

ADVANCES IN RADIATION PLANT BREEDING

REPORTS indicating that the method of inducing mutations in plants by radiation is making an important contribution to plant breeding were given at a symposium organized by the International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization of the United Nations (FAO) held in Pullman, Washington, USA, from 14 to 18 July 1969.

It was shown that at least 65 new varieties of plants evolved by the method have now been released to growers (though many others are expected soon), nearly 40 of them during the past five years. They include rice and wheat varieties with improved qualities of resistance to disease and weather, higher protein content and increased yield. Economic gains have been made in producing castor-oil in India and peppermint, the yield of which had been seriously threatened, in USA. An interesting, and commercially valuable, aspect has been the successful introduction of the technique into breeding ornamental plants.

Practical demonstrations of results obtained were provided by Washington State University, where the symposium was held. A large variety of plants bred by the method had been grown and were on view in the grounds.

Dr. Björn Sigurbjörnsson, of the FAO/IAEA Joint Division of Atomic Energy in Food and Agriculture, spoke of the progress made in the five years since the last symposium on the subject. At that time many doubts were expressed, but now success had been achieved. The efforts of dedicated scientists over four decades were now paying off in millions of hectares of superior crops throughout the world. He quoted as examples the highest-yielding rice variety in Japan, Reimei, a mutant which succeeded in competition with varieties resulting from the most intensive conventional breeding efforts. Doubling of the protein content of rice, which had been reported, also indicated the potential of the method for quality improvements.

The recent shortening by three weeks of the growing period of rice in Hungary of a neutron-induced mutant could have far-reaching effects on the extent of rice cultivation in Europe.

Mutant varieties of durum wheat had shown superior performance in trials in more than a score of countries in Asia, Africa and Europe. The FAO was recommending that some countries replace their entire wheat acreage with the new mutants.

Ingenious applications had, he said, successfully adapted one of the high-yielding Mexican dwarf wheats to local preferences in India, drastically speeding up acceptance by Indian consumers.

Mutation breeding had made a great contribution to the breakthrough called the Green Revolution, which had alleviated the problem of providing enough high-quality food to keep pace with the population growth. More important, it had drawn attention to the significance of plant breeding and the number of characteristics of high-yielding varieties which needed improvement. This was a role for which induced mutations were especially suited.

M. S. Swaminathan (India), one of the world leaders in the work, drew attention to a problem in growing castor-oil seeds which had severely affected growers. This was that it took 270 days to mature and thus was at the mercy of changes in the rainy season. As a result of inducing mutants by radiation a new variety had been evolved maturing in 120 days and was now being grown by farmers. They were now assured of a good crop every year. In addition the soil used was released for an extra 150 days for other crops such as rice, of which some varieties mature also in 120 days.

First positive results of applying the method to maize were reported by C. O. Gardner (USA), who said that yield might possibly be increased beyond the highest yet known. This could be the most important development with maize since the 1920's and was the first example of using the method effectively on open-pollinated, rather than self-pollinating, crops.

One of the best-known flavours in the world, peppermint, might have been lost but for use of the radiation method to develop resistance against disease. M. J. Murray (USA) reported that after fourteen years of fighting against a disease which could destroy whole crops it was found that use of radiation to produce plants to resist it was the only possible solution. Other methods had produced plants which resisted the disease but altered the flavour to spearmint. Use of the radiation method had saved an extremely important market.

New varieties listed, according to information available to the FAO/IAEA Joint Division up to 1 July 1969, are in types of bread

and durum wheat, barley, oats, rice, soybean, peas, beans, peanuts, rape, mustard, tobacco, peach, and a number of flowers. The countries where they have been developed are Argentina,

Austria, China, Czechoslovakia, India, Indonesia, Italy, Japan, the Netherlands, Sweden, USSR, UK and USA.—[Courtesy: International Atomic Energy Agency.]

METABOLISM OF *p*-COUMARIC ACID BY *STREPTOMYCES*—FORMATION OF CAFFEIC ACID AS INTERMEDIATE

A. M. D. NAMBUDIRI, P. V. SUBBA RAO AND J. V. BHAT

Microbiology & Pharmacology Laboratory, and Biochemistry Department, Indian Institute of Science, Bangalore-12

p-COUMARIC acid (4-hydroxy cinnamic acid) is regarded as an important aromatic compound involved in the biosynthesis of lignin in higher plants.^{1,2} This compound has been reported to be an intermediate in the metabolism of tyrosine by basidiomycete fungi.³ This communication outlines the hitherto unknown metabolism of *p*-coumaric acid by a strain of *Streptomyces nigrifaciens*.

The organism was isolated in this laboratory by enrichment culture from soil and was maintained on agar slopes containing malt extract (3%), peptone (0.5%), yeast extract (0.1%) and agar (1.5%). Cells grown on liquid medium of the same composition, without agar, were used in metabolic studies with the substrates.

Washed cells suspended in 100 ml. of 0.01 M sodium phosphate buffer, pH 7 containing 50 mg. of *p*-coumaric or caffeic acid were incubated at room temperature (25–28° C.) on a shaker. Samples (10 ml.) were drawn at 6 hr. intervals and the phenolic acids were extracted in ether (X3) after acidifying to pH 2. After removing the solvent, the residue was taken in 1 ml. ethyl acetate and the metabolites were identified by paper chromatography.^{4–6} The identity of the compounds were further confirmed by comparing the u.v. spectra with those of the authentic samples.

On incubation with *p*-coumaric acid, caffeic acid was identified as the major intermediate. On prolonged incubation for more than 24 hr. traces of protocatechuic acid was also detected on the chromatogram. One compound which gave a pink spot turning violet on spraying with diazotized *p*-nitroaniline and moving almost with the solvent front (rf. 0.77) in formic acid-water (1 : 49) could not be identified. When caffeic acid was the substrate, the medium contained protocatechuic acid in detectable amounts.

It is apparent from the above results that *S. nigrifaciens* first hydroxylates the aromatic ring of *p*-coumaric acid to form caffeic acid which is further β -oxidized yielding protocatechuic acid. Though the conversion of *p*-coumaric acid to caffeic acid is common in plants,^{2,7} it is rarely met with in micro-organisms. Reduction of the double bond in the side-chain of *p*-coumaric acid was observed by Whiting and Carr⁸ with *Lactobacillus pastorianum* var. *quinicus*, though according to Finkle *et al.*,⁹ *Aerobacter* sp. anaerobically decarboxylated the compound resulting in the formation of the corresponding styrene. Fungi, in general, oxidize the propane side-chain first to form *p*-hydroxybenzoic acid and this in its turn gets hydroxylated to form protocatechuic acid.^{3,10} However, the initial hydroxylation of the aromatic ring of *p*-coumaric acid leaving the side chain intact by *S. nigrifaciens* is similar to the observation made by Power *et al.*¹¹ with the fungus *Lentinus lepideus*, though the further conversion of caffeic acid by this organism is not known. Detailed investigations on this species will be reported elsewhere.

1. Towers, G. H. N., In *Biochemistry of Phenolic Compounds*, Edited by J. B. Harborne, Academic Press, New York, 1964, p. 249.
2. Brown, S. A., *Ann. Rev. Plant Physiol.*, 1966, **17**, 223.
3. Moore, K., Subba Rao, P. V. and Towers, G. H. N., *Biochem. J.*, 1968, **106**, 507.
4. Ibrahim, R. K. and Towers, G. H. N., *Arch. Biochem. Biophys.*, 1960, **87**, 125.
5. Reio, L., *J. Chromatog.*, 1958, **1**, 338.
6. —, *Ibid.*, 1960, **4**, 458.
7. Vaughan, P. F. R. and Butt, V. S., *Biochem. J.*, 1967, **104**, 65.
8. Whiting, G. C. and Carr, J. G., *Nature, Lond.*, 1959, **184**, 1427.
9. Finkle, B. J., Lewis, J. C., Corse, J. and Ludin, R. E., *J. Biol. Chem.*, 1962, **237**, 2926.
10. Nambudiri, A. M. D., Subba Rao, P. V. and Bhat, J. V. (Submitted for publication).
11. Power, D. M., Towers, G. H. N. and Neish, A. C., *Can. J. Biochem.*, 1965, **43**, 1397.