Regenerated plants (40) with developed roots were transferred to soil (pots) with 80% survival.

Regeneration of plants from protoplasts through encapsulation in alginate beads is reported in dicots[14,15]. The present technique has been applied successfully to indica rice for the first time. This protocol of protoplast culture and plant regeneration can be successfully exploited for gene transfer in rice by direct DNA uptake.

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# Modified procedure for bromide estimation with ion-selective electrode for predicting nitrate movement in soil

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**Because of the similar behaviour, bromide ($Br^-$) could be used as a tracer for nitrate ($NO_3^-$) in soil-water systems. However, successful use of $Br^-$ depends upon accurate recovery of added $Br^-$ from soil and detection by instrument. In the present study efficiency of different extraction procedures for $Br^-$ estimation was examined with ion-selective electrode. These procedures gave variable recoveries of added $Br^-$. The method which gave the highest recovery (90–93%) of the added $Br^-$ was selected and modified to obtain precise and near 100% recovery in an Alfisol and a Vertisol of ICRISAT Centre, near Hyderabad.**

LEACHING of fertilizer-derived $NO_3^-$ in soils can be measured accurately with $^{15}N$ techniques but is cost prohibitive. A tracer bromide ($Br^-$), which is similar in charge and behaviour to $NO_3^-$ in soil-water systems[1,2] may provide the basis for an alternative technique of $NO_3^-$ estimation. $Br^-$ has several other characteristics to its advantages – it is in low background concentration, nonreactive with soil constituents[3], nontoxic to plants[4] biologically conserved[5], less likely to contaminate the environment[6], easy to analyse, and inexpensive.

Development of ion-selective electrodes has rendered measurement of $Br^-$ more precise. However, successful use of $Br^-$ as a tracer for $NO_3^-$ movement in the soil depends upon accurate recovery of added $Br^-$ from soils and accurate detection of it by $Br^-$ ion-selective electrode. We therefore conducted experiments to evaluate several procedures used by past researchers for $Br^-$ extraction from soils[6–12]. These procedures involved the use of different soil-to-water (or soil-to-electrolyte) ratios, shaking time, etc. Our objective was to identify and refine the most appropriate procedure to obtain accurate recovery of added $Br^-$ from Alfisols and Vertisols at ICRISAT Centre, Patancheru, near Hyderabad.

These procedures gave variable recoveries of added $Br^-$ (88–95%). The method which gave the highest recovery in both Alfisol (90%) and Vertisol (93%) involved shaking of 25 g soil samples with 49 ml distilled water and 1 ml of 5 M $NaNO_3$ as an ion strength adjuster for 30 min, followed by filtration[7]. This method was selected and further modified to improve the accuracy of $Br^-$ recovery, using four alternative physico-chemical treatments applied to the filtrate[13]. The method which gave the most accurate $Br^-$ recovery (98–100%) (Table 1) involved addition of 0.5 ml of $H_2O_2$ (30% w/v) into the filtrate and heating for 10 min on a water bath at 80–85°C. After cooling, $Br^-$ was estimated by ion-selective electrode (PHM85 precision pH meter, Radiometer, Copenhagen). The excellent performance of this method may be due to the elimination of some interfering ions, particles of organic substances or dissolved gases, which

Table 1. Precision and accuracy of modified method in recovering added $Br^-$ with bromide ion electrode

| Bromide added ($\mu g\ g^{-1}$)* | Bromide recovery ($\mu g\ g^{-1}$)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Alfisol | | | | Vertisol | | | |
| | Range | Mean | SE | CV (%) | Range | Mean | SE | CV (%) |
| 10 | 9 5–10 0 | 9 77 | ±0 09 | 2 21 | 9 6–10 6 | 9 97 | ±0 13 | 3 19 |
| 100 | 96 4–100 0 | 97 83 | ±0 62 | 1 55 | 97 5–102.5 | 99 83 | ±0.67 | 1 65 |

*Results based on six determinations

if present in traces in the filtrate can considerably reduce the sensitivity of the ion-selective electrode[14]. This analytical procedure was also found to be highly stable and precise (Table 1). Hence, it can be used more successfully for studying the movement of $NO_3^-$ in these soils.

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# Surface ultrastructure of *Beauveria bassiana* infecting silkworm *Bombyx mori* Linn.

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**The surface ultrastructure investigations on entomopathogenic fungi, *Beauveria bassiana* infecting silkworm *Bombyx mori* Linn. reveal that the infecting stage, i.e. oval or spherical conidia are formed on host integument from aerial hyphae. The vegetative hyphae form a network inside the integument and further divide in haemolymph. The crystals of varying size, formed of ammonium and magnesium oxalate have also been observed on integument and in haemolymph.**

THE disease, white muscardine caused by entomopathogenic fungi, *Beauveria bassiana* (Balsamo) Vuillemin in silkworm (*Bombyx mori* L.) has been responsible for considerable silkworm crop loss in the recent past. The disease is contagious in silkworm and infects the integument, digestive tract, and haemolymph[1–6]. The life cycle[3,4] and histological observations on oral infection[5,6] of *B. bassiana* infecting *B. mori* have been studied earlier. However, no attention has been paid so far on surface ultrastructure study on *B. bassiana* infecting *B. mori* in order to generate further information. Therefore, in the present paper, SEM has been used as a tool to investigate the different stages of life cycle and route of infection of *B. bassiana* infecting *B. mori* to confirm the findings generated by earlier workers based on visual and light microscopy observations.

Third instar larvae of *B. mori* ($NB_{18}$) were surface infected with $4 \times 10^5$ spore/ml and reared on mulberry leaves at $25 \pm 1^\circ C$ temperature and 60–70% RH. On the seventh day of post infection, larvae were dissected to process the infected integument, digestive tract and trachea. The tissue was fixed in 2.5% glutaraldehyde prepared in cacodylate buffer for 2 h, dehydrated in ethanol series, critically dried, coated with gold, mounted onto copper stubs and scanned under JEOL 100 CX II at 20 kV. Further, a few critically dried samples were also randomly fractured to observe under electron micro-