesis of cell wall constituents. At a low concentration of cytosolic Ca\(^{2+}\), cell wall deposition was reduced, mainly as a result of the reduction in lignin and non-cellulosic polysaccharide deposition. High concentrations of Ca\(^{2+}\) stimulated non-cellulosic polysaccharide and lignin deposition. The results revealed that following the increase of intracellular Ca\(^{2+}\) concentration in apical bud cells under the influence of SD photoperiod, cell walls were thickened and stained densely. At the same time, plasmodesmata were constricted, sealed, blocked, and/or even disappeared. The elevation in cytosolic Ca\(^{2+}\) may, therefore, be triggering the synthesis of non-cellulosic polysaccharide (such as callose) and lignin deposition in cell walls, leading to thickened cell walls and changes in plasmodesmata, which in turn may lead to events associated with growth cessation and dormancy development in the buds of poplar.

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