

Root apices as plant command centres: the unique ‘brain-like’ status of the root apex transition zone

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Although plants are generally immobile and lack the most obvious brain activities of animals and humans, they are not only able to show all the attributes of intelligent behaviour but they are also equipped with neuronal molecules, especially synaptotagmins and glutamate/glycine-gated glutamate receptors. Recent advances in plant cell biology allowed identification of plant synapses transporting the plant-specific neurotransmitter-like molecule, auxin. This suggests that synaptic communication is not limited to animals and humans but seems to be widespread throughout plant tissues. Root apices seated at the anterior pole of the plant body show many features which allow us to propose that they, especially their transition zones, act in some way as “brain-like” command centres. The opposite posterior pole harbours sexual organs and is specialized for plant reproduction. Last but not least, we propose that vascular tissues represent highways for plant nervous activity allowing rapid exchange of information between the growing points of above-ground organs and the “brain-like” zones in the root apices.

Key words: actin, action potentials, auxin, intelligence, neurotransmitter, root, synapse.

Introduction

There is a long history of studies on plant intelligence starting with ARISTOTLE in about 280 BC, who was convinced that plants have a soul and feelings, and culminating with Charles DARWIN'S (1880) statement, in his influential book ‘The Power of Movement in Plants’, that the root apex acts like a diffuse brain, resembling brains of

lower animals. On page 573, Charles DARWIN, assisted by his son, Francis, wrote about the root apex with its “...*brain being seated within the anterior end of the body, receiving impressions from the sense-organs, and directing the several movements*” (DARWIN, 1880). Although studies on plant ‘neurobiology’ continue up to the present day (BOSE, 1926; SIMONS, 1992; ROSHCHINA, 2001), they have been pushed to the extreme pe-

riphery of plant biology, as though they were considered scientifically incorrect and embracing some type of parapsychology. Here, we wish to show that this view is incorrect and we, for the first time, discuss critically the new data on ‘nervous plant biology’ obtained both from electrophysiology as well as from cell and molecular biology. Our conclusion is that there is highly specialized group of cells in the root apex, which has almost all the attributes of a “brain-like” tissue.

Historically, plants and animals were considered to be organized on contrasting principles due to the immobility of plants. But the history of cell doctrine, elaborated preferentially by means of observations upon plant material and later fully confirmed for animals (HARRIS, 1999), is a nice example of how originally contrasting ideas have finally converged together. Our present concept, that “brain-like” attributes are a defining feature of a highly specialised zone of the root apex, is another step in showing that plants and animals, despite obvious superficial differences, are much closer to each other than would have been usually considered. The discovery of these features of nervous-like activities in plants also closes the gap noticed in an attempt to harmonise the number of biological sub-systems necessary for the processing of matter, energy and information in both plants and animals (BARLOW, 1999).

Plant intelligence: information acquisition, learning and memory for adaptation and more

Currently, there is a general agreement that higher plants are not only able to receive diverse signals from the environment but that they also possess mechanisms for rapid signal transmission. Moreover, plants can effectively process information obtained from their surroundings and can show learning behaviour which involves goal-seeking, error-assessment, and memory mechanisms (THELLIER et al., 1982; KNIGHT et al., 1998; GOH et al., 2003; TREWAVAS, 2002, 2003). Plants can also communicate this information with neighbouring plants (DICKE & BRUIN, 2001; BRUIN & DICKE, 2001). Intriguingly, herbivory of above-ground organs induces emission of chemical volatile-based signals from roots and this information is then received by roots of neighbouring plants (DICKE & DIJKMAN, 2001). There are also other examples of underground information transfer between plants (CHAMBERLAIN et al., 2001). Plant intelligence is closely linked with phenotypic plasticity, allowing effective adaptive behaviour in

the face of an ever changing environment (GOH et al., 2003). Intelligent plant behaviour is obviously designed to maximize fitness in the given environment (TREWAVAS, 2003). These learning-memory processes are closely associated with rhythmic diurnal and ultradian oscillations of ion fluxes which are sensitive towards environmental factors and stresses (AMZALLAG, 1997; SHABALA, 2003; SHABALA & NEWMAN, 1997a, 1998). However, plant bodies can, and do, reach extreme sizes: sequoia trees, for example, are surely the largest of land organisms. This size necessitates rapid means of long-distance communication in order to harmonize activities of under-ground roots and above-ground shoots without undue delay.

Plant telecommunication: action potentials in long-distance plant communication

There are numerous examples of action potentials in plants and, currently, it is accepted that action potentials occur in all plants, not only in those plants such as insectivores which show excitable and rapid movements (PICKARD, 1973; SIMONS, 1981; GOLDSWORTHY, 1983; DAVIES, 1987). In fact, it is worth recalling that the very first report (in 1873) of an action potential was made on plants; it came from John BURDON-SANDERSON who discovered this bio-electrical phenomenon in leaf traps of *Dionea* (BURDON-SANDERSON, 1873). The plant action potential is a negative potential wave with characteristic shape, amplitude, and length. It shows all the characteristics known from animal neuronal action potentials, such as stable propagation velocity, propagation without decrement, all-or-none character of responses, and excitation showing periodicity (DZIUBINSKA et al., 1983; ZAWADZKI, 1980; ZAWADZKI et al., 1991). Ultra-fast plant action potentials can reach the speed characteristic for action potentials in animal nerves (VOLKOV et al., 2000; LABADY et al., 2002; SHVETSOVA et al., 2002). In addition, and similar to those of animal neuronal cells, plant action potentials are closely associated with calcium transients (BEILBY, 1984; WARD et al., 1995). Furthermore, transmitted electrical signals were reported to induce calmodulin gene expression (VIAN et al., 1996). Plant action potentials can be induced by wounding as well as by several environmental stimuli: for example, mechanical stress, temperature, light, gravity, and even in response to the plant hormone, auxin (DAVIES & SHUSTER, 1981; RHODES et al., 1996; PICKARD, 1984; DAVIES, 1987; WILDON et al., 1992; SHIMMEN, 2001a). Moreover, action po-

tentials induce morphogenetic responses similar to those of plant hormone auxin (FRACHISSE et al., 1985; GOLDSWORTHY & RATHORE, 1985). Interestingly in this respect, several data discussed in more detail below suggest that auxin acts as a neurotransmitter-like (BALUŠKA et al., 2003a) and morphogen-like (BHALERAO & BENNETT, 2003) ancient signal molecule of plants.

Classical examples of plant telecommunication are those elicited by wounding and pathogen attack. For instance, the wounding of tomato and grapevine plants results in systemic electrical signal transduction (RHODES et al., 1996; MANCUSO, 1999). Another spectacular example of telecommunication in plants is the rapid development of systemic acquired resistance (SAR) after pathogen attack (ALVAREZ et al., 1998; PETERSEN et al., 2000). A local 'immunizing' infection in one part of the plant rapidly produces a non-specific resistance to the pathogen throughout the plant body. In addition, plant cells monitor and sense several physical parameters of the environment, such as light and gravity, rapidly transmitting this information radially to adjacent cells or along the apical-basal axis of the plant organ in question (TANADA et al., 1980; BEHRENS et al., 1985; COLLINGS et al., 1992; FROMM & ESCHRICH, 1993; BISCHOFF et al., 1997; WEISENSEEL & MEYER, 1997; SCHÜTZ & FURUYA, 2001).

Plant telecommunication is based on rapid propagation of electrical signals (MANCUSO, 1999; SHIMMEN et al., 2001a,b) which are often followed with rapid changes of gene expression (WILDON et al., 1992; STANKOVIC & DAVIES, 1996; VIAN et al., 1996). This suggests that electrical signals can induce genetic reprogramming. Moreover, action potentials can induce release of the stress plant hormone, ethylene (DZIUBINSKA et al., 2003) and an ancient gaseous signal, nitric oxide (S. MANCUSO, S. MUGNAI, D. VOLKMANN & F. BALUŠKA, unpublished data) in distant non-stimulated plant parts. Thus, rapid action potentials are well-known phenomena, but as yet there is no cell biological explanation for this form of plant telecommunication.

Plant synapses: the case of plant root apices

Although action potentials are well accepted in diverse plant species and organs, it was formerly almost impossible to link these bioelectrical telecommunication phenomena to nervous-like plant activities as plants were considered to lack synapses, neurons, and brains. However, all this seems about to change dramatically in the

face of our discovery that cross-walls of the transition zone in root apex, and possibly all end-poles to some extent, have properties (BALUŠKA et al., 2003a,b,c) which fulfill the recently updated, broader definition of synapses (DUSTIN & COLMAN, 2002). Basically, recent advances in cellular immunology have revealed that signalling interactions between T-cells and antigen-presenting the target cells in animal immune system culminate in the formation of actin-based immunological synapses which, in many respects, resemble neuronal synapses (DUSTIN & COOPER, 2000; DAS et al., 2002; DUSTIN & COLMAN, 2002; FULLER et al., 2003; HUPPA et al., 2003). Because of this, these authors updated the definition of synapses. In the new definition, synapses are characterized as actin-based asymmetric adhesion domains specialised for rapid cell-to-cell communication which is accomplished by vesicle trafficking (DUSTIN & COLMAN, 2002). Our detailed analysis of plant end-poles, especially in the transition zone of the root apex, reveals that these domains can also be considered as actin-based synapses (BALUŠKA et al., 2000, 2001, 2003a,b,c, 2004; BARLOW et al., 2004; WOJTASZEK et al., 2004). Interestingly, besides actin, these plant synapses accumulate large amounts of plant-specific unconventional myosin of class VIII (BALUŠKA et al., 2000, 2003c, 2004), a molecule apparently involved in plant endocytosis (BALUŠKA et al., 2004).

If the end-poles represent plant synapses, then there is also the need for some cell type to serve like a classical neuron. In animal brains, the immobility of neuronal cell bodies necessitates production of numerous elongated processes, axons, which seek out partner cells and thereby organise neuronal synapses in the brain. Plant cells are basically tubular in shape and they typically contact each other at their end-poles, also known as cross-walls (plant synapses), to form lengthy cell files which compose the basic units of plant tissues (BALUŠKA et al., 2003b). Obviously, plant cells equipped with rigid walls (WOJTASZEK et al., 2004), which guarantee their elongated tubular shapes (BALUŠKA et al., 2003b), do not need to extend long processes like axons in order to find the partner cells. This characteristic feature of cell-to-cell interactions within plant tissues might be the reason why plants do not possess any of the classical microtubule-associated proteins (MAPs) characteristic of axon-extending neurons (LLOYD & HUSSEY et al., 2001; HUSSEY et al., 2002; MEAGHER & FECHHEIMER, 2003).

A further similarity with neuronal and immunological synapses (DUSTIN & COOPER, 2000;

DUSTIN & COLMAN, 2002; DAS et al., 2002; FULLER et al., 2003) is that plant synapses perform cycles of regulated exocytosis/endocytosis. These are driven by actin polymerization (GELDERNER et al., 2001, 2003; GREBE et al., 2003; BOONSICHIRAI et al., 2003). Importantly, plant synapses, in conformity with neuronal and immunological synapses, are highly enriched with actin (BALUŠKA et al., 1997, 2000) and an intact actin cytoskeleton is important for polar auxin transport (MUDAY, 2000; MUDAY & MURPHY, 2002). This feature might also be related to the signalling across plant synapses because the actin cytoskeleton has been proposed to act as an electronic integration device specialized for noise-to-signal enhancement and allowing the amplification of coherent signals together with a reduction of random noise (GARTZKE & LANGE, 2002).

Auxin is plant neurotransmitter-like signalling molecule

If plant cells are interconnected *via* synaptic contacts specialised for transfer of electrical signals, then the immediate question concerns the nature of the necessary neurotransmitters. Surprisingly, plants contain many neuronal neurotransmitters (ROSHCHINA, 2001; for extensive discussion see below) although their roles in plant cell-to-cell communication remains so far unexplored. In addition to these classical neurotransmitters present in plant cells, the plant hormone, auxin, resembles these neurotransmitter molecules in many respects (BALUŠKA et al., 2003a). For instance, extracellular auxin elicits a range of electrical responses in plants (CLELAND, 1977; BATES & GOLDSMITH, 1983; VOROBIEV & MANUSADZIANAS, 1983; PICKARD, 1984; GOLDSWORTHY & RATHORE, 1985; BÖTTGER & HILGENDORF, 1988; MILLER & GOW 1989; GOLDSWORTHY & MINA, 1991; MINA & GOLDSWORTHY, 1991; ZIMMERMANN et al., 1994). Moreover, auxin modulates the activities of diverse ion channels (BÖTTGER & HILGENDORF, 1988; ZIMMERMANN et al., 1994; THOMINE et al., 1997; BECKER & HEDRICH, 2002) which are essential for the propagation of electric signals (WARD et al., 1995) and for the maintenance of other properties of the plasma membrane (e.g. ZBELL & WALTER-BACK, 1988). Auxin also elicits oscillations of cytosolic free calcium and of pH (FELLE, 1998) and the electrical response of the plasma membrane to external auxin suggests a direct involvement of the plasma membrane H^+ -ATPase (FELLE et al., 1991). In fact, auxin activates

the plasma membrane H^+ -ATPase (KINOSHITA & SHIMAZAKI, 1999) and induces calcium transients which are similar to those induced by gravistimulation while, conversely, auxin transport inhibitors such as NPA and TIBA interfere with gravi-induced calcium responses (PLIETH & TREWAVAS, 2002). Importantly, auxin transport is sensitively modulated *via* gravity (FRIML et al., 2002a; OTTENSCHLÄGER et al., 2003) as well as as several other environmental factors (SCHRADER et al., 2003). In accordance with the neurotransmitter-like transport and action of auxin, its transport is sensitive towards F-actin drugs (MUDAY, 2000; MUDAY & MURPHY, 2002) but not towards drugs affecting microtubules (HASENSTEIN et al., 1999).

Electrical stimulation of growth and polarity of plant cells requires the presence of exogenous auxin (GOLDSWORTHY & RATHORE, 1985). Importantly in this respect, auxin exerts many of its actions on plant cells from the outside (DIEKMANN et al., 1995; TIAN et al., 1995; STEFFENS et al., 2001) and this involves its presumptive receptor, auxin-binding protein 1 (ABP1), the localization of which is still unclear (RÜCK et al., 1993; DIEKMANN et al., 1995; BAULY et al., 2000; STEFFENS et al., 2001; NAPIER et al., 2002). Importantly, ABP1 is evidently essential for assembly and correct maintenance of plant synapses because mutation of ABP1 in *Arabidopsis thaliana* and antisense suppression of ABP1 in tobacco BY-2 cells, results in both aberrant cross-wall (plant synapse) formation and irregular cell files (CHEN et al., 2001). These features confer neurotransmitter-like features upon auxin and its transport system (BALUŠKA et al., 2003a, BARLOW et al., 2004). Recent research suggests that polar transport of auxin is accomplished *via* vesicular secretion linked to endocytotic and recycling processes (BALUŠKA et al., 2003a). This would imply that besides its hormone- and morphogen-like properties (JONES, 1998; FRIML, 2003; BHALERAO & BENNETT, 2003), auxin also shows neurotransmitter-like behavior resembling the neurotransmitter glutamate of neuronal synapses (BALUŠKA et al., 2003a).

Strong support for the neurotransmitter-like action of auxin comes from experimental studies on polar transport of auxin in pine cambium (WODZICKI, 1993; WODZICKI & WODZICKI, 1981; WODZICKI et al., 1979, 1999). These authors showed that external application of auxin on the apical part of cambium segments induces wave-like pattern of efflux of auxin from basal parts of these segments. Intriguingly, the rate of this auxin-induced signal propagation is several times quicker

than auxin transport itself and it can be propagated also against the main direction of the polar transport of auxin. The essential importance of auxin for plant life, related to its neural-like nature besides its hormone and morphogen properties, is evidenced also from data revealing the very ancient nature of this rather small but extremely powerful signalling molecule (COOKE et al., 2002, 2003; POLI et al., 2003).

Root apices act as plant command centres

As already mentioned in the Introduction, Charles DARWIN was the first to propose that a diffuse plant brain is localized within root apices at the anterior pole of the plant body (DARWIN, 1880). Our preliminary data are fully in agreement with this, at that time purely speculative, notion. Intuitively, there are several good reasons why, during evolution, plants placed their “brain-like” tissues within root apices buried deeply underground. First of all, the soil environment is much more stable when compared with the air environment with respect to both temperature and humidity, and it is protected from atmospheric ozone as well as solar UV radiation. Next, roots are protected also from the many destructive animals that feed on plants. Last but not least, the stem-pole of the plant body (its posterior end) bears organs of sexual reproduction whereas the opposite root-pole (the anterior end) is then logically the site of “brain-like” activities. Importantly, root apices are composed of three distinct zones, the interplay of which allows their effective exploration of soil (BALUŠKA et al., 1994, 2001) in search of both nutrients and soil water. Interestingly in this respect, one single mutation in *TOPLESS* locus transforms shoot apices into root apices in developing *Arabidopsis* embryos (LONG et al., 2002).

Root cell elongation is much more rapid than the elongation of shoot cells, and this property does not allow any cell division in the region of rapid elongation. In contrast, cell cycling and cell elongation occur concomitantly in shoot apices. The clear separation of division and elongation regions in root apices allowed us to identify a unique zone, the so-called transition zone, interpolated between the two more obvious regions (BALUŠKA et al., 1994, 2001). Cells of this transition zone show a unique cytoarchitecture, with centralised nuclei surrounded by perinuclear microtubules radiating towards the cell periphery (BALUŠKA et al., 2001). This configuration, we suppose, is optimally suited both for the perception of signals and for their transmission to and from the nuclei. As

these cells are not occupied with the demanding tasks of either cell division or rapid cell elongation, they can focus all their resources upon perception and processing of environmental signals and developmental cues. Interestingly, root apices act as sites for perception of the low-temperature stimulus (GOULAS et al., 2003) as well as of drought (BLAKE & FERRELL, 1977), and transmit this information to aerial plant parts and shoot apices. Root apices serve also for plant-to-plant communication *via* emitting and receiving of volatiles induced by the herbivore attack of above-ground organs (CHAMBERLAIN et al., 2001; DICKE & DIJKMAN, 2001).

In accordance with the view that root apices harbour plant “brain-like” tissue, it is well known that polar auxin transport is accomplished along very complex pathways in root apices, with the root cap acting as some kind of redistribution centre (SABATINI et al., 1999; FRIML et al., 2002a,b). In fact, auxin transport drives root apex patterning (FRIML et al., 2002b; JIANG & FELDMAN, 2003; BHALERAO & BENNETT, 2003; BARLOW et al., 2004). In accordance with these features, root apices are equipped with high numbers of actin-enriched and auxin-transporting plant synapses (BARLOW et al., 2004). Moreover, an auxin maximum (SABATINI et al., 1999; JIANG & FELDMAN, 2003) is localised at the quiescent centre and root cap statocytes.

Importantly, this auxin maximum responds rapidly to gravistimulation (RASHOTTE et al., 2001; OTTENSCHLÄGER et al., 2003; BOONSIRICHAI et al., 2003) and to exposures of extracellular auxin (OTTENSCHLÄGER et al., 2003). In this latter regard, even minimal levels of external auxin applied to roots elicit inhibition of growth while much higher auxin levels applied to shoots stimulate their growth in above-ground plant tissues. External auxin induces also dramatic redistribution of cortical microtubules (BLANCAFLOR & HASENSTEIN, 1995; BALUŠKA et al., 1996b). Similar dramatic responses of microtubules were also reported from root apices exposed to glutamate, and glutamate-receptors were shown to be critical for this response (SIVAGURU et al., 2003). Intriguingly, neurotoxic cation aluminium (DELEERS et al., 1986; TROMBLEY, 1998) treatment mimics glutamate treatment (SIVAGURU et al., 2003) and aluminium is also known to inhibit the basipetal transport of auxin (KOLLMEIER et al., 2000). Glutamate-gated calcium fluxes are rather prominent in root apices whereas calcium fluxes show only small responses to external glutamate in leaves and cotyledons (DENNISON & SPALDING,

2000). Even more importantly, the distal part of the transition zone is the most aluminium-sensitive zone of the whole plant (SIVAGURU et al., 1998, 1999; KOLLMEIER et al., 2000) and it is also known to be the root region most sensitive towards exogenous auxin and calcium (ISHIKAWA & EVANS, 1992, 1993; BALUŠKA et al., 1996a,b). Interestingly, cells in the distal part of the transition zone respond to aluminium exposure by inhibition of respiration and by depletion of ATP, as well as by production of reactive oxygen species (YAMAMOTO et al., 2002, 2003; BOSCOLO et al., 2003).

The high auxin-sensitivity of root apices is in agreement with the large number of active plant synapses in root apices, especially in the transition zone which acts as a “brain-like” tissue. In fact, the transition zone turns out to be critical sensory zone receiving of and responding to a large number of external signals and developmental cues (BALUŠKA et al., 1994, 2001). The zonation of growing root apices (BALUŠKA et al., 1994, 2001) allows synchronization of cellular activities and electrical responses. Just like brain, it represents the largest sink for oxygen and also shows rhythmic oscillations in the uptake of oxygen, potassium, and calcium (Stefano MANCUSO, Alina SCHICK, Dieter VOLKMANN & František BALUŠKA, unpublished data). Such behaviour of anatomically grouped root cells resemble the synchronous and oscillatory patterning of anatomically grouped neurons that drive the sensorimotor networks in brains (ENGEL et al., 2001; HARRIS et al., 2003). If some of these oscillatory physiological features are coupled to cell growth control, then they could be related to nutational movements of roots (BARLOW et al., 1994; SHABALA & NEWMAN, 1997b).

Importantly, our preliminary work reveals that the rhythmic oscillations of oxygen uptake into the transition zone of maize root apex extremely rapidly (within few seconds) to gravistimulation, as well as to wounding and other stress treatments applied selectively to the shoot apex of young maize seedlings (S. MANCUSO, A. SCHICK, D. VOLKMANN & F. BALUŠKA, unpublished data). Intriguingly, single root cell measurements within the transition zone show that increased oxygen uptake is registered almost immediately, with a lag-phase of 2–3 seconds, following the arrival of action potentials elicited by local stimulation of the shoot apex. This situation is almost identical to that recorded for single neurons in animal brain (THOMPSON et al., 2003). However, the big question remains unanswered. What

cellular processes drive this rapid transmission of signals between shoot and root apices, inducing almost instantaneous response of cells within the transition zone of the root apex? Do plants have nervous tissues that could be specialised for this task?

Vascular bundles as assemblies of plant nerves?

The plant vascular system is composed of long continuous strands of highly elongated cells which interconnect all roots and shoots of a given plant body (BERLETH & MATTSSON, 2000; BERLETH et al., 2000). Similarly like neurons which are supported by glial cells, xylem and phloem elements of plant vascular bundles are supported by neighbouring parenchymatic cells. Traditionally, this system is thought to secure the long-distance transport of solutes and assimilates, as well as to provide the above-ground part of the plant body with mechanical support (ESAU, 1954). Recent data, however, reveal that plant homologues of ionotropic glutamate receptors are expressed preferentially in vascular tissues (KIM et al., 2001; TURANO et al., 2002). Moreover, vascular tissues are enriched also with auxin (SAUGY & PILET, 1985) as well as actin and actin-binding proteins (PARTHASARATHY et al., 1985; BALUŠKA et al., 1997; KLAHRE & CHUA, 1999; MUN et al., 2002). In support of nerve-like roles of vascular bundles, neurotransmitters like acetylcholine and serotonin activate ion pumps in cells of xylem parenchyma (ZHOLKEWICH et al., 2003). Interestingly in this respect, vascular tissues were reported to conduct light directly to root apices and induce photomorphogenic responses within them (SUN et al., 2003)

A very attractive possibility is that, besides the above mentioned more apparent tasks, the cell files of vascular bundles act as highly effective channels for plant telecommunication, and so serve in the capacity of plant nerves. Intriguingly, development of veins and vascular bundles is primarily controlled *via* the plant hormone auxin (BERLETH et al., 2000; BERLETH & MATTSSON, 2000; SACHS, 2000, 2003; BERLETH & SACHS 2001; DENGLER, 2001) which emerges as a plant neurotransmitter (BALUŠKA et al., 2003a; see also above). Polar transport of auxin is essential for the formation of new vascular strands as well as for their continuity throughout plant organs, auxin itself inducing vascular development, and auxin being transported along the vascular strands. The central role of polar auxin transport in plant polarity extends

also towards neural long-distance communication based upon the neurotransmitter-like properties of auxin (BALUŠKA et al., 2003a,b; BARLOW et al., 2004). Importantly, vascular bundles active in transport terminate in the “brain-like” transition zone where phloem accomplishes unloading of shoot-derived assimilates (OPARKA & SANTA CRUZ, 2000) and of neurotransmitter-like auxin (SWARUP et al., 2001). This feature surely makes the neuron-like cells of the transition zone well supplied with both auxin and the nutrition necessary for their energy-demanding “brain-like” activities.

Diffuse nervous system in plants?

Plants clearly lack any centralised control site and resemble colonies of social insects, like ants. Their development is proposed to be driven more by a collective specification than by central planning (SACHS, 2003). Similarly, as in social insect networks (FEWELL, 2003) and also in neuronal networks of the central nervous system (LAUGHLIN & SEJNOWSKI, 2003), plants build complex patterns of organs *via* large-scale phenomena driven by a limited set of processes based on non-linear dynamics (SACHS, 2003; FEWELL, 2003; LAUGHLIN & SEJNOWSKI, 2003). In his influential book, Charles DARWIN proposed that root apices, seated at the anterior pole of the plant body, represent plant brains which in turn resemble the diffuse brains of lower animals (DARWIN, 1880). Moreover, root and shoot apices are considered generally to represent so-called “dominant centres” of plants which sense environmental signals and developmental cues, and which communicate together *via* long-distance signalling pathways (POLEVOI, 2001). Indeed, a recent breakthrough article revealed that the diffuse nerve net of hemichordates is anatomically patterned despite lacking a centralised system (LOWE et al., 2003). In this respect, the diffuse nerve net of lower animals resembles the patterned venation of leaves and other organs of higher plants (SACHS, 2000, 2003). Obviously, the diffuse but patterned nerve net of hemichordates later became centralised during the evolution of the chordate lineage (LOWE et al., 2003; HOLLAND, 2003). Sessile plants, continuously exposed to actions of ever changing environmental factors and constant gravity, have retained the rather diffuse nervous system which fits better with their sessile life-style and which allows the flexible development driven by a collective specification.

Nervous molecules in plants

Many neurologically active compounds are natural plant products, of which some of the most notorious examples are nicotine, cocaine, caffeine, marijuana, morphine, and cannabis. This list is however much more longer (ROSHCHINA, 2001) and still growing. Besides the 20 or so genes for ionotropic glutamate receptors (GluRs) in *Arabidopsis* (LAM et al., 1998; CHIU et al., 1999; TURANO et al., 2001; DAVENPORT, 2002), plants express several of their agonists (MONAGHAN et al., 1989; ROSS et al., 1989; COPANI et al., 1991; BETTLER & MULLE, 1995; BRENNER et al., 2000, 2003). Recently, *N*-arachidonyl ethanolamine (NAE) anandamide, a neuroactive lipid mediator, which is the ligand of neuronal cannabinoid receptors, have been both isolated from plants and shown to regulate diverse plant processes in plants *via* the same class of cannabinoid receptors (CHAPMAN, 2000; BLANCAFLOR et al., 2003; TRIPATHY et al., 2003).

In addition to these classical neuronal agonists, plants apparently synthesize and use for synaptic-like cell-to-cell communication diverse classical neurotransmitters such as glutamate, glycine, histamine, acetylcholine, dopamine, γ -aminobutyric acid (GABA), and ATP. While glutamate and glycine were shown to gate Ca^{2+} -permeable channels in plants (DENNISON & SPALDING, 2000; DUBOS et al., 2003), glutamate was also reported to rapidly depolarize the plasma membrane in a process mediated by glutamate receptors (SIVAGURU et al., 2003). Overexpression of AtGluR2 gene impaired calcium utilization and enhanced sensitivity of *Arabidopsis* seedlings against stress (KIM et al., 2001). In addition, the putative glutamate receptor protein, AtGluR1.1, functions as a regulator of carbon and nitrogen metabolism (KANG & TURANO, 2003). Extracellular ATP, which acts as a neurotransmitter in brains (SILINSKY & REDMAN, 1996; VIZI et al., 2000; KHAKH, 2001; DEMIDCHIK et al., 2003), is reported to cause depolarisation of the plasma membrane potential of growing root hairs (LEW & DEARNALEY, 2000), to increase cytoplasmic calcium in root cells, as well as to exert many signalling functions coordinate the responses of plant cells to environmental stimuli (DEMIDCHIK et al., 2003). Intriguingly, extracellular ATP also inhibits polar transport of auxin and impairs root gravitropism (TANG et al., 2003).

Two other relatively well studied classical neurotransmitters in plants, both of which have some well-documented roles in plant signalling

and stress response, are acetylcholine and GABA. Acetylcholine mediates phytochrome-based signalling (JAFFE, 1970; BOSSEN et al., 1991) and also promotes cell swelling (TRETYN et al., 1990a, b) as well as cell elongation (EVANS, 1972). Interestingly, acetylcholine is especially abundant at the stele-cortex interface of maize mesocotyls (MOMONOKI, 1992) and activates ion pumps in cells of xylem parenchyma of maize root apices (ZHOLKEWICH et al., 2003). Much more is known on the roles of GABA: apparently, it mediates adaptation of plants to diverse stresses including hypoxia, cold, water stress, mechanical stress, and pathogen attack (STREETTER & THOMPSON, 1972; WALLACE et al., 1984; RHODES et al., 1986; MENEGUS et al., 1989; ROBERTS et al., 1992; RAMPUTH & BOWN, 1996; SHELP et al., 1995, 1999; REGGIANI & LAORETI, 2000). For example, a 10- to 25-fold increase of GABA was recorded within 1 to 4 minutes of mechanical stimulation (RAMPUTH & BOWN, 1996). GABA also regulates growth of plant cells (EVANS, 1972; KATHIRESAN et al., 1998), and exogenous GABA induces up to a 14-fold increase of ethylene biosynthesis (KATHIRESAN et al., 1997). Synthesis of GABA is stimulated by the lowering of cytoplasmic pH (CARROLL et al., 1994; CRAWFORD et al., 1994) which, interestingly, also regulates the gravisensitivity and gravitropism of root apices (SCOTT & ALLEN, 1999; FASANO et al., 2001; BOONSICHI-RAI et al., 2003) as well as root water transport under anoxia (TOURNAIRE-ROUX et al., 2003; HOLBROOK & ZWIENIECKI, 2003). Interestingly, anaerobic induction of GABA accumulation is mediated *via* signalling pathways involving heterotrimeric proteins and phospholipase C (REGGIANI & LAORETI, 2000).

Nevertheless, it might well be that GABA simply participates in stress-induced metabolism without having any role in cell-to-cell signalling. But a recent breakthrough study revealed that GABA helps tip-growing pollen tubes to navigate towards ovules deeply buried within female tissues (PALANIVELU et al., 2003). Obviously, GABA is acting as a signalling molecule able to provide direction for tip-growth of cells and to transmit stress information from cell-to-cell within plant tissues, the latter leading to an adaptation process in plants (BOUCHÉ et al., 2003). Because GABA also mediates the navigation of migrating neuroblasts and neurons (BARKER et al., 1998; BEHAR et al., 2001), and because axon growth resembles tip-growing plant cells (PALANIVELU & PREUSS, 2000), it is very attractive to propose that GABA acts not only as a universal neu-

rotransmitter but also functions in plant cell-to-cell communication. In support of this notion is the finding that plants cells can secrete GABA (CHUNG et al., 1992) and this might stimulate adjacent cells to secrete GABA too, as was shown for auxin in wood cambium (WODZICKI, 1993; WODZICKI et al., 1979, 1999). Given the great number of reports on physiological responses of plant cells to external GABA, one is tempted to suggest that GABA acts as neurotransmitter-like molecule in plants. However, well-designed experimental studies are needed to confirm this attractive scenario.

As a big surprise, the *Arabidopsis* genome project has revealed that plants also have the potentiality to synthesise other nervous molecules, such as synaptotagmins and copines (CRAXTON, 2001; TOMSIG & CREUTZ, 2002). These molecules are essential for the calcium-mediated regulation of secretion in neurons (NAKAYAMA et al., 1999; YOSHIHARA et al., 2002). However, regulated exocytosis is typical for cell-to-cell communication throughout multicellular eukaryotes. Therefore, it should not be a surprise that these proteins are absent in unicellular yeast which perform only constitutive exocytosis (CRAXTON, 2001; TOMSIG & CREUTZ, 2002). Another class of calcium- and phospholipid-binding proteins regulating secretion and present in both animals and plants – but missing from yeast – are the annexins (DELMER & POTIKHA, 1997; DONNELLY & MOSS, 1997; BRAUN et al., 1998; GERKE & MOSS, 2002). Annexins are present in multicellular but not unicellular fungal organisms (BRAUN et al., 1998). Analysis of the genome of the moss, *Physcomitrella patens*, shows the presence of synaptotagmins (DIDIER SCHAEFER, personal communication), suggesting an important role for these calcium sensors in the regulation of secretion in lower plants too. As yet, there are no functional data available on plant synaptotagmins, but recent studies on plant copines have revealed that expression of their genes is regulated in response to pathogen attack and abiotic stimuli (JAMBUNATHAN & MCNELLIS, 2003). Moreover, copine mutants of *Arabidopsis* show low temperature-dependent seedling dwarfism (HUA et al. 2003) as well as increased resistance to pathogen attack (JAMBUNATHAN et al., 2001).

Outlook

This overview of data that concern the nervous aspects of higher plants makes it clear that, although plants are generally immobile and

lack the most obvious brain activities of animals and humans, they not only are able to show many attributes of intelligent behaviour but they also are equipped with several critical molecules, especially with synaptotagmins (CRAXTON, 2001) and glutamate-/glycine-gated glutamate receptors (DAVENPORT, 2002; DUBOS et al., 2003; SIVAGURU et al., 2003), that could support synapse-like cell-to-cell communication in plants. Indeed, the recent advances in plant cell biology allowed identification of plant synapses (BALUŠKA et al., 2003b,c; BARLOW et al. 2004), leading to a breakthrough in nervous plant biology. It is increasingly obvious that synaptic communication is not limited to brains of animals and humans but that it is widespread throughout plant tissues also. Moreover, root apices show many features which allow us to propose that they, especially their transition zones, act in some way as plant brains. On the other hand, stelar tissue is specialised not simply for the transport of solutes and assimilates but also brings about the transport of neurotransmitter-like and morphogen-like auxin (BALUŠKA et al., 2003a; BHALERAO & BENNETT, 2003). Moreover, the vascular bundles support the transmission of action potentials suggesting that they act as a highway for plant nervous activity. It is obvious that stelar tissues are enriched with actin and nervous molecules like glutamate receptors as well as acetylcholine. However, more needs to be learned about plant neurotransmitter-like molecules and their receptors. For future studies, it will be critical to learn more concerning plant synapses and the nerve-like character of vascular tissue. Cell and molecular biology should reveal the molecules which drive assembly and maintenance of plant synapses, while electrophysiology should be at the forefront of attempts to reveal the impact of signalling networks on synaptic cell-to-cell communication in plants. Current methodological advances in plant cell biology give excellent perspectives to all these efforts. Obviously, the most exciting times are lying ahead which should revolutionise plant science. They will also lead to a more sensitive appreciation of the plant kingdom, as well as emphasising the common features that bind together the community of living things.

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References

- ALVAREZ, M. E., PENNELL, R. I., MEIJER, P.-J., ISHIKAWA, A., DIXON, R. A. & LAMB, C. 1998. *Cell* **92**: 773–784.
- AMZALLAG, G. N. 1997. *Austral. Plant Physiol.* **24**: 579–586.
- BALUŠKA, F., BARLOW, P. W. & KUBICA, Š. 1994. *Plant & Soil* **167**: 31–42.
- BALUSKA, F., BARLOW, P. W. & VOLKMANN, D. 1996a. *Plant Cell Physiol.* **37**: 1013–1021.
- BALUSKA, F., BARLOW, P. W. & VOLKMANN, D. 1996b. *Bot. Acta* **109**: 25–34.
- BALUŠKA, F., VITHA, S., BARLOW, P. W. & VOLKMANN, D. 1997. *Eur. J. Cell Biol.* **72**: 113–121.
- BALUŠKA, F., BARLOW, P. W. & VOLKMANN, D. 2000. Actin and myosin VIII in developing root cells, pp. 457–476. In: STAIGER, C. J., BALUŠKA, F., VOLKMANN, D. & BARLOW, P. W. (eds), *Actin: a dynamic framework for multiple plant cell functions*, Kluwer Academic Publishers, Dordrecht, The Netherlands
- BALUŠKA, F., VOLKMANN, D. & BARLOW, P. W. 2001. *J. Plant Growth Regul.* **20**: 170–181.
- BALUSKA, F., ŠAMAJ, J. & MENZEL, D. 2003a. *Trends Cell Biol.* **13**: 282–285.
- BALUSKA, F., WOJTASZEK, P., VOLKMANN, D. & BARLOW, P. W. 2003b. *BioEssays* **25**: 569–576.
- BALUŠKA, F., ŠAMAJ, J., WOJTASZEK, P., VOLKMANN, D. & MENZEL, D. 2003c. *Plant Physiol.* **136**: 482–491.
- BALUSKA, F., ŠAMAJ, J., HLAVACKA, A., KENDRICK-JONES, J. & VOLKMANN, D. 2004. *J. Exp. Bot.* **55**: 463–473.
- BARKER, J. L., BEHAR, T., LI, Y. X., LIU, Q. Y., MA, W., MARIC, D., MARIC, I., SCHAFFNER, A. E., SERAFINI, R., SMITH, S. V. ET AL. 1998. *Prospect. Dev. Neurobiol.* **5**: 305–322.
- BARLOW, P. W. 1999. *Adv. Space Res.* **23/12**: 1975–1986.
- BARLOW, P. W., PARKER, J. S. & BRAIN, P. 1994. *Adv. Space Res.* **14**: 149–158.
- BARLOW, P. W., VOLKMANN D. & BALUŠKA, F. 2004. Polarity in roots. In: LINDSEY, K. (ed), *Polarity in Plants*, Blackwell, Oxford, UK, pp. 192–241.
- BATES, G. W. & GOLDSMITH, M. H. M. 1983. *Planta* **159**: 231–237.
- BAULY, J. M., SEALY, I. M., MACDONALD, H., BREARLEY, J., DROGE, S., HILLMER, S., ROBINSON, D. G., VENIS, M. A., BLATT, M. R., LAZARUS, C. M. & NAPIER, R. M. 2000. *Plant Physiol.* **124**: 1229–1238.
- BECKER, D. & HEDRICH, R. 2002. *Plant Molec. Biol.* **49**: 349–356.
- BEHAR, T. N., SMITH, S. V., KENNEDY, R. T., MCKENZIE, J. M., MARIC, I. & BARKER, J. L. 2001. *Cortex* **11**: 744–753.
- BEHRENS, H. M., GRADMANN, D. & SIEVERS, A. 1985. *Planta* **163**: 463–472.
- BEILBY, M. J. 1984. *Plant Cell Environm.* **7**: 415–421.

- BERLETH, T. & MATTSSON, J. 2000. *Curr. Opin. Plant Biol.* **3**: 406–411.
- BERLETH, T. & SACHS, T. 2001. *Curr. Opin. Plant Biol.* **4**: 57–62.
- BERLETH, T., MATTSSON, J. & HARDTKE, C. S. 2000. *Trends Plant Sci.* **5**: 387–393.
- BETTLER, B. & MULLE, C. 1995. *Neuropharmacology* **34**: 123–139.
- BHALERAO, R. P. & BENNETT, M. J. 2003. *Nat. Cell Biol.* **5**: 939–943.
- BISCHOFF, F., MILLAR, A. J., KAY, S. A. & FURUYA, M. 1997. *Plant J.* **12**: 839–849.
- BLAKE, J. & FERRELL, W. K. 1977. *Physiol. Plant.* **39**: 106–109.
- BLANCAFLOR, E. B. & HASENSTEIN, K. H. 1995. *Protoplasma* **185**: 75–82.
- BLANCAFLOR, E. B., HOU, G. & CHAPMAN, K. D. 2003. *Planta* **217**: 206–217.
- BOONSIRICHAI, K., SEDBROOK, J. C., CHEN, R., GILROY, S. & MASSON, P. H. 2003. *Plant Cell* **15**: 2612–2625.
- BOSCOLO, P. R. S., MENASSI, M. & JORGE, R. A. 2003. *Phytochem.* **62**: 181–189.
- BOSE, J. C. 1926. *The nervous mechanism of plants*. Longmans, Green and Company, London, New York.
- BOSSEN, M. E., TRETYN, A., KENDRICK, R. E. & VREDENBERG, W. J. 1991. *J. Plant Physiol.* **137**: 706–710.
- BÖTTGER, M. & HILGENDORF, F. 1988. *Plant Physiol.* **86**: 1038–1043.
- BOUCHÉ, N., LACOMBE, B. & FROMM, H. 2003. *Trends Cell Biol.* **13**: (in press).
- BRAUN, E. L., KANG, S., NELSON, M. A. & NATVIG, D. O. 1998. *J. Mol. Evol.* **47**: 531–543.
- BRENNER, E. D., MARTINEZ-BARBOZA, N., CLARK, A. P., LIANG, Q. S., STEVENSON, D. W. & CORUZZI, G. M. 2000. *Plant Physiol.* **124**: 1615–1625.
- BRENNER, E. D., STEVENSON, D. W. & TWIGG, R. W. 2003. *Trends Plant. Sci.* **8**: 446–452.
- BRUINE, J. & DICKE, M. 2001. *Biochem. Syst. Ecol.* **29**: 1103–1113.
- BURDON-SANDERSON, J. 1873. *Proc. R. Soc. Lond.* **21**: 491–496.
- CARROLL, A. D., FOX, G. G., LAURIE, S., PHILLIPS, R., RATCLIFFE, R. G. & STEWART, G. R. 1994. *Plant Physiol.* **106**: 513–520.
- CHAMBERLAIN, K., GUERRIERI, E., PENNACCHIO, J., PETTERSSON, J., PICKETT, P. A., POPPY, G. M., POWELL, W., WADHAMS, L. J. & WOODCOCK, C. M. 2001. *Biochem. Syst. Ecol.* **29**: 1063–1074.
- CHAPMAN, K. D. 2000. *Chem. Phys. Lipids* **108**: 221–230.
- CHEN, J.-G., ULLAH, H., YOUNG, J. C., SUSSMAN, M. R. & JONES, A. M. 2001. *Genes Dev.* **15**: 902–911.
- CHIU, J., DESALLE, R., LAM, H.-M., MEISEL, L. & CORUZZI, G. 1999. *Mol. Biol. Evol.* **16**: 826–838.
- CHUNG, I., BOWN, A. W. & SHELPE, B. J. 1992. *Plant Physiol.* **99**: 659–664.
- CLELAND, R. E., PRINS, H. B. A., HARPER, J. R. & HIGINBOTHAM, N. 1977. *Plant Physiol.* **59**: 395–397.
- COLLINGS, D. A., WHITE, R. G. & OVERALL, R. L. 1992. *Plant Physiol.* **100**: 1417–1426.
- COOKE, T. J., POLI, D. B., SZTEIN, A. E. & COHEN, J. D. 2002. *Plant. Molec. Biol.* **49**: 319–338.
- COOKE, T. J., POLI, D. B., & COHEN, J. D. 2004. Did auxin play a crucial role in the evolution of novel body plans during the late Silurian-early Devonian radiation of land plants? In: HEMSLEY, A. R. & POOLE, I. K. (eds), *Evolution of Plant Physiology*, Academic Press, London, UK, (in press).
- COPANI, A., CANONICO, P. L., CATANIA, M. V., ARONICA, E., BRUNO, V., RATTI, E., VAN AMSTERDAM, F. T. M., GAVIRAGHI, G. & NICOLETTI, F. 1991. *Brain. Res.* **558**: 79–86.
- CRAWFORD, L. A., BOWN, A. W., BREITKREUZ, K. E. & GUINEL, F. C. 1994. *Plant Physiol.* **104**: 865–871.
- CRAXTON, M. 2001. *Genomics* **77**: 43–49.
- DARWIN, CH. (assisted by F. DARWIN) 1880. *The Power of Movements in Plants*. John Murray, London.
- DAS, V., NAL, B., ROUMIER, A., MEAS-YEDID, V., ZIMMER, C., OLIVO-MARIN, J.-C., ROUX, P., DAUTRY-VARSAT, A. & ALCOVER, A. 2002. *Immunol. Rev.* **189**: 123–135.
- DAVENPORT, R. 2002. *Ann. Bot.* **90**: 549–557.
- DAVIES, E. 1987. *Plant Cell Environm.* **10**: 623–631.
- DAVIES, E. & SCHUSTER, A. 1981. *Proc. Natl. Acad. Sci. USA* **78**: 2422–2426.
- DELEERS, M., SERVAIS, J. P. & WÜLFERT, E. 1986. *Biochim. Biophys. Acta* **855**: 271–276.
- DELMER, D. P. & POTIKHA, T. S. 1997. *Cell Mol. Life Sci.* **53**: 546–553.
- DEMIDCHIK, V., NICHOLS, C., OLIYNYK, M., DARK, A., GLOVER, B. J. & DAVIES, J. M. 2003. *Plant Physiol.* **133**: 456–461.
- DENGLER, N. G. 2001. *J. Plant Growth Regul.* **20**: 1–13.
- DENNISON, K. L. & SPALDING, E. P. 2000. *Plant Physiol.* **124**: 1511–1514.
- DICKE, M. & BRUIN, J. 2001. *Biochem. Syst. Ecol.* **29**: 981–994.
- DICKE, M. & DIJKMAN, H. 2001. *Biochem. Syst. Ecol.* **29**: 1075–1087.
- DIEKMANN, W., VENIS, M. A. & ROBINSON, D. G. 1995. *Proc. Natl. Acad. Sci. USA* **92**: 3425–3429.
- DONNELLY, S. R. & MOSS, S. E. 1997. *Cell Mol. Life Sci.* **53**: 533–538.
- DUBOS, C., HUGGINS, D., GRANT, G. H., KNIGHT, M. R. & CAMPBELL, M. M. 2003. *Plant J.* **35**: 800–810.
- DUSTIN, M. L. & COOPER, J. A. 2000. *Nat. Immunol.* **1**: 23–29.
- DUSTIN, M. L. & COLMAN, D. R. 2002. *Science* **298**: 785–789.
- DZIUBINSKA, H., PASZEWSKI, A., TREBACZ, K. & ZAWADZKI, T. 1983. *Physiol. Plant.* **57**: 279–284.

- DZIUBINSKA, H., FILEK, M., KOSCIELNIAK, J. & TREBACZ, K. 2003. *J. Plant Physiol.* **160**: 1203–1210.
- ENGEL, A. K., FRIES, P. & SINGER, W. 2001. *Nat. Rev. Neurosci.* **2**: 704–716.
- ESAU, K. 1954. *Biol. Rev.* **29**: 46–86.
- EVANS, M. L. 1972. *Plant Physiol.* **50**: 414–416.
- FASANO, J. M., SWANSON, S. J., BLANCAFLOR, E. B., DOWD, P. E., KAO, T. & GILROY, S. 2001. *Plant Cell* **13**: 907–921.
- FELLE, H. H. 1998. *J. Exp. Bot.* **49**: 987–995.
- FELLE, H. H., PETERS, W. & PALME, K. 1991. *Biochim. Biophys. Acta* **1064**: 199–204.
- FEWELL, J. H. 2003. *Science* **301**: 1867–1870.
- FRACHISSE, J.-M., DESBIEZ, M.-O. & THELLIER, M. 1985. *Physiol. Plant.* **64**: 48–52.
- FRIML, J., WISNIEWSKA, J., BENKOVA, E., MEDGEN, K. & PALME, K. 2002a. *Nature* **415**: 806–809.
- FRIML, J., BENKOVA, E., BLILOU, I., WISNIEWSKA, J., HAMANN, T., LIUNG, K., WOODY, S., SANDBERG, G., SCHERES, B., JÜRGENS, G. & PALME, K. 2002b. *Cell* **108**: 661–673.
- FRIML, J. 2003. *Curr. Opin. Plant Biol.* **6**: 7–12.
- FROMM, J. & ESCHRICHT, W. 1993. *J. Plant Physiol.* **141**: 673–680.
- FULLER, C. L., BRACIALE, V. L. & SAMELSON, L. E. 2003. *Immunol. Rev.* **191**: 220–236.
- GARTZKE, J. & LANGE, K. 2002. *Am. J. Physiol. Cell Physiol.* **283**: 1333–1346.
- GELDNER, N., FRIML, J., STIERHOF, Y. D., JÜRGENS, G. & PALME, K. 2001. *Nature* **413**: 425–428.
- GELDNER, N., ANDERS, N., WOLTERS, H., KEICHER, J., KORNBARGER, W., MULLER, P., DELBARRE, A., UEDA, T., NAKANO, A. & JÜRGENS, G. 2003. *Cell* **112**: 219–230.
- GERKE, V. & MOSS, S. E. 2002. *Physiol. Rev.* **82**: 331–371.
- GOH, C.-H., NAM, H. G. & PARK, Y. S. 2003. *Plant J.* **36**: (in press).
- GOLDSWORTHY, A. 1983. *J. Theor. Biol.* **103**: 645–648.
- GOLDSWORTHY, A. & RATHORE, K. S. 1985. *J. Exp. Bot.* **36**: 1134–1141.
- GOLDSWORTHY, A. & MINA, M. G. 1991. *Planta* **183**: 368–373.
- GOULAS, E., DILY, F. L. & OURRY, A. 2003. *J. Plant Physiol.* **160**: 893–902.
- GREBE, M., XU, J., MÖBIUS, W., UEDA, T., NAKANO, A., GEUZE, H. J., ROOK, M. B. & SCHERES, B. 2003. *Curr. Biol.* **13**: 1378–1387.
- HARRIS, H. 1999. *The birth of the cell*. Yale University Press, New Haven and London.
- HARRIS, K. D., CSICSVARI, J., HIRASE, H., DRAGOI, G. & BUSZAKI, G. 2003. *Nature* **424**: 552–556.
- HASENSTEIN, K. H., BLANCAFLOR, E. B. & LEE, J. S. 1999. *Physiol. Plant.* **105**: 729–738.
- HOLBROCK, N. M. & ZWIENIECKI, M. A. 2003. *Nature* **425**: 361.
- HOLLAND, N. D. 2003. *Nat. Rev. Neurosci.* **4**: 1–11.
- HUA, J., GRISAFI, P., CHENG, S.-H. & FINK, G. R. 2001. *Genes Dev.* **15**: 2263–2272.
- HUPPA, J. B., GLEIMER, M., SUMEN, C. & DAVIS, M. M. 2003. *Nat. Immunol.* **4**: 749–755.
- HUSSEY, P. J., HAWKINS, T. J., IGARASHI, H., KALORITI, D. & SMERTENKO, A. 2002. *Plant Molec. Biol.* **50**: 915–924.
- ISHIKAWA, H. & EVANS, M. L. 1992. *Plant Physiol.* **100**: 762–768.
- ISHIKAWA, H. & EVANS, M. L. 1993. *Plant Physiol.* **102**: 1203–1210.
- JAMBUNATHAN, N. & MCNELLIS, T. W. 2003. *Plant Physiol.* **132**: 1370–1381.
- JAMBUNATHAN, N., SIANI, J. M. & MCNELLIS, T. W. 2001. *Plant Cell* **13**: 2225–2240.
- JAFFE, M. J. 1970. *Plant Physiol.* **46**: 768–777.
- JONES, A. M. 1998. *Science* **282**: 2201–2202.
- JIANG, K. & FELDMAN, L. J. 2003. *J. Plant Growth Regul.* **21**: 432–440.
- KANG, J. & TURANO, F. J. 2003. *Proc. Natl. Acad. Sci. USA* **100**: 6872–6877.
- KATHIRESAN, A., MIRANDA, J., CHINNAPPA, C. C. & REID, D. M. 1997. *Plant Physiol.* **115**: 129–135.
- KATHIRESAN, A., MIRANDA, J., CHINNAPPA, C. C. & REID, D. M. 1998. *Plant Growth Regul.* **26**: 131–137.
- KHAKH, B. S. 2001. *Nat. Rev. Neurosci.* **2**: 165–174.
- KIM, S. A., KWAK, J. M., JAE, S. K., WANG, M.-H. & NAM, H. G. 2001. *Plant Cell Physiol.* **42**: 74–84.
- KINOSHITA, T. & SHIMAZAKI, K. 1999. *EMBO J.* **18**: 5548–5558.
- KLAHRE, U. & CHUA, N.-H. 1999. *Plant Mol. Biol.* **41**: 65–73.
- KNIGHT, H., BRANDT, S. & KNIGHT, M. R. 1998. *Plant J.* **16**: 681–687.
- KOLLMEIER, M., FELLE, H. H. & HORST, W. J. 2000. *Plant Physiol.* **122**: 945–956.
- LABADY, A., THOMAS, D., SHVETSOVA, T. & VOLKOV, A. G. 2002. *Bioelectrophysiology* **57**: 47–53.
- LAM, H.-M., CHIU, J., HSIEH, M.-H., MEISEL, L., OLIVEIRA, I. C., SHIN, M. & CORUZZI, G. 1998. *Nature* **396**: 125–126.
- LAUGHLIN, S. B. & SEJNOWSKI, T. J. 2003. *Science* **301**: 1870–1874.
- LEW, R. R. & DEARNALEY, J. D. W. 2000. *Plant Sci.* **153**: 1–6.
- LLOYD, C. W. & HUSSEY, P. J. 2001. *Nat. Rev. Mol. Cell Biol.* **2**: 40–47.
- LONG, J. A., WOODY, S., POETHING, S., MEYEROWITZ, E. M. & BARTON, M. K. 2002. *Development* **129**: 2797–2806.
- LOVE, C., WU, M., SALIC, A., EVANS, L., LANDER, E., STANGE-THOMANN, N., GRUBER, C. E., GERHART, J. & KIRSCHNER, M. 2003. *Cell* **113**: 853–865.
- MANCUSO, S. 1999. *Aust. J. Plant Physiol.* **26**: 55–61.
- MEAGHER, R. B. & FECHHEIMER, M. 2003. *The Arabidopsis cytoskeletal genome*. In: Meyerowitz, E. M. & Somerville, C. R. (eds.), *The Arabidopsis book*, American Society of Plant Biologists (<http://www.aspb.org/publications/arabidopsis>).
- MENEGUS, F., CATTARUZZA, L., CHERSI, A. & FRONZA, G. 1989. *Plant Physiol.* **90**: 29–32.

- MILLER, A. L. & GOW, N. A. R. 1989. *Plant Physiol.* **89**: 1198–1206.
- MINA, M. G. & GOLDSWORTHY, A. 1991. *Planta* **186**: 104–108.
- MOMONOKI, Y. S. 1992. *Plant Physiol.* **99**: 130–133.
- MONAGHAN, D. T., BRIDGES, R. J. & COTMAN, C. W. 1989. *Annu. Rev. Pharmacol. Toxicol.* **29**: 365–402.
- MUDAY, G. K. 2000. *J. Plant Growth Regul.* **19**: 385–396.
- MUDAY, G. K. & MURPHY, A. S. 2002. *Plant Cell* **14**: 293–299.
- MUN, J. H., LEE, S. Y., YU, H. J., JEONG Y. M., SHIN, M. Y., KIM, H. LEE, I. & KIM, S. G. 2002. *Gene* **292**: 233–243.
- NAKAYAMA, T., YAOI, T. & KUWAJIMA, G. 1999. *J. Neurochem.* **72**: 373–379.
- NAPIER, R. M., DAVID, K. M. & PERROT-ROCHENMANN, C. 2002. *Plant Molec. Biol.* **49**: 339–348.
- OPARKA, K. J. & SANTA CRUZ, S. 2000. *Annu. Rev. Plant Physiol. Plant Molec. Biol.* **51**: 323–437.
- OTTENSCHLÄGER, I., WOLFF, P., WOLVERTON, C., BHALERAU, R. P., SANDBERG, G., ISHIKAWA, H., EVANS, M. & PLAME, K. 2003. *Proc. Natl. Acad. Sci. USA* **100**: 2987–2991.
- PALANIVELU, R. & PREUSS, D. 2000. *Trends Cell Biol.* **10**: 517–524.
- PALANIVELU, R., BRASS, L., EDLUND, A. F. & PREUSS, D. 2003. *Cell* **114**: 47–59.
- PARTHASARATHY, M. V., PERDUE, T. D., WITZTUM, A. & ALVERNAN, J. 1985. *Am. J. Bot.* **72**: 1318–1323.
- PETERSEN, M., BRODERSEN, P., NAESTED, H., ANDREASSON, E., LINDHART, U., JOHANSEN, B., NIELSEN, H. B., LACY, M., AUSTIN, M. J., PARKER, J. E., SHARMA, S. B., KLESSIG, D. F., MARTIENSSON, R., MATTSSON, O., JENSEN, A. B. & MUNDY, J. 2000. *Cell* **103**: 1111–1120.
- PICKARD, B. G. 1973. *Bot. Rev.* **39**: 172–201.
- PICKARD, B. G. 1984. *Plant Cell Environm.* **7**: 171–178.
- POLEVOI, V. V. 2001. *Russ. J. Plant Physiol.* **48**: 545–555.
- POLI, D. B., JACOBS, M. & COOKE, T. J. 2003. *Am. J. Bot.* **90**: 1405–1415.
- PLIETH, C. & TREWAVAS, A. J. 2002. *Plant Physiol.* **129**: 786–796.
- RAMPUTH, A.-I. & BOWN, A. W. 1996. *Plant Physiol.* **111**: 1349–1352.
- RASHOTTE, A. M., DELONG, A. & MUDAY, G. K. 2001. *Plant Cell* **13**: 1683–1697.
- REGGIANI, R. & LAORETI, P. 2000. *Plant Cell Physiol.* **41**: 1392–1396.
- RHODES, D., HANDA, S. & BRESSAN, R. A. 1986. *Plant Physiol.* **82**: 890–903.
- RHODES, J. D., THAIN, J. F. & WILDON, D. C. 1996. *Planta* **200**: 50–57.
- ROBERTS, J. K. M., HOOKS, M. A., MIAULLIS, A. P., EDWARDS, S. & WEBSTER, C. 1992. *Plant Physiol.* **98**: 480–487.
- ROSHCHINA, V. V. 2001. *Neurotransmitters in plant life*. Enfield, Science Publishers Inc.
- ROSS, S. M., ROY, D. N. & SPENCER, P. S. 1989. *J. Neurochem.* **53**: 710–715.
- RÜCK, A., PALME, K., VENIS, M. A., NAPIER, R. M. & FELLE, H. 1993. *Plant J.* **4**: 41–46.
- SABATINI, S., BEIS, D., WOLKENFELT, H., MURFETT, J., GUILFOYLE, T., MALAMY, J., BENFEY, P., LEYSER, O., BECHTOLD, N., WEISBEEK, P. & SCHERES, B. 1999. *Cell* **99**: 463–472.
- SACHS, T. 2000. *Plant Cell Physiol.* **41**: 649–656.
- SACHS, T. 2003. *BioEssays* **25**: 897–903.
- SAUGY, M. & PILET, P. E. 1985. *Plant Sci. Lett.* **37**: 93–99.
- SCHRADER, J., BABA, K., MAY, S. T., PALME, K., BENNETT, M., BHALERAU, R. P. & SANDBERG, G. 2003. *Proc. Natl. Acad. Sci. USA* **100**: 10096–10101.
- SCHÜTZ, I. & FURUYA, M. 2001. *Planta* **212**: 759–764.
- SCOTT, A. C. & ALLEN, N. S. 1999. *Plant Physiol.* **121**: 1291–1298.
- SHABALA, S. 2003. *Plant & Soil* **255**: 217–227.
- SHABALA, S. N. & NEWMAN, I. A. 1997a. *Physiol. Plant.* **100**: 917–926.
- SHABALA, S. N. & NEWMAN, I. A. 1997b. *Physiol. Plant.* **101**: 770–776.
- SHABALA, S. N. & NEWMAN, I. A. 1998. *J. Membr. Biol.* **161**: 45–54.
- SHELP, B. J., WALTON, C. S., SNEDDEN W. A., TUIN, L. G., ORESNIK, I. J. & LAYZELL, D. B. 1995. *Physiol. Plant.* **94**: 219–228.
- SHELP, B. J., BOWN, A. W. & MCLEAN, M. D. 1999. *Trends Plant Sci.* **4**: 446–452.
- SHIMMEN, T. 2001a. *Aust. J. Plant Physiol.* **28**: 567–576.
- SHIMMEN, T. 2001b. *Plant Cell Physiol.* **42**: 366–373.
- SHVETSOVA, T., MWESIGWA, J., LABADY, A., KELLY, S., THOMAS, D., LEWIS, K. & VOLKOV, A. G. 2002. *Plant Sci.* **162**: 723–731.
- SILINSKY, E. M. & REDMAN, R. S. 1996. *J. Physiol.* **492**: 815–822.
- SIMONS, P. 1981. *New Phytol.* **87**: 11–37.
- SIMONS, P. 1992. *The action plant*. Blackwell Publishers, Oxford, England.
- SIVAGURU, M. & HORST, W. J. 1998. *Plant Physiol.* **116**: 155–163.
- SIVAGURU, M., BALUŠKA, F., VOLKMAN, D., FELLE, H. H. & HORST, W. J. 1999. *Plant Physiol.* **119**: 1073–1082.
- SIVAGURU, M., PIKE, S., GASSMANN, W. & BASKIN, T. I. 2003. *Plant Cell Physiol.* **44**: 667–675.
- STANKOVIC, B. & DAVIES, E. 1996. *FEBS Lett.* **390**: 275–279.
- STEFFENS, B., FECKLER, C., PALME, K., CHRISTIAN, M., BÖTTGER, M. & LÜTHEN, H. 2001. *Plant J.* **27**: 591–599.
- STREETER, J. G. & THOMPSON, J. F. 1972. *Plant Physiol.* **49**: 572–578.
- SUN, Q., YODA, K., SUZUKI, M. & SUZUKI, H. 2003. *J. Exp. Bot.* **54**: 1627–1635.
- SWARUP, R., FRIML, J., MARCHANT, A., LJUNG, K., SANDBERG, G., PALME, K., & BENNETT, M. 2001. *Genes Dev.* **15**: 2648–2653.

- TANADA, T. & VINTEN-JOHANSEN, C. 1980. *Plant Cell Environm.* **3**: 127–130.
- TANG, W., BRADY, S. R., SUN, Y., MUDAY, G. K. & ROUX, S. J. 2003. *Plant Physiol.* **131**: 147–154.
- THELLIER, M., DESBIEZ, M. O., CHAMPAGNAT, P. & KERGOSIEN, Y. 1982. *Physiol. Plant.* **56**: 281–284.
- THOMINE, S., LELIEVRE, F., BOUFFLET, M., GUERN, J. & BARBIER-BRYGOO, H. 1997. *Plant Physiol.* **115**: 533–542.
- THOMPSON, J. K., PETERSON, M. R. & FREEMAN, R. D. 2003. *Science* **299**: 1070–1072.
- TIAN, H., KLÄMBT, D. & JONES, A. M. 1995. *Proc. Natl. Acad. Sci. USA* **270**: 26962–26969.
- TOMSIG, J. L. & CREUTZ, C. E. 2002. *Cell. Mol. Life Sci.* **59**: 1467–1477.
- TOURNAIRE-ROUX, C., SUTKA, M., JAVOT, H., GOUT, E., GERBEAU, P., LUU, D.-T., BLIGNY, R. & MAUREL, C. 2003. *Nature* **425**: 393–397.
- TRETYN, A., KENDRICK, R. E., BOSSEN, M. E. & VREDENBERG, W. J. 1990a. *Planta* **182**: 473–479.
- TRETYN, A., BOSSEN, M. E. & KENDRICK, R. E. 1990b. *J. Plant Physiol.* **136**: 24–29.
- TREWAVAS, A. 2002. *Nature* **415**: 841.
- TREWAVAS, A. 2003. *Ann. Bot.* **92**: 1–20.
- TRIPATHY, S., KLEPPINGER-SPARACE, K., DIXON, R. A. & CHAPMAN, K. D. 2003. *Plant Physiol.* **131**: 1781–1791.
- TROMBLEY, P. Q. 1998. *J. Neurophysiol.* **80**: 755–761.
- TURANO, F. J., PANTA, G. R., ALLARD, M. W. & VAN BERKUM, P. 2001. *Mol. Biol. Evol.* **18**: 1417–1420.
- TURANO, F. J., MUHITCH, M. J., FELKER, F. C. & MCMAHON, M. B. 2002. *Plant Sci.* **163**: 43–51.
- VIAN, A., HENRY-VIAN, C., SCHANTZ, R., LEDOIGT, G., FRACHISSE, J.-M., DESBIEZ, M.-O. & JULIEN, J.-L. 1996. *FEBS Lett.* **380**: 93–96.
- VIZI, E. S., NITAHARA, K., SATO, K. & SPERLAGH, B. 2000. *J. Autonom. Nerv. Syst.* **81**: 278–284.
- VOLKOV, A. G., COLLINS, D. J. & MWESIGWA, J. 2000. *Plant Sci.* **153**: 185–190.
- VOROBIEV, L. N. & MANUSADZINAS, L. 1983. *Physiol. Plant.* **59**: 651–658.
- WALLACE, W., SECOR, J. & SCHRADER, L. E. 1984. *Plant Physiol.* **75**: 170–175.
- WARD, J. M., PEI, Z.-M. & SCHROEDER, J. I. 1995. *Plant Cell* **7**: 833–844.
- WEISENSEEL, M. H. & MEYER, A. J. 1997. *Planta* **203**: 98–106.
- WILDON, D. C., THAIN, J. F., MINCHIN, P. E. H., GUBB, I. R., REILLY, A. J., SKIPPER, Y. D., DOHERTY, H. M., O'DONNELL, P. J. & BOWLES, D. J. 1992. *Nature* **360**: 62–65.
- WODZICKI, T. J. 1993. *Acta Soc. Bot. Pol.* **66**: 355–364.
- WODZICKI, T. J. & WODZICKI, A. B. 1981. *Physiol. Plant.* **53**: 176–180.
- WODZICKI, T. J., WODZICKI, A. B. & ZAJĄCZKOWSKI, S. 1979. *Physiol. Plant.* **46**: 97–100.
- WODZICKI, T. J., WODZICKI, A. B. & ADAMCZYK, J. 1999. *Acta Soc. Bot. Pol.* **68**: 201–209.
- WOJTASZEK, P., VOLKMANN, D. & BALUSKA, F. 2004. Polarity and cell walls. In: LINDSEY, K. (ed), *Polarity in plants*, Blackwell, Oxford, UK, pp. 72–121.
- YAMAMOTO, Y., KOBAYASHI, Y., DEVI, S. R., RIKISHI, S. & MATSUMOTO, H. 2002. *Plant Physiol.* **128**: 63–72.
- YAMAMOTO, Y., KOBAYASHI, Y., DEVI, S. R., RIKISHI, S. & MATSUMOTO, H. 2003. *Plant & Soil* **255**: 239–243.
- YOSHIHARA, M. & LITTLETON, J. T. 2002. *Neuron* **36**: 897–908.
- ZAWADZKI, T. 1980. *J. Exp. Bot.* **31**: 1371–1377.
- ZAWADZKI, T., DAVIES, E., DZIUBINSKA, H. & TREBACZ, K. 1991. *Physiol. Plant.* **83**: 601–604.
- ZBELL, B. & WALTER-BACK, C. 1988. *J. Plant Physiol.* **133**: 353–360.
- ZIMMERMANN, S., THOMINE, S., GUERN, J. & BARBIER-BRYGOO, H. 1994. *Plant J.* **6**: 707–716.
- ZHOLKEWICH, V. N., ANISKIN, D. N. & DUSTMAMATOV, A. G. 2003. *Dokl. Biol. Sci.* **392**: 138–141.

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