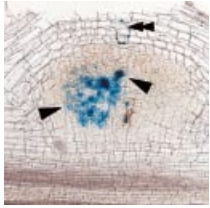


# ContentSelect

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

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## Symbiosis gets the nod

In the early days of genetic manipulation it was frequently suggested that it would be possible to transfer nitrogen-fixing capability to non-leguminous crop plants. However, doubts were soon raised about the ability of non-leguminous plants to provide the highly regulated environment of the root nodule in which  $N_2$ -fixation occurs. Despite the role of the host plant, host genes involved in establishing the symbiosis have been the focus of less attention than the *nod* genes of the bacterial symbiont, but this gap is being filled by the type of work described by **Tsyganov and associates (St Petersburg, Russia and Aachen, Germany; pp. 357–366)**. This group has previously reported that more than 40 pea genes (the *Sym* genes) are involved in establishing the symbiosis. This paper focuses on the initiation phase, using mutants that cannot develop root nodules (*Nod*<sup>-</sup>). The first indication of an 'infection' by *Rhizobium* is root hair curling in response to the bacterium. Even this apparently simple response involves several genes, some of which also participate in the next steps: root hair curling leads to formation of the infection thread (by which the bacteria enter the root) and at the same time cortical cells are induced to divide in an organized manner to form the nodule primordium. These two events are kept in step first because some genes are involved in both processes, and secondly because there is signalling between the two processes. By the time the nodule primordium has been established, at least 15 host genes have been involved. The picture is further complicated as we recall that there is also cross-talk between the products of these genes and those of the *Rhizobium* symbiont. So, while these results emphasize the difficulties of transferring  $N_2$ -fixation to non-legumes, they do provide background information for enhancement of the process in legumes themselves.

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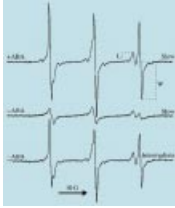


## Drop dead gorgeous

In some plant species, successful pollination is followed by a very marked decrease in the attractiveness of flowers to pollinators. The flowers may close to prevent access; the petals may change or lose colour, become senescent and drop off as the plant diverts resources away from flowering to embryogenesis. An extensive survey of the plant kingdom shows that this sequence is mainly, but not completely, confined to dicots; within the dicots it does *not* occur in those species where the female phase precedes the male phase, or in species where the flowers are in any case very short-lived. In those species that do exhibit this marked change in floral attractiveness, a key question is the nature of the signalling system that perceives a successful pollination event and leads to petal senescence. Based on other forms of plant senescence, a possible candidate for triggering petal death is the hormone ethylene. **Van Doorn (University of Wageningen; pp. 375–383)** has tested this idea by exposing non-pollinated flowers of about 200 different species to physiological concentrations of ethylene. In general, he found that treatment with ethylene mimicked the effect of pollination, suggesting that endogenous ethylene is indeed involved in the loss of floral attractiveness. In those species that do not show this sequence of events, ethylene either had no effect or induced an onset of rapid senescence. Focusing specifically on those plants that exhibit the marked change in floral attractiveness, the results show that ethylene triggers the changes more rapidly than pollination. The likely explanation for this is that in normal pollination there are steps 'upstream' of ethylene synthesis in the signal transduction pathway. The nature of those steps is not yet known but should surely be the subject of further research as we seek to understand the processes involved in successful seed set.

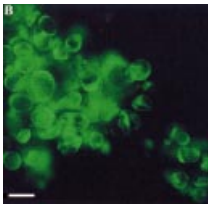
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*Continued overleaf*



### Drying to survive

Many plant species can now be propagated by generation of somatic embryos — embryos that arise not from a sexual fusion but from individual cells within a callus culture. However, although they are morphologically similar, somatic and sexual embryos differ significantly in their desiccation tolerance. Embryos inside seeds can exist for extended periods with moisture contents as low as 10 %. During natural embryogenesis and seed maturation, the abscisic acid (ABA) content of the embryo increases, and it is known that this prevents precocious germination. However, it is now clear from research with mutants of *Arabidopsis* that ABA is also important for acquisition of desiccation tolerance by the embryo. Somatic embryos, by contrast, have very poor desiccation tolerance, and very low germination percentages are observed after they have been dried down to the water contents of normal dry seeds. Germination percentages may be improved slightly if drying is rapid but are still much lower than those of normal embryos. The obvious question, and one of those addressed by **Sreedhar and colleagues (Guelph and Wageningen; pp. 391–400)**, is whether ABA can induce desiccation tolerance in somatic embryos. Based on their results, the answer is a resounding ‘Yes!’ Transfer of 22 d somatic embryos to 20  $\mu\text{M}$  ABA dramatically increases desiccation tolerance as measured by germination rate after desiccation. But what are the cellular effects of ABA? Using several sensitive biophysical techniques, the authors have obtained evidence that in desiccation-intolerant embryos, membranes are more damaged, lipid phase transitions are more chaotic (possibly resulting from oxidative damage) and proteins have more  $\beta$ -sheet (perhaps resulting from partial proteolysis) than in tolerant embryos. Based on these and other data, the authors conclude that the role of ABA is to decrease metabolic activity in the embryo to a level at which desiccation damage is minimized. How ABA does this is another question.



### Green light for protoplast fusion

Fusion of protoplasts has been seen as a means of achieving hybridization between related species which, despite that relatedness, are not inter-fertile. In the past, much research effort has been invested in protoplast fusion as a route to crop improvement, and there has been some success with a limited number of genera, including *Petunia* and *Citrus*. Today, although GM has diverted attention from protoplast fusion, the latter still has potential applications. A major bottleneck in the process is the regeneration of plants from the fused protoplasts. Furthermore, even when apparently successful regeneration has been achieved, the resulting plants may be chimeric or may even have totally lost one of the parental genomes. Markers are needed to follow the fate of the two parental lines through fusion, culture and regeneration. Ideal markers will be those that can be readily and quickly detected by non-destructive techniques. One such potential marker is Green Fluorescent Protein (GFP), encoded by a gene isolated from the jellyfish, *Aequorea victoria*. Essential for its use as a marker in protoplast fusion is its ease of detection by illuminating live cells or tissues at the appropriate excitation wavelength. A group working at the Agricultural Research Institute in Valencia (**Olivares-Fuster *et al.*; pp. 491–497**) has fused transgenic protoplasts of citrange (a *Citrus–Poncirus* hybrid), expressing the *GFP* gene, with protoplasts of *Citrus reticulata*. GFP was monitored from initial fusion through to establishment of plants. Expression of the *GFP* gene was of course indicative of only one parental line; the presence of both was confirmed by the use of DNA markers. However, it is noted that different forms of GFP are available that fluoresce at different wavelengths; it would thus be possible to use this very practicable technique to check the presence of both parental lines and so monitor the success of the somatic hybridization.