In this issue

Symbiosis in sugarcane

The term biological nitrogen fixation, fixing of atmospheric nitrogen into a form suitable for consumption by plants, is typically associated with symbiotic bacteria colonizing the nodules of the leguminous plants. This apart, symbionts can fix nitrogen in non-leguminous plants, and examples of free-living nitrogen fixers are also known.

Muthukumarasamy *et al.* (**page 137**) review the nitrogen fixers in non-leguminous plants, and symbiosis of *Gluconacetobacter diazotrophicus* with sugarcane plants in particular, with special reference to growth, physiology and genetics of the bacteria. According to an estimate, up to 80% of the plant nitrogen in certain sugarcane varieties are derived from endo-symbiotic bacteria. The diazotrophic endosymbionts could populate up to 10^6–10^7 cells/g fresh tissue of sugarcane. No nodules or specialized structures are found in sugarcane plants colonized by the diazotrophic bacteria. The point of entry primarily is at the site of emergence of lateral roots, but occasionally bacteria are noticed to enter through the damaged stomata in sugarcane leaves. Electron microscopic studies confirmed the presence of these bacteria in apoplast and xylem vessels of sugarcane. Association of *G. diazotrophicus* is a symbiosis of monocotyledonous non-leguminous plant sugarcane and a diazotroph. This type of colonization by *G. diazotrophicus* had been termed 'obligate endophyte' by some, and 'opportunistic endophyte' by others. *G. diazotrophicus* has been isolated from at least 9 plants.

As a microbe, *G. diazotrophicus* exhibits several interesting features. A Gram-negative, acid-tolerant obligate aerobe, it can occur as single cells, in pairs, or as a chain without endospores. The bacteria grow on high sucrose (10%) containing medium and at low pH (3.0 or so). It can fix nitrogen under micro-aerophilic conditions, up to 50% of the fixed nitrogen being excreted to the benefit of the host plant in a usable form. There are several unique features about the nitrogen fixation by *G. diazotrophicus*. Like other nitrogen-fixers, they have a cluster of *nif* (nitrogen fixation) genes, though these are not required for colonization. Unlike several other bacteria, *nifY* gene is absent; and a gene required for chemotaxis, *mcpA*, is associated with the *nif* gene cluster.

G. diazotrophicus could be used with nitrate fertilizers since nitrate does not inhibit nitrogen fixation. Nitrogenase is protected against inhibition by oxygen at high sucrose concentration (10%). Apparently, by fermenting sugars in the nutrient medium that reduces the pH below 3.0, they are antagonistic to the red rot pathogen in sugarcane, *Colletrotrichum falcatum*. They are also tolerant to several antibiotics like streptomycin, erythromycin, and rifampicin.

Sugarcane is a major cash crop in India, Brazil, Cuba, the Caribbean, Mexico, and parts of Florida. 50% of the global demand for sugar is met from the sugarcane plantations. Endophytes described in this paper will, of course, not substitute for the needs of 11.4 million metric tonnes of chemical fertilizers in India, but the work is definitely a significant step towards employing eco-friendly biofertilizers.

S.G.

Insecticidal proteins

Realizing the hazards for the environment caused by chemical insecticides, strategies for the Integrated Pest Management (IPM) were advocated by the US Department of Agriculture since 1994. IPM strategies aimed at developing natural or biological alternatives for controlling pests, weeds, and diseases of commercially important plants. One of such approaches had been to make the so-called insecticidal proteins from bacteria that could eliminate harmful pests.

Two of the insecticidal proteins available for field trials are avidin and insecticidal crystal protein (ICP). Avidin inhibits larval growth by binding to biotin that is essential for growth of insects. Traditional source of avidin had been chicken eggs, but lately avidin is being produced from transgenic corn. Avidin is a broad spectrum insecticide, whereas ICP works on a narrower range of insects, primarily lepidopteran, dipteran, and coleopteran insects. ICP has no known toxicity to beneficial insects, human, cattle and other vertebrates. These features made ICP the most widely used insecticidal protein. This protein when expressed in potato (1993), tomato (1989), tobacco (1987), cotton (1990), corn (1993) and rice (1993) confers resistance to various pests. Bhattacharya *et al.* (**page 146**) report the expression of ICP in a transgenic cabbage cultivar, Golden Acre, that confers significant resistance to diamond-back moth.

ICP was originally isolated as a proteinaceous crystalline inclusion produced by Gram-positive bacteria *Bacillus thuringiensis* (*Bt*) during the process of sporulation. The gene was later cloned from several strains of *Bt* and the gene products identified as proteins of various molecular weights (28, 64–68, 83, 132 kDa). In some strains, the 132 kDa protein is produced as a pro-toxin that is cleaved to form the 65 kDa toxin. To improve expression of the gene in plant cells, chemically synthesized genes were cloned to improve translatability of the mRNA and to design the codon usage specific for the plant hosts (US Patent No. 05380831). Bhattacharya *et al.* report results from a transgenic cabbage cultivar incorporating a synthetic *cryIA*(*b*) gene that expresses ICP significantly inside the plant cells.

A synthetic *cryIA*(*b*) gene coding for the *Bt* insecticidal protein of Bt was transferred to cabbage cultivar 'Golden Acre' by co-cultivating hypocotyl explants with *Agrobacterium tumefaciens* vectors carrying the gene. Immunoblot analysis confirmed high-level expression of

81.3 kDa Cry protein in the transgenic plants. It was shown that a synthetic gene designed for monocot codon usage can be expressed in dicotyledonous cabbage plants. Expression resulted in a significant insecticidal activity in the transgenics against the larvae of diamond-back moth (*Plutella xylostella*). *Bt*-proteins produced in transgenic plants caused rapid cessation of larval-feeding activity and subsequent inhibition of their development. At the whole plant stage, *Bt*-expressing plants were sufficiently protected against damage from diamond-back moth attack.

The authors conclude that Indian cabbage cultivar, Golden Acre, can now join the ranks of genetically modified pest-resistant plants that could be tried on the field in an integrated pest management program.

S.G.

FISHing in genomes

Fluorescent *in situ* hybridization (FISH) is the state-of-the-art molecular technique in physical mapping of the genome. It is used for identifying specific region on a chromosome with a suitable molecular probe for hybridization and has found use in medical genetics for studying chromosomal aberrations in human syndromes and in evolutionary genetics for studying phylogenetic relationships. The C-banding technique, the traditional heterochromatin banding technique based on differential Leishman staining of chromosomal matter, had been a powerful tool in cytogenetics in producing characteristic chromosomal fingerprints.

Dhar *et al*. (**page 150**) describe the C-banding pattern in *Plantago ovata* that is an important commercial source of psyllium (isabgol) used primarily as a laxative. *P. ovata* genome consists of the diploid set of 8 chromosomes. C-banding pattern in somatic chromosomes of the plant was analysed in a sample prepared from the root tips of germinating seeds. Finer mapping of the chromosomes was carried out by FISH technique using rRNA probes. The results obtained by the two different techniques are in agreement. This primary source of information is to be used by researchers interested in genetic manipulation of the plant.

Lavania (**page 124**) advocates the use of similar 'mapping' and 'painting' techniques to study chromosomal diversity and evolutionary familial relationship, emphasizing the study of 'ploidy' in plant genome, and the flux of repetitive sequences in the genomes of a population.

S.G.

Warming at the turn of the millennium

Why did the harp-string break?
I tried to force a note that was beyond
It's power, that is why the harp-string
Is broken

— Rabindra Nath Tagore

Himalaya (Sanskrit hima + alaya): Home of the Snow

Global warming is a gradual phenomenon that has caused concern at the turn of the millennium. Presumably human action, helpless or reckless, is likely to raise the average surface temperature of the planet we inhabit. The total rise in temperature is likely to be 1.4–5.8°C by the end of the 21st century.

Compared to the global warming caused over a geological time scale, magnetic storms are abrupt worldwide geomagnetic disturbances, believed to be brought by bright eruptions from the Sun's chromosphere. Incidentally, Alexander von Humboldt (1769–1859), a naturalist, was a pioneer in observing such storms by setting up observatories in the Russian and the British Empire.

Chakrabarty *et al.* (**page 167**) discuss the aftermath in the thermosphere during the recovery phase of a geomagnetic storm in the Earth's magnetosphere. Based on the observations made on the Mount Abu, the authors argue that thermospheric temperature at low latitudes oscillates with a 'response time delay' of about 12.5 h, deviating considerably from the predictions made for the high latitude zones. Such changes are caused by the natural 'geomagnetic forces', but the ablation of snow in the Himalayas could be indirectly attributed to man-made 'industrialization'. Kulkarni *et al.* (**page 120**) discuss the field-data obtained from the Indian Remote Sensing Satellite to comment on the effects of snow ablation in the Himalayas, apparently caused by global warming, on the drainage pattern in the Beas basin and the Baspa river. Global warming has invaded the 'Home of the Snow'.

S. Ganguli