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XANTHOMONAS CAMPESTRIS PV. *JUGLANDIS* —A NEW REPORT FROM INDIA

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THE walnut is an important fruit crop of hills of India. The bacterial blight of walnut (*Juglans regia*) caused by *Xanthomonas campestris* pv. *juglandis* is recorded from India. The disease, observed in the orchard of the Govt. Fruit Research Station, Pithoragarh during May and June, was noted in all the ten varieties of walnut. The fruit infection (%) was Xenia 47.16; Tutle 31 25; Tutle 16 16.23; RX Giant 20; Hartley 42.85; Franquette 39.39; Conveymytle 16.15; Payne 39; G. Seedling 37.50; and Blackmore 33.73.

No infection was found on local varieties of walnut. In Switzerland the fruit infection was reported[1] to be very high i.e. Esterhazy 70–90% and Franquette 50%.

The young leaves seem to be most vulnerable to infection during mid May. The water-soaked translucent spots appear on the leaves along the midrib and sometimes on the leafsheath. These spots gradually increase in diameter, turn brown to black and form circular to irregular patches. The disease spreads to new leaves and sometimes large areas are formed due to the coalescence of a number of small spots leading to the death and shedding of leaves. Symptoms also appear on petiole and fruits (figure 1). Infection causes considerable reduction in the fruit size and premature fall of fruits. During the advanced stage dry spots become rougher, raised, cracked and the entire fruit decays. The bacterium is rod-shaped (0.2–0.5 × 1.2–2 μ in size), forms no spores and capsule, and is gram-negative and aerobic. Colonies on beefagar are circular, straw yellow to pale yeliow and slightly raised.

Figure 1. Infected fruits of walnut by *Xanthomonas campestris* pv. *juglandis*.

The pathogen has been identified as *Xanthomonas campestris* pv. *juglandis* (IMI 317492 and 317493).

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TRANSFER OF BIOLOGICALLY FIXED NITROGEN FROM SOIL TO RICE PLANT

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PADDY soils favour the activities of free-living nitrogen fixers significantly contributing to nitrogen economy[1,2], $^{15}N_2$ incorporation studies on rice plants under water culture conditions reveal that less than 10% of the fixed nitrogen was translocated to the plant tissues[3]. However, a total of 19–25% of the nitrogen fixed in the rice rhizosphere was transferred to the roots, leaves, stems and ears of the rice plant during $^{15}N_2$ exposure[4]. Considerable losses of nitrogen can also occur in waterlogged rice soils than in aerobic soils[5,6]. Evidently, information regarding the availability, transfer and distribution of biologically fixed nitrogen to rice is limited and conflicting. The present study was, therefore, aimed at demonstrating the transfer and distribution of biologically fixed nitrogen to the rice plant employing ^{15}N-tracer technique.

The transfer of biologically fixed nitrogen to the

rice plant was studied in a pot experiment. Alluvial soil (1.5 kg/desiccator) amended with 1% glucose under submerged conditions was placed in two desiccators and exposed to a gas mixture of the following composition: 30% N_2 with 60 at. % abundance ^{15}N (Prochem, London), 20% O_2 and 50% Ar. The sealed desiccators were wrapped with black paper and incubated in the dark (to arrest the growth of autotrophs) for 30 days to enrich the soil with ^{15}N through biological dinitrogen fixation. At the end of incubation, the total nitrogen in the soil was estimated by Kjeldahl method and the acidified distillates were used to determine the ^{15}N enrichment of the soil following mass spectrometry[7].

The ^{15}N enriched soil was air-dried for 10 days and transferred to pots before four seedlings of rice (15 to 20-day-old cv. Vijaya) were transplanted. The plants were raised to maturity without addition of supplemental nitrogen, harvested and separated into roots, leaves and grain. The total nitrogen and the at. % excess ^{15}N in the leaves, roots, grain and in the soil after harvest were determined. The per cent ^{15}N derived from fixed (labelled) nitrogen in different parts of the plant and in the soil after harvest was also calculated.

Since the available reports indicate that nitrogen fixation was low in unamended soils compared to soils amended with organic matter[8–10], the soil was amended with glucose to achieve higher biological assimilation of the labelled nitrogen from $^{15}N_2$. Appreciable nitrogen fixation occurred in the glucose amended soil exposed to $^{15}N_2$ for 30 days. While the total nitrogen was 1232 μg/g soil, the excess at. % of ^{15}N in the soil was 0.253. According to Rinaudo *et al*[11] nitrogen fixation due to bacterial activity in the rice rhizosphere was of the order of 2 to 5 (acetylene reduction assay) and 1 to 3 μg N/g/day (Kjeldahl method). The soil thus enriched with biologically fixed nitrogen, mainly through the activity of non-symbiotic dinitrogen fixers, was utilized for tracing the labelled nitrogen in the subsequent rice crop grown on this soil. Rice seedlings when transplanted to this soil developed symptoms of wilting perhaps due to production of organic acids and other phytotoxic substances known to accumulate during the anaerobic metabolism of glucose added initially. It was, therefore, necessary to air-dry the soil for 10 days before the rice seedlings were transplanted. The plants grew well till maturity probably due to detoxification of the soil during air-drying. The grain yield was, however, low because no supplemental nitrogen was added to prevent dilution of the labelled nitrogen in the soil.

Since the isotope could be detected in substantial amounts in different parts of the rice plant (table 1), it is evident that the fixed nitrogen from the soil was transferred to different parts of the rice plant. The distribution pattern revealed that the grain derived a major portion (32%) of the fixed nitrogen followed by the roots (10%) and the leaves (4%). Thus, about 46% of the fixed nitrogen was transferred to different parts of the rice plant which, in part, could be ascribed to rapid immobilization of the fixed nitrogen in the soil type used for the present study. In contrast, Ito *et al*[3] reported the translocation of only less than 10% of fixed nitrogen in rice under water culture conditions while 19–25% was shown to be transferred from the rice rhizosphere to the roots, stems and ears of the rice plants[4]. Nevertheless, the at. % excess observed in different parts of the rice plant is well within the reported range in the higher plants[12]. Stewart[13] provided evidence for the release of fixed nitrogen into the soil either on autolysis or in the form of extracellular materials from growing cells. A substantial portion (16%) of the fixed

Table 1 *Distribution pattern of fixed nitrogen in different parts of rice plant*

Part of the plant	Dry weight (g/pot)	Total N (mg/pot)	At % excess ^{15}N in the sample	^{15}N derived from fixed nitrogen (%)
Leaf	3.9	10.9	0.0106	4.2
Root	2.2	6.9	0.025	9.8
Grain	1.3	11.2	0.080	31.6
Soil after harvest	1500	1892	0.040	15.8

Values are the averages of two samples.

nitrogen was left in the soil after harvest which could be available to the successive crop.

Although a major portion of the fixed nitrogen was recovered in different parts of the plant, and in the soil after harvest, about 40% of the fixed nitrogen could not be accounted for. In fact, there are several reports of significant loss of nitrogen from flooded rice soils. For instance, Datta *et al*[14] observed that 24% of ammonium sulphate was lost through denitrification mainly as N_2 gas. Further, according to Becking[6], in general, only 10–50% of the nitrogen in the plant was derived from fertilizer nitrogen and 50–90% of the applied fertilizer nitrogen was probably lost to the system. This loss in rice soils is considerable since in other aerable crop soils and soil types, nitrogen losses (by denitrification) as measured by tracer techniques range from 10 to 40%[5,15]. Factors and mechanisms of significant nitrogen loss from the flooded rice soil used in the present study are not clear. Nevertheless, it may be reasonable to presume denitrification, operating in such systems, as the major means of nitrogen loss. Thus, the present study demonstrates the transfer of considerable amounts of biologically fixed nitrogen from the soil to the rice plant.

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IN VITRO PLANTLET FROM INTERNODE AND INFLORESCENCE AXIS OF *BRASSICA OLERACEA* VAR. *BOTRYTIS*

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Brassica oleracea var *botrytis* is an important vegetable crop grown throughout the world. The cell and tissue culture techniques are employed in the improvement of various crop plants[1,2]. Attempts have been made to induce differentiation and success has been reported from the callus[3,4], suspension[4], leaf lemina, mid-rib[5] segments and curd[6,7] explants. However, there is no report of differentiation from the internode and inflorescence axis. In the present communication differentiation and plantlet formation in this variety have been discussed.

Seeds of *Brassica oleracea* var. *botrytis* procured from the Agricultural Research Station, Durgapura, Jaipur were grown in experimental beds. Vegetative internodes and inflorescence axis were collected before and after flowering respectively. After surface-sterilization with 0.1% mercuric chloride and thorough washings, small segments (10 mm long) of explants were cultured on MS medium[8] supplemented with Kn (kinetin), BAP (benzyl-aminopurine) with or without IBA (indole butyric acid) and NAA (naphthalene acetic acid). All the growth substances were added to the medium before autoclaving at 1.06 kg/cm^2 pressure for 15 min. All the cultures were incubated in a growth room at 26 ± 2°C under continuous light (3000 lux) and observations were recorded daily up to 30th day. Five replicates of each treatments were kept and all the experiments were repeated twice.

Kn and BAP (0.5–5 mg/l) induced callus from the cut ends of both the explants after one week of