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Rice biotechnology: Somatic hybridisation for improv ed salinity tolerance and xylem colonisation by rhizobia for endophytic nitrogen fixation

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Abstract. At the Arles Seminar in September 1996 on Mediterranean Rice Research Activities, it was highlighted that rice is now regarded as a model plant for biotechnological developments. At this meeting on The Future of Water Management for Rice in Mediterranean Countries it is now timely to highlight studies on the somatic hybridisation of rice for improved salinity tolerance and on the xylem colonization of rice by rhizobia for endophytic nitrogen fixation. Somatic hybrid plants were obtained following the electrofusion of rice cell suspension-derived protoplasts with non-dividing leaf protoplasts of *Porteresia coarctata*, a saline tolerant wild species. One of the somatic hybrid plants had an allohexaploid chromosome complement and full-chromosome complements of both *Oryza sativa* and *P. coarctata*, as determined using genomic *in situ* hybridisation. We have also investigated whether rhizobia, whilst unable to nodulate a non-legume such as rice, might nevertheless be able to colonize the xylem of roots of rice, following entry at lateral root cracks. We have shown that extensive colonization of the xylem of both primary and lateral rice roots can be detected using rice grown under controlled conditions and inoculated with *Azorhizobium caulinodans* ORS571 (pXLGD4) which carries the *lac*Z gene and produces a blue precipitate in the presence of X-Gal. Studies are now being undertaken to determine the detailed pathway of xylem invasion following crack entry, and also to determine the possible role of xylem colonization in endophytic nitrogen fixation in rice.

Key Words. *Porteresia coarctata* – *Azorhizobium caulinodans* – Diazotrophs.

Introduction

In any consideration of water management for rice in Mediterranean Countries, ways of breeding rice for improved salinity tolerance and investigating the possibilities of incorporating nitrogen fixation capacity into rice merit detailed analysis, particularly the opportunities arising from plant biotechnology. It is well established that soil salinity markedly suppresses the growth of rice, and that rice suffers from a mismatch between nitrogen used and nitrogen supplied as fertilizers, resulting in a heavy loss of applied nitrogen fertilizers with consequent nitrate pollution of ground water. Two recent biotechnological advances offer new approaches to these two major challenges. Firstly, the production of intergeneric somatic hybrids of rice and the highly salt tolerant species, *Porteresia coarctata* will now enable an assessment in breeding programmes of the use of *P. coarctata* for the introduction into rice of genes for salinity tolerance.

Secondly, the development of procedures for the inoculation of rice with the diazotroph *Azorhizobium caulinodans*, and the endophytic establishment of this nitrogen-fixing rhizobial strain in the rice plant, is now offering exciting opportunities for investigating the possibilities of incorporating nitrogen fixation capacity into rice.

I – Somatic hybrids of rice (Oryza sativa (+) Porteresia coarctata)

Porteresia coarctata (previously called *Oryza coarctata*) is a halophytic species, closely related taxonomically to *Oryza sativa*, which can withstand total submergence in sea water for periods of at least 10 hours per day (Bal and Dutt, 1986). Pre-zygotic incompatibilities have resulted in the species being recalcitrant to sexual hybridisation with *O. sativa* in most sexual crossing attempts; there are, however, a few reports of successful sexual crosses using special procedures, including embryo rescue (Jena 1994, Fararooq *et al.,* 1996, Brar *et al.,* 1997).

It is well established that pre-zygotic sexual incompatibilities in plants can be overcome using somatic protoplast fusion coupled with plant regeneration from the heterokaryons formed by interspecies protoplast fusions (Kumar and Cocking, 1987). Production of heterokaryons by the fusion of mesophyll protoplasts of *Porteresia coarctata* (2n = 4x = 48) and cell suspension derived protoplasts of *Oryza sativa* (2n = 2x = 24) was reported in 1990 (Finch, Slamet and Cocking) and recently, following plant regeneration, somatic hybrid plants have now been produced (Jelodar *et al.,* 1999). One of the somatic hybrid plants had an allohexaploid chromosome complement of 72 (2n = 6x = 72) and, following examination of its vegetative clones by genomic *in situ* hybridisation (GISH), it was confirmed as an amphiploid somatic hybrid with 72 chromosomes, containing full chromosome complements of both *O. sativa* (24 chromosomes) and *P. coarctata* (48 chromosomes). It will now be possible to assess the potential of this novel amphiploid somatic hybrid (*O. sativa* (+) *P. coarctata*) in breeding programmes for the introduction into rice of genes for salinity tolerance.

Such a breeding programme to introgress genes from highly salt tolerant *P. coarctata* into rice is more realistic than attempts at this stage to produce transgenic rice plants. Salinity tolerance is a complex trait involving many genes, the genetic basis of which is poorly understood. As discussed by Flowers *et al.*, 1997, transformation of plants with what appear to be the simplest of the characters for salinity tolerance does not necessarily have the hoped for consequences. As recently highlighted by Swaminathan (1998) rice, unlike wheat, has a wide range of adaptation to growing conditions, which is an asset in an era of global change. Anticipatory research for meeting the challenges of climate change is equally important. Swaminathan (1998) has pointed out the need for more work to be done with saline tolerant species like *Porteresia coarctata* to meet these challenges arising from climate change.

II – Endophytic establishment of Azorhizobium caulinodans in rice

Nitrogen is the nutrient that most frequently limits agricultural production; improved water management and reducing the inputs of nitrogenous fertilizers in rice production are closely linked. Recent advances in understanding symbiotic *Rhizobium*-legume interactions at the molecular level, the discovery of endophytic interactions of nitrogen-fixing organisms with non-legumes and the ability to introduce genes into rice by transformation have stimulated researchers world-wide to harness opportunities for nitrogen fixation and improved nitrogen nutrition in rice (Ladha *et al.,* 1997).

Fresh impetus to the attempted endophytic establishment of nitrogen fixing bacteria (diazotrophs) in rice has arisen from our discovery that *Azorhizobium caulinodans* produces nitrogen fixing nodules on the legume *Sesbania rostrata* and colonizes the xylem of the roots of this legume (O'Callaghan *et al.,* 1997). This led us to investigate whether this rhizobial strain, whilst not nodulating the non-legume rice, might nevertheless be able to colonize the xylem of the roots of rice. Surface-sterilized rice seeds of the Indian varieties, ADT36 and CR1009, were germinated and grown aseptically in tubes containing nitrogen-free Fåhræus medium (0.8% w/v agar) with 5×10^{-5}M naringenin, inoculated with *Azorhizobium caulinodans* (pXLGD4) carrying a constitutive *lac*Z reporter gene, and maintained in a growth chamber.

Azorhizobium caulinodans expressing *lac*Z were readily located in plant tissue, as they produced a dark blue precipitate in the presence of X-Gal. Plants were fixed in glutaraldehyde and processed for light and electron microscopy. Toluidine blue-stained sections of roots of plants inoculated with *Azorhizobium caulinodans* (pXLGD4) showed numerous bacteria in the xylem of primary and emergent lateral roots, with electron microscopy confirming the presence of bacteria in the xylem elements. Electron microscopy verified the presence of bacteria in the xylem, in those regions where the blue precipitate had been observed by light microscopy (Gopalaswamy *et al.,* 1998).

The xylem of sugarcane has been suggested as a potential site of nitrogen fixation by diazotrophs, providing both the low pO2 for nitrogenase activity and a site for metabolic exchange (Boddey *et al.,* 1995). This ability of rice to interact with the diazotroph *Azorhizobium caulinodans* resulting in xylem invasion may similarly provide a niche for the establishment of endophytic symbiotic nitrogen fixation. If so, rice like sugarcane may be enabled to be grown with significant reductions in inputs of nitrogenous fertilizers (and with associated improvements in reduced water pollution) without loss of yield.

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