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John Bryant takes a closer look at some of this month's Original Articles

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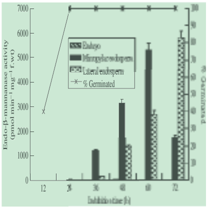
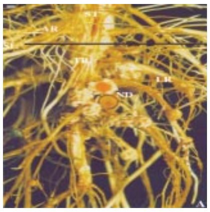


Reinforced cells do not prevent breakout

We have noted before that, in some hard-seeded species, the endosperm cells in mature seeds are extensively thickened with mannans and/or galactomannans and that enzymic hydrolysis of these walls recycles monosaccharides for seedling growth. Further, since the endosperm surrounds the embryo, these cells form a barrier to germination and it is not clear whether the hydrolysis of the cell wall polymers is a prerequisite for germination. This is the question addressed by **Gong *et al.*, Guelph, Canada (pp. 1165–1173)**. They have looked at the timing of radicle emergence and its relationship to the activity of endo-β-mannanase in several species in which the endosperm cell walls are thickened in this way. The taxonomic range of the species investigated was very broad but the picture that emerges is consistent across the range: germination, as indicated by radicle emergence, is not dependent on endo-β-mannanase activity. Instead, the hydrolysis of the cell walls is a post-germination event. How then does the radicle break out? Microscopic examination of the germinating seeds revealed two mechanisms. In most species studied, including fenugreek (*Trigonella foenum-graecum*), carob (*Ceratonia siliqua*) and coffee (*Coffea arabica*), there is a region of the endosperm, located under the micropyle and over or surrounding the tip of the radicle, where the cell walls are not thickened. It is here that the emerging radicle breaks out. In the second mechanism, seen in date (*Phoenix dactylifera*), there is a ring of unthickened cells, underlying the operculum in the enclosing testa; the endosperm fractures round this ring, allowing the emerging radicle to push its way through the endosperm as if removing a lid. Both mechanisms imply exquisite positional controls of the endosperm cell wall thickening during seed development and provide further examples of the amazing range of variation on the basic angiosperm lifestyle.



Emergency air supply piped to nodules

Although the nitrogenase enzyme involved in N-fixation is inhibited by oxygen, the overall process has a high demand for respiratory energy and is thus very sensitive to anoxia. This is nicely shown by the work of **Thomas *et al.*, Campinas, Brazil (pp. 1191–1198)** on N-fixing root nodules in soybean. Flooding the root system led to oxygen availability being reduced by approx. 90 % and an almost complete disappearance of the metabolic products of N-fixation (ureides and glutamine). However, there was also a rapid morphological response: adventitious roots, derived from divisions of the pericycle, appeared in the area of the stem–root junction; aerenchyma formed in the same region as well as in the taproot, lateral roots, the newly formed adventitious roots and at a later stage in the less deeply submerged nodules. Over a period of 21 days, three different mechanisms for aerenchyma formation were observed. In the taproot and lateral roots, the initial formation of aerenchyma was by lysigeny of cortical cells, but the cortex was later displaced by a spongy parenchyma (secondary aerenchyma) formed by cell division in the pericycle. In adventitious roots, aerenchyma was initially formed by separation of cell layers (schizogeny) followed by lysigeny of cortical cells and eventually, after about 21 days, secondary aerenchyma was also formed. By contrast, in both the stem–root junction and in nodules, aerenchyma arose almost exclusively via cell division (from the pericycle in the former; from the phellogen in the latter). Aerenchymatous nodules had a very different appearance from non-flooded nodules but nevertheless possessed a normal complement of leghaemoglobin, suggesting a capability for N-fixation. Evidence that they actually did fix N was that between 7 and 10 days after the start of the flooding treatment, ureides and glutamine were once again detectable in significant amounts in the xylem. The formation of aerenchyma had thus led to the restoration of normal nodule function.

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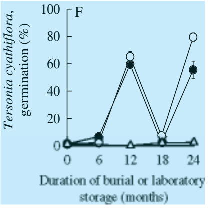
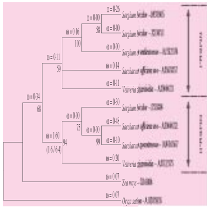


Smoke in the water

There are plant species in many parts of the world whose seeds germinate after fire. The mechanisms for this are varied but in the two fire ephemerals, *Actinotus leucocephalus* and *Tersonia cyathiflora*, studied by **Katherine Baker and colleagues in Australia (pp. 1225–1236)**, organic chemicals in smoke appear to be the key signal. However, these species also exhibit other facets of seed dormancy. To show this, the authors buried seeds in either recently burnt or unburnt soil and exhumed them at intervals over a period of 2 years to study the factors that affected germination. Here we focus on the seasonal variation in response to dormancy-breaking chemicals supplied as smoke-water. The data for *T. cyathiflora* are very clear: at no time did the seeds germinate in plain water; smoke-water was an absolute requirement, whether the seeds had been buried in burnt or unburnt soil. However, imposed on this response to smoke-water was a very clear seasonal pattern. Seeds exhumed in spring did not germinate; seeds exhumed in autumn did. Thus there was a seasonal cycling between total dormancy and germinability. Seeds stored dry in the laboratory did not germinate in any season; burial in soil was essential. In *A. leucocephalus*, the picture was more complex. Firstly, seeds stored in the laboratory showed an increasing ability over 24 months to germinate in smoke-water (burial was not necessary to break dormancy) with no seasonal variation. Secondly, seeds buried in non-burnt soils germinated in plain water. However, these seeds and those buried in burnt soils (which required smoke-water) showed the same seasonal pattern of germinability as seen in *T. cyathiflora*. In *A. leucocephalus*, the seasonal variation could be obtained by exposing seeds in the laboratory to cycles of moisture and temperature variation. This was not successful in *T. cyathiflora*, in which other factors, as yet unknown, must play a major role.



A pair of genes

Readers of this journal know very well that genome sizes vary enormously between different species, based mainly on large differences in the amount of non-coding DNA sequences. However, there is also some variation, albeit relatively small, between species in respect of gene numbers. Why then does one species possess, and by implication need, 10 % more genes than another? The conundrum is beautifully illustrated by the work of **Rondeau *et al.* (La Réunion and Lausanne, France, pp. 1307–1314)** on the evolution of the gene(s) encoding plastid NADP-dependent malate dehydrogenase (NADP-MDH). This enzyme is involved in shuttling malate out of the plastid but in C_4 plants it also has a key role in the initial fixation of CO_2. The question thus arises as to whether C_4 plants have two separate genes encoding NADP-MDH or whether both functions are covered by one gene. The authors have used RT–PCR to amplify the NADP-MDH sequences from a number of species in the sub-family Panicoideae of the family Poaceae. This sub-family contains both C_3 and C_4 members. Phylogenetic analysis of the sequence data show a number of features, of which we focus here on the existence of two forms of the gene: NMDH-I and NMDH-II. These appear to have arisen by duplication of an ancestral gene followed by sequence divergence. Does the possession of two genes relate to the ability to carry out C_4 photosynthesis? Certainly several C_4 species possess and express both NMDH-I and II while the C_3 *Oryza sativa* possesses only a NMDH-II-like gene. However, this neat story is spoiled by a classic C_4 species, *Zea mays*, which also possesses only a NMDH-II-like gene. So, within the same sub-family there are species that possess two genes covering two roles and at least one species that possesses only one gene to cover the same two roles. Functional genomics can thus reveal some very intriguing situations.

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