found that at full transpiration, the amount of ^{32}P in shoots in mycorrhizal plants was almost 2.3 times that at low transpiration. The higher ^{32}P activity in L-4 pairs of leaves in all treatments may be due to their presence close to the roots.

The study confirms the hypothesis[7] that the use of a mixture of two compatible species of VAM fungi can prove to be a better alternative to a species of VAM fungi universally adapted to different agro-climatic conditions. The results also indicate that the increased growth in plants inoculated with dual inocula compared to those inoculated with single inocula of VAM fungi may be due to the increased transfer of P to the shoot from the root.

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Somatic tissues leading to embryogenesis in cumin

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Seeds of cumin (*Cuminum cyminum* L.) were germinated on Murashige and Skoog's (MS) medium supplemented with benzyladenine (BA) (8 mg/l) and kinetin (1 mg/l). From the germinated seedlings, various explants (roots, hypocotyls and cotyledons) were excised and inoculated on MS medium supplemented with 8 mg/l of BA. Out of all the explants, hypocotyls were found to be the source to somatic embryos. However, separation of embryogenic clumps was difficult on solidified medium; thus, on maturation root poles remained suppressed. Nevertheless, complete regeneration was observed in 10% of the cultures.

PLANT regeneration via somatic embryogenesis is preferred over organogenesis due to various advantages, one distinct advantage being that somatic embryos are bipolar structures bearing both root and shoot apices. Somatic embryogenesis is a pre-requisite in crop improvement. It was reported that embryogenesis has been observed in all the Apiacean spices, except in cumin[1]. Nevertheless, investigations in cumin led to rhizogenesis, caulogenesis and complete plantlet formation through callus[2].

Cumin is an important spice used as a flavouring agent for culinary purposes. Its oil has significant medicinal properties (antispasmodic, antihysteric, stomachiachic, astringent and cooling). The technique of tissue culture has applications in propagation and improvement of crops. The present investigation is the first report on somatic embryogenesis leading to regeneration in cumin.

Cumin seeds were surface-sterilized and inoculated on MS medium[3], solidified with 0.8% agar, incorporated with BA (8.0 mg/l) and kinetin (1.0 mg/l).

Explants from two-week-old seedlings were inoculated on MS medium supplemented with BA (8.0 mg/l).

From the mixed type of calli, simple callus (non-differentiating + embryo initials) and clumps of embryo initials were carefully separated and inoculated onto MS medium with the same BA concentration.

Seedlings obtained from seeds inoculated on BA-containing-medium differed from normal seedlings in their entire thickness. From the various organs, i.e. roots, cotyledons and hypocotyls, cotyledons showed necrosis, roots formed pale yellow callus which did not

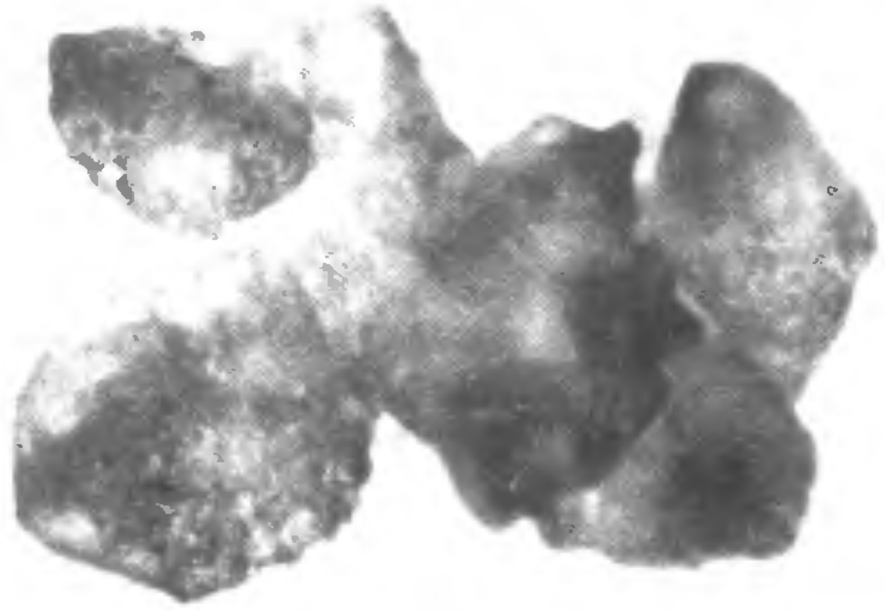

Figure 1. Group of proembryo initials

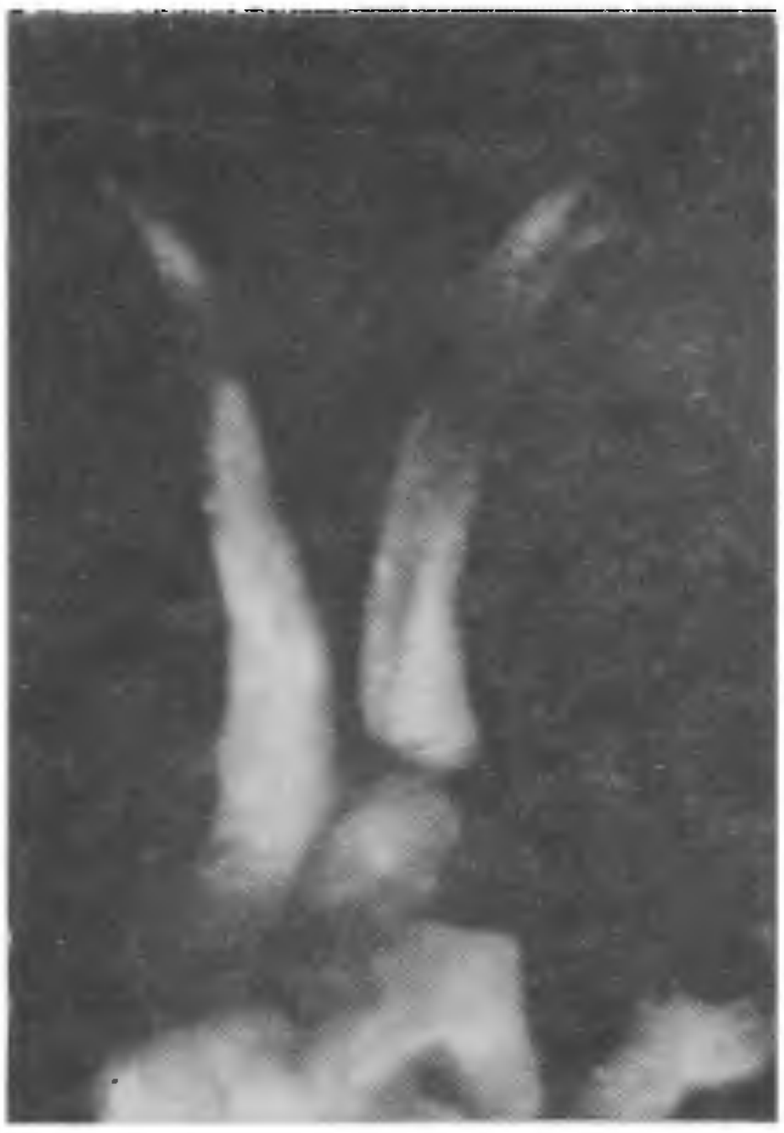

Figure 2. Dicotyledonary embryos with distinct shoot and root poles.

differentiate, whereas hypocotyls were able to produce somatic embryos.

The proembryo clumps were separated from the rest of the callus mass (Figure 1). Passing from the globular and heart-shaped stages, the embryos reached maturity, attaining a torpedo shape. However, separation of embryos was a difficulty continuously faced (Figure 1, where embryos are seen to be lying in clusters because of the solid medium) and it could be achieved in only 10% of the cultures where distinct shoot and root poles could be observed (Figure 2), while the rest of the cultures could produce only shoots as the root poles remained suppressed.

Hypocotyl, which has proved to be a productive source of embryogenic callus in our experiments, has been reported earlier also to be so[1,3,5].

The present study strongly supports the need of cytokinins for somatic embryogenesis. For the Apiacean members, auxins have been stated as a requisite for embryogenesis[6–9]. On the contrary, auxins were not found to be essential for the induction of embryogenesis.

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Fertile plants regenerated from mesophyll protoplasts of cold-tolerant rice

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A protocol for regeneration of fertile plants from leaf-sheath-derived protoplast of *Oryza sativa* (L.), which is a pre-requisite for utilization of genetic manipulations at the cellular level without or with very little creation of genetic variation, is reported. Isolated protoplasts from inner leaf sheath of 7-day-old seedlings were cultured in modified N6 medium in the presence of feeder cells. The presence of feeder cells and reduction of osmoticum played an important role in obtaining the sustained divisions of mesophyll protoplasts. Among the three procedures, i.e. protoplast cultured in liquid medium, in 0.15% agarose and on top of a cellulose nitrate membrane in the presence of feeders, the latter procedure exhibited the highest number of protoplast-derived calli (protocalli). The protocalli obtained from 0.15%-agarose-cultured protoplasts (without membrane) produced a larger number of plantlets.

REPRODUCIBLE plant regeneration from protoplasts is an essential pre-requisite for genetic manipulations such as somatic hybridization, cytoplasmic recombination and direct uptake of DNA. Plant regeneration from protoplasts has been achieved through callus and suspensions in most of the gramenease, i.e. rice[1–5], wheat[6], sugarcane[7,8], pearl millet[9], maize[10] and barley[11]. It is generally observed that the establishment of cell culture in cereals is not only difficult, but they also tend to become less regenerable and accumulate genetic changes[12,13] on prolonged culture. Due to the above reasons, extensive efforts have been made[14] since 1974 to induce sustained divisions and regeneration of plants from mesophyll protoplasts in cereals. Recently, Gupta and Pattanayak[15] have reported the regeneration of plants from mesophyll protoplasts of rice. The reported protocol of Gupta and Pattanayak[15] is not repeatable[4,16]. In this paper, we report a simple and reproducible procedure for induction of