signalling system, such as phospholipase D, play an early role during cytokinin signalling to the *ARR5* gene promoter (G.A. Romanov *et al.*, unpublished). Cytokinin signalling will certainly turn out to be complex. However, now that the actors are known, a range of genetic and molecular tools should rapidly unravel further hidden secrets of cytokinin signalling.

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Targeting detoxification pathways: an efficient approach to obtain plants with multiple stress tolerance?

Dorothea Bartels

A serious factor limiting the engineering of stress tolerance has been our ignorance about the function of stress-induced genes. A stress-activated novel aldose–aldehyde reductase was cloned from alfalfa. The ectopic expression of this gene in tobacco resulted in tolerance to oxidative stress and dehydration. Physiological analysis suggested that aldose reductase probably functions by reducing the level of reactive aldehydes. This provides a promising perspective for the development of crop plants with improved stress tolerance.

Environmental stress conditions are major factors limiting plant productivity and plant distribution[1]. The increasing world population necessitates that crop plants be grown in areas that are prone to being negatively effected by abiotic stress conditions such as water deficit, saline soils, low temperatures or heavy metal contamination. The nature of the damage at the plant cellular level caused by abiotic stress such as high salt concentrations, dehydration or freezing is not entirely clear. However, an important consequence of damage might be the production of reactive oxygen species (ROS) that cause cellular injury, such as lipid peroxidation or protein and nucleic acid modification[2] (Fig. 1). Many research activities have been initiated to reveal resistance mechanisms that enable plants to avoid or tolerate stress. Molecular studies have focused on the identification of genes that are activated by different forms of stress and might thus contribute to tolerance[3–7]. The results of current research projects indicate that stress tolerance is a complex quantitative trait and that no real diagnostic marker has been reported yet. In

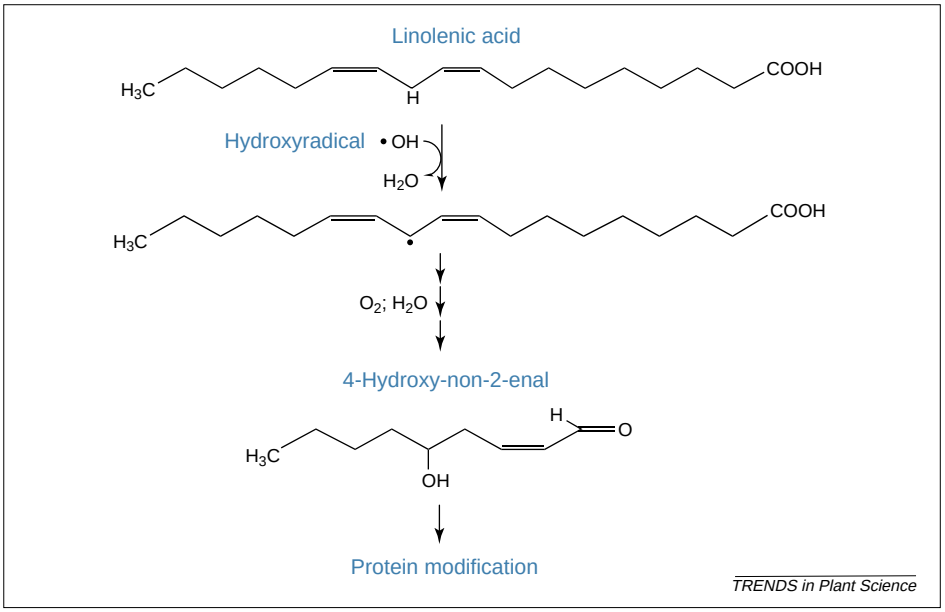

Fig. 1. Possible peroxidation pathway of linolenic acid. The hydroxy radical takes a hydrogen-atom from carbon 11, which leads to an electron deficiency among carbons 9 to 13. Chain breakage reactions might subsequently occur to produce aldehydes, for example, 4-hydroxynon-2-enal. If the aldehyde accumulates, it will react with other cellular compounds such as proteins.

spite of the characterization of many stress-responsive genes, the functions of few genes have been established[5]. These facts make it difficult to design a 'transgenic approach' to improve stress tolerance.

Aldo-keto reductase gene superfamily

A group of stress-activated genes that could not be related to a biochemical pathway belongs to the family of aldose–aldehyde reductases, a subgroup of the aldo-keto reductase superfamily[8]. Aldose–aldehyde reductases form a large gene family and have been described for many organisms including plants. As putative substrates for these enzymes, many aldehyde compounds have been identified and are supposed to act in diverse pathways, but the physiological target of most of them is unknown. Oberschall and colleagues[9] have shown elegantly that a novel plant NADPH-dependent aldo-keto reductase might function in a detoxification pathway and thus prevent stress damage. The authors isolated the gene MsALR encoding an aldose–aldehyde reductase from alfalfa. This gene is closely related to genes of other origins: for example, human aldehyde and aldose reductase genes[8]; a barley gene that was shown to be associated with the acquisition of desiccation tolerance in barley embryos[10]; and an aldose reductase gene from the desiccation-tolerant resurrection plant *Xerophyta viscosa*[11], which was expressed only under dehydration conditions.

Engineering stress tolerance

The transcript of the *MsALR* gene was present in all tissues of an alfalfa plant, and it accumulated to higher levels in response to a wide range of stress treatments: osmotic stress, heavy metal (cadmium), the plant hormone ABA, or chemicals generating free radicals. The recombinant alfalfa aldose reductase protein exhibits specific enzymatic activities: it reduces aldose and aldehyde substrates using NADPH as a cofactor but not NADH. In its biochemical properties, the alfalfa enzyme resembles the human aldose reductase in several aspects (substrate preference or inhibition studies) rather than the recombinant barley aldose reductase[8,12,13]. In mammalian cells, aldo-keto reductases can reduce 4-hydroxynon-2-enal (HNE), which is a major lipid peroxide degradation product[13] (Fig. 1). 4-hydroxynon-2-enal is a cytotoxic lipid aldehyde, which is reactive with proteins. Interestingly, the recombinant alfalfa protein could also metabolize 4-hydroxynon-2-enal efficiently, indicating a possible detoxification potential. Although the alfalfa protein was less active than the human protein, 4-hydroxynon-2-enal was preferred over glyceraldehyde as a substrate. This observation prompted Oberschall and colleagues[9] to test whether the recombinant protein can also be effective in transgenic plants. For this the MsALR cDNA was ectopically expressed in tobacco plants under the control of the CaMV 35S promoter[9]. The transgenic tobacco plants overexpressing the alfalfa aldose reductase showed reduced damage (measured by chlorophyll fluorescence) when exposed to oxidative stress agents such as paraquat (a herbicide that produces ROS) or hydrogen peroxide. The plants were also more tolerant to heavy metal, salt or dehydration stress. The transgenic tobacco plants recovered better from damage caused by water deficit than the untransformed wild-type control plants. This improved tolerance is probably because of a decrease in the level of ROS. This assumption was confirmed: the concentration of reactive aldehydes were considerably lower in tobacco plants over-producing alfalfa aldose reductase than in non-transformed plants. The experiment strongly supports the notion that stress tolerance is due to the elimination of aldehydes by reducing them to non-reactive molecules.

Although aldose reductase transcripts have previously been associated with stress tolerance in plants, mainly from expression profiles, direct proof was still missing. The work by Oberschall *et al.*[9] identified a possible physiological role for a group of aldose reductase genes and how they contribute to stress protection. If it is possible to target the same detoxification pathway efficiently in agriculturally and horticulturally important plants, then the discovery of improved stress tolerance will have a wide and important potential in its application. It is important with respect to application that tolerance to a broad range of stressors has been obtained. Evidence that preventing or diminishing the accumulation of ROS in response to stress is a promising way to engineer stress tolerance is also supported by other approaches[14]. Overexpressing antioxidant enzymes or ROS-scavenging enzymes is one possibility for the induction of functional detoxification systems, for example, transgenic tobacco or alfalfa plants expressing Mn-superoxide dismutase tend to have reduced injury from water-deficit stess[15]. Transgenic plants that overproduce osmolytes such as mannitol, fructans, proline or glycine-betaine also show increased resistance to some forms of abiotic stress[16,17]. It is hypothesized that the synthesized osmolytes act as ROS scavengers.

Conclusions

Preventing oxidative stress or reducing the level of the reactive molecules appears to be a promising approach to obtain plants with diverse tolerance to abiotic stress. Engineering crop plants that can cope with oxidative molecules could have a broad application in agriculture. The examples discussed in this article show that there are several pathways that can be used to obtain stress-tolerant plants. Because there are still many stress-activated genes with unknown functions, future experiments might discover further pathways that lead to reduced levels of reactive molecules. Therefore, as yet unidentified stress-induced genes could have the potential to engineer plants with improved stress tolerance.

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Techniques & Applications

Functional imaging of plants by magnetic resonance experiments

Walter Köckenberger

Microimaging based on magnetic resonance is an experimental technique that can provide a unique view of a variety of plant physiological processes. Particularly interesting applications include investigations of water movement and spatially resolved studies of the transport and accumulation of labelled molecules in intact plant tissue. Some of the fundamental principles of nuclear and electron magnetic resonance microimaging are explained here and the potential of these techniques is shown using several representative examples.

Only a few techniques make it possible to map physical and chemical parameters in intact, living plants. Imaging methods based on magnetic resonance are among the most versatile techniques within this group. The information that is available from the use of nuclear magnetic resonance (NMR) techniques includes the *in vivo* distribution of metabolites, water flow in the vascular conduits and physical properties such as water diffusion and relaxation mechanisms in different cellular compartments. In addition, electron paramagnetic resonance (EPR) techniques can be used to detect free stable radicals in plant tissue.

Noninvasive images of virtual transverse sections

Several nuclides, such as ^{1}H, ^{13}C, ^{15}N and ^{17}O, have angular momentum (nuclear spin) and a magnetic moment. These two nuclear properties are a prerequisite for any NMR experiment. In microimaging experiments with plants, images are frequently formed from the dominant signal of the protons bound in the water molecule. However, it is also possible to detect protons in metabolites with much lower concentrations or to use ^{13}C nuclei in an imaging experiment. The principles underlying the detection of nuclei by NMR are summarized in Box 1. In essence, the application of a strong magnetic field creates a weak sample magnetization, which can be manipulated by irradiating with appropriate radio-frequency pulses. The sample magnetization can then be detected through an induction of a weak voltage in a coil placed around the sample. The frequency components of the time-dependent signal can be extracted by Fourier analysis and represented in a spectrum.

If the sample consists of one type of nuclei, such as protons bound in the water molecule, and a homogeneous external magnetic field is applied, there is only one frequency component in the spectrum (Fig. 1a). The signal can be spatially encoded by exploiting one of the most fundamental principles of magnetic resonance, which states that the resonance frequency is proportional to the interacting local magnetic field. Therefore, if a magnetic field gradient is applied across the sample, the local magnetic field becomes spatially dependent and the detected signal

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