digests of 3 of the 4 plasmids. Also shown are restriction digests of pRSFO885 and other plasmids. It was found that the molecular weights estimated from the *Bam*HI fragments were lower than the estimates obtained by using the *Eco*RI fragments. This discrepancy could be resolved if the small *Bam*HI fragment of pRSFO885 was considered to be present in duplicate (data not shown). Duplication of this fragment is required probably because both the *Bam*HI sites in pRSFO885 are in vital segments of plasmid DNA and the only way to hold an insert stably at a *Bam*HI site would be by having a fragment duplicated such that both sites are left intact and a third new site is available for insertion of foreign DNA. A schematic representation of this is shown in figure 2. It should be noted that the three *Bam*HI fragments have to be in the correct orientation for an extra *Bam*HI site to be available for insertion of foreign DNA in the presence of two functional *Bam*HI sites.

The four plasmids, and a few others, were tested for their stability. As shown in table 2, the four chimeric plasmids derived from *Bam*HI-cut pRSFO885 are more stable than the plasmids derived from pJ1–8 (with the exception of pKuvr1)[17]. The fact that all four plasmids carried only the small fragment in duplicate may be purely a coincidence.

A new principle of cloning DNA emerges from this i.e. even when the cutting site (of *Bam*HI) in the DNA may be in a gene with a vital function, the problem can be overcome by duplication of the intervening plasmid segments in the construct.

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TRANSFER OF SALINE TOLERANCE FROM ONE STRAIN OF RICE TO ANOTHER BY INJECTION OF DNA

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SALINITY and drought are regarded as the major problems facing rice production in the world. The moderately saline-tolerant variety of rice, Pokkali, is widely cultivated in the brackish water areas of Kerala, India and the sensitive variety of rice IR-20, is cultivated in the irrigated areas of the country. The tall and long-duration rice variety Pokkali cannot be adopted for cultivation under upland conditions.

Direct injection of genomic DNA of Pokkali into developing floral tillers of variety IR-20 produced transgenic seeds that were similar to Pokkali in husk colour, germinated well in 0.2 M NaCl, and had a 4–6-fold higher proline content. Transgenic seeds expressing chimeric genes have practical use in plant breeding to improve the quality and yield of cereal crops.

Genomic DNA (75 μg) isolated from rice variety Pokkali[1] and purified by gel filtration through a

Biogel A5m column[2], in 1 ml of 10 mM Tris–HCl, 1 mM EDTA, pH 8.5, was injected into each of 4 floral tillers of rice variety IR-20 about 75 days after transplantation at the zero auricular stage when the inflorescence is about 2 cm. After about 45 days, mature seeds from injected and uninjected tillers were collected, dehusked, and spread on germination papers soaked in water or 0.2 M NaCl at 30°C for 36 h in the dark. About 13% of Pokkali and 17 out of 623 seeds from the injected tillers of IR-20, germinated. None of the 603 IR-20 seeds from uninjected tillers germinated in 0.2 M NaCl. Individual seeds were homogenized in 0.5 ml of 3% (v/v) sulphosalicylic acid, and an equal volume of a mixture of glacial acetic acid and 6 M orthophosphoric acid in the ratio 3:2 (v/v) containing 2.5% (w/v) ninhydrin was added. The mixture was heated on a boiling water bath for 1 h and cooled on ice. The proline–ninhydrin complex was extracted with 1 ml of toluene, and proline was estimated from absorbance at 520 nm[3].

The amino acid proline is known to act as osmoprotectant in cellular adaptation of plants to osmotic stress caused by drought or salinity[4–6]. Tolerance tc salinity was tested by the ability of rice seeds to germinate and accumulate proline when kept in high concentration of NaCl solution. Over 66, 30 and 13% of the seeds of the rice variety Pokkali germinated in 0.1, 0.15 and 0.2 M NaCl respectively at 30°C in the dark. Only 5% of the IR-20 seeds germinated in 0.1 M NaCl, above which there was no germination. However, the vigour of germination as shown by the weight and shoot and root length of Pokkali seeds germinated in 0.2 M NaCl was lower than that of those germinated in the absence of salt, showing the stress caused by salinity on germination. The amount of proline was more than three-fold higher in Pokkali seeds germinated in 0.2 M NaCl than in seeds germinated in the absence of salt, while the variety IR-20 did not show such an increase (table 1).

Table 1 *Proline content of seeds of rice varieties IR-20 and Pokkali and of transgenic seeds*

	Proline (nmoles)	
Variety	0 M NaCl	0.2 M NaCl
IR-20	13.9±0.6	14.3±1.5
Pokkali	28.4±3.6	97.1±8.5
Transgenic	—	90.6±7.5

Each value is the average for 6 seeds.

Direct delivery of DNA into living plant tissues has been developed to circumvent the problems of generation of plants from transformed protoplasts and the host-range restrictions of *Agrobacterium tumefaciens*. Use of high-velocity microprojectiles for delivery of DNA into *Allium cepa* epidermal cells was tried successfully[7]. DNA from Sea island cotton injected into axial placenta of glandless upland cotton about a day after pollination seems to have been expressed and inherited[8]. A plasmid containing aminoglycoside phosphotransferase gene under the control of nopaline synthase promoter was injected into developing floral tillers of rye plants; a few transgenic kanamycin-resistant seedlings which expressed the enzyme activity were obtained[9]. We obtained 927 mature seeds from IR-20 rice tillers that received Pokkali DNA by injection. These seeds were smaller than those of normal IR-20, and the colour of the husk of about 50% of the seeds was similar to that of seeds of Pokkali (figure 1). The aleurone layer of the seeds from injected IR-20 was darker than that of normal IR-20 seeds, but not as dark as that of Pokkali seeds. Of 623 such seeds, 17 germinated well in 0.2 M NaCl. The vigour of germination was comparable to that of Pokkali seeds germinated in 0.2 M NaCl. None of the 603 seeds from IR-20 tillers that did not receive Pokkali DNA germinated in 0.2 M NaCl. The amount of proline in six germinated (0.2 M NaCl) seeds from injected IR-20 was 124, 53, 120, 95, 60 and 89 nmoles.

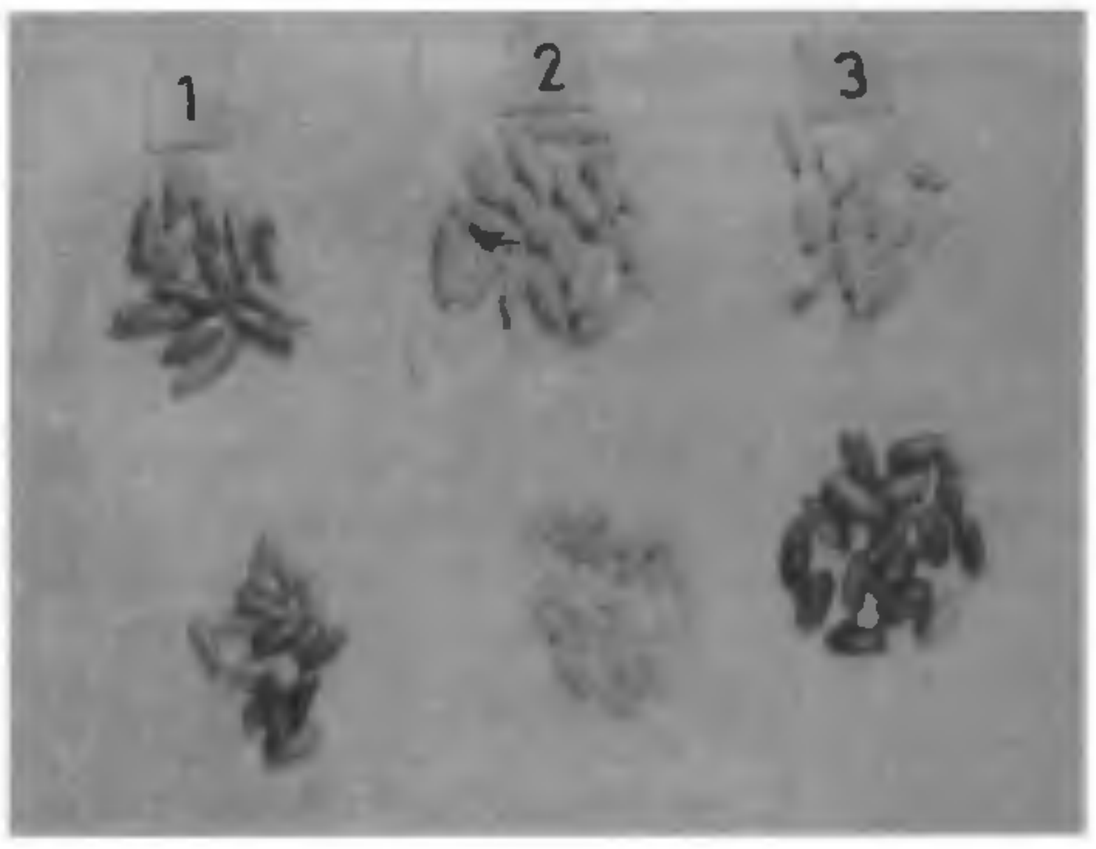

Figure 1. Seeds of (1) IR-20 plants that received Pokkali DNA, (2) normal IR-20, (3) Pokkali. Top row, with husk; bottom row, dehusked.

which are 4–8-fold higher than that of normal IR-20 seeds (table 1).

In various organisms, like bacteria, algae, crustaceans and higher plants, accumulation of proline in cells during water stress was found to prevent cell dehydration. The gene for γ-glutamyl kinase, which is the key enzyme in the biosynthesis of proline, may become insensitive to feedback inhibition by proline, resulting in overproduction of proline in Pokkali and transgenic rice seeds. Transfer of DNA from the same or a different species of plant by direct injection into floral tillers may find wide application in the generation of transgenic plants with specific qualities.

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