severe decline in the abundance and diversity of forest birds was noted in a Oak forest at a nature reserve in New Jersey, USA[5]. Similar population decline was also observed at eight other sites in eastern USA[5]. One feature common to all these sites was their isolation in relatively small patches of forests surrounded by a 'matrix' of residential areas or farmland. This dramatic decline in forest birds was not found in forests larger than 100 hectares[5,6]. Robinson and coworkers[3] have recently demonstrated that the decline of the forest birds was due to the adverse effects of forest fragmentation on the nesting success of these birds. Fieldwork showed that small fragmented forests are an unfavourable environment for nesting because of loss of eggs and nestlings to predators, such as racoons and feral cats and due to parasitism by cowbirds. An important conclusion that emerges from their study is that small forests are unfavourable for nesting not because of the habitat characteristics of the forest itself but because of the features of the surrounding landscape or the 'matrix'. If the 'matrix' has few nest predators or cowbirds, then nesting success will be similar for large and small forests. On the other hand, if the features of the 'matrix' are such that attract cowbirds, racoons or feral cats, then nesting success will be low. Robinson and coworkers determined nest predation and brood parasitism in nine different landscapes ranging from 90% agricultural to more than 90% forested. They showed that in landscapes fragmented by agricultural fields, levels of nest predation and brood parasitism were so high that population of forest birds in such areas is population 'sink', where reproduction is too low to sustain population. Areas with large forests have low rates of predation and parasitism and therefore serve as population 'sources' which may provide surplus birds to fragmented areas.

In fact, the influence of the 'matrix' can occasionally even override the negative impact of fragmentation. This is seen from the results of the experiments carried out in the Biological Dynamics of Forest Fragment Project (BDFFP) near Manaus, Brazil[4]. The findings from this 10-year long study conducted in the Brazilian Amazon, presented by Mandy Tocher of the University of Canterbury in Christchurch, New Zealand, at the meeting of the Biological Society of America, at Snowbird Utah, USA, (30 July through 3 August 1995) surprised the ecologists since it showed that frogs actually became more diverse after patches of forests were isolated. However, results of other BDFFP experiments showed that there was a well-marked decline in diversity in birds, bees, wasps and beetles after isolation of forest patches. The crucial role of the 'matrix' and its interaction with the ecology of different species is considered to be the most important finding of these experiments. It is suspected that the land outside (pastures, farmland and secondary growth) was permeable to frogs so that isolated fragments were not really fragments as far as frogs were concerned. Thus it appears that in fragmented habitats, the characteristic features of the landscape 'matrix' may be friendly for the species inside the patches, such as those permitting migration of keystone species, or may be hostile, if they promote predators and parasites of the species inhabiting the fragmented habitats.

In view of growing awareness regarding environmental issues and the realization that conservation measures should be focused on threatened habitat types and not just on individual species types[2], this is an important finding. If protected habitats are sliced by roads, human settlements, etc., the result will be fragmented patches of the habitat where conservation still may not work if the patches are too small and the landscape 'matrix' features do not favour the species living within the reserve.

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SCIENTIFIC CORRESPONDENCE

Variability in miniplasmids of *Xanthomonas campestris* pv. *malvacearum*

XANTHOMONAS CAMPESTRIS pv. *malvacearum* (*Xcm*) is one of the most important bacterial pathogens causing, on an average, yield loss of 20–30% on tetraploid cotton in India. Widespread occurrence of several highly virulent races of the pathogen, in this subcontinent, has thrown several cultivars out of cultivation[1]. Primary infection of seedling originating from internal seed infection is a significant factor in disease epidemiology[2]. Detection and rapid identification of seed-borne inoculum is necessary to develop need-based management practices. The importance of this has become more evident with the increased international movement of germplasm and commercial seed in recent years. The traditional techniques for detection of pathogen are accurate but too slow and generally cannot be applied on a large scale. Recently, plasmid-based detection methods (PCR, DNA-hybridization) are gaining importance as these are not only rapid but also very sensitive (can detect as less as ten bacteria in infected seed)[3–6]. *Xcm* contain plasmids[7,8], and pathogenicity of the most virulent race-32 (neutralizes five blight resistant genes or *B*-genes) was lost in plasmid-cured

strains[8]. In the present study plasmid profiles of different races of *Xcm* were determined in order to identify the common plasmids which, in future, could be used as probes for detection of the pathogen.

Four highly virulent races of *Xcm*, viz. race-32, race-31, race-30 and race-26 were used for plasmid profile studies. Plasmids were isolated by the methods of Birnboim and Doly[9], with a slight modification in two steps, viz. a different medium (nutrient broth) and an additional step (washing of cells with 2% NaCl solution). NaCl was necessary for clearing the exopolysaccharide of the bacterial cells. Agarose (highly purified, EEO: 0.15–0.20, gelling temperature 40–42°C; SRL Pvt Ltd, Bombay) gel (0.7%) prepared in Tris-acetate EDTA buffer was used for electrophoretic separation of plasmid DNA and DNA-marker (1 kb DNA ladder; GIBCO BRL 5615 SA). Kilobase length (kb) of plasmid was calculated by comparing with the mobility of the DNA markers.

Eight different plasmids of 60, 40, 10, 8.5, 5.5, 4, 3.7 and 2.5 kb were identified from 62 isolates of *Xcm*, representing four races (Table 1). Race-32, race-31 (Figure 1) and race-26 (Figure 2) contained five plasmids each while race-30 (Figure 1) contained only four plasmids. Two large plasmids of 60 kb and 40 kb were uniformly present in all the 62 isolates. The frequency distribution of other plasmids was 64% (10 kb), 37% (8.5 kb), 35% (5.5 kb), 23% (4 kb), 77% (3.7 kb) and 43% (2.5 kb), indicating highly variable occurrence of the miniplasmids. A miniplasmid (3.7 kb) of wide occurrence (in 77% isolates) was selected from different races for further charcterization. Complete digestion of the 3.7 kb plasmid from four strains of *Xcm* (*Xcm*-202, *Xcm*-239, *Xcm*-297 and *Xcm*-306) was done by *Eco*RI. In all these four

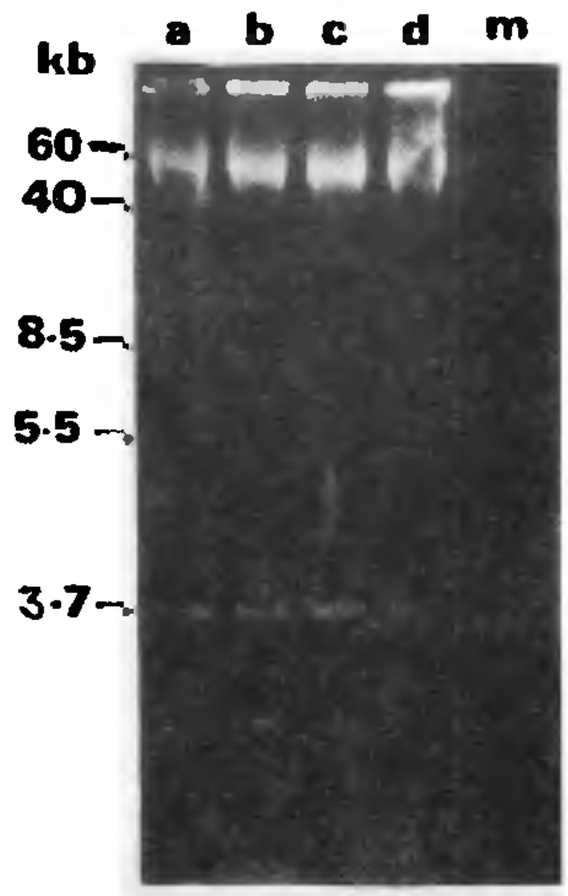

Figure 2. Plasmid profiles (undigested) of *Xcm* race-26. All the isolates (lanes a, b, c and d) contain five plasmids uniformly. Lane m, 1 kb DNA ladder.

Table 1. Plasmids in some highly virulent races of *Xanthomonas campestris* pv. *malvacearum*

Xcm	Plasmids present (size in kb)	*B*-genes neutralized
Race-32	60, 40, 10, 4, 2.5	B_2, B_4, B_7, B_{In}, B_N
Race-31	60, 40, 10, 3.7, 2.5	B_2, B_7, B_{In}, B_N
Race-30	60, 40, 10, 3.7	B_2, B_4, B_{In}, B_N
Race-26	60, 40, 8.5, 5.5, 3.7	B_2, B_{In}, B_N

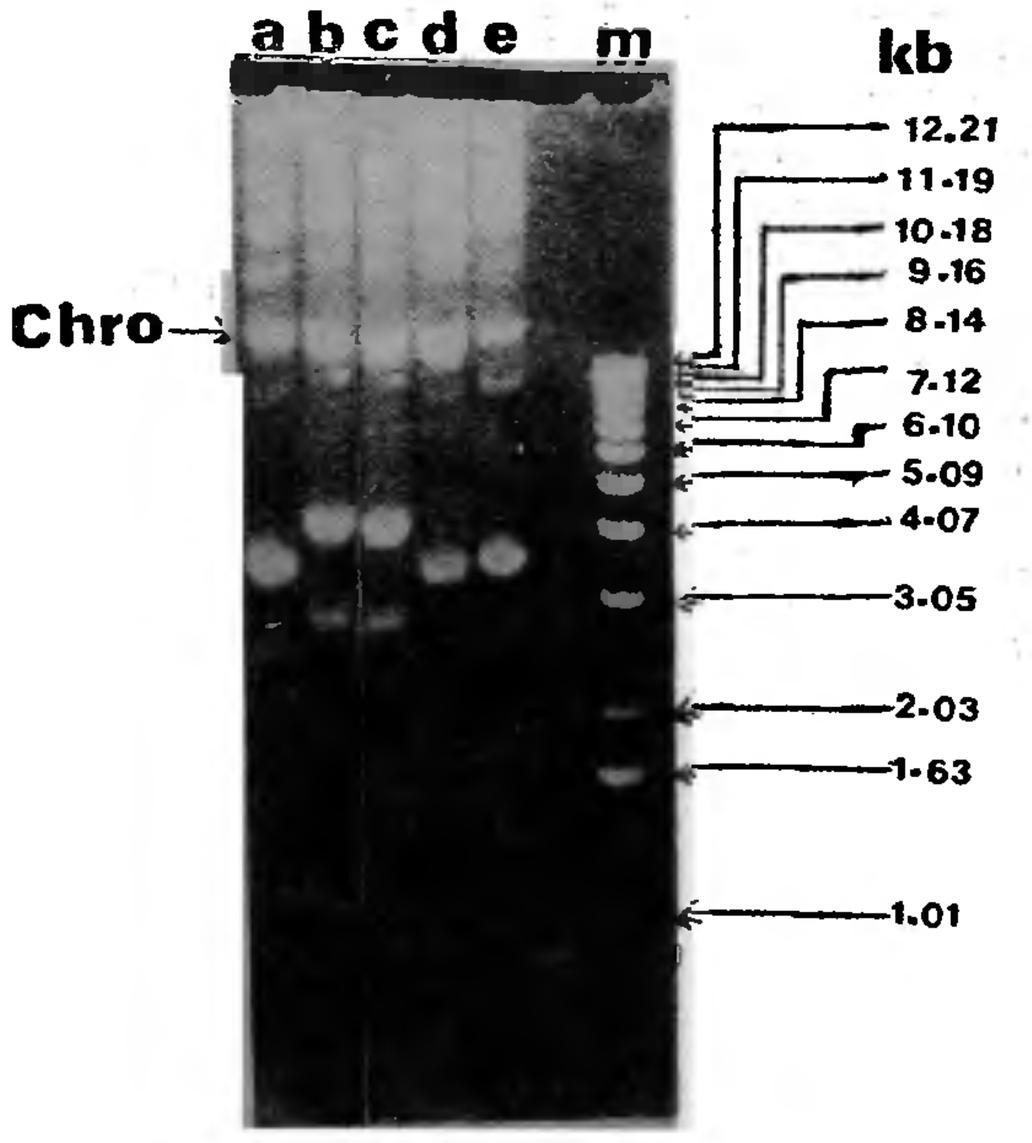

Figure 1. Plasmid profiles (undigested) of *Xcm* race-31 (lane a), race-32 (lanes b and c) and race-30 (lanes d and e). Race-31 and race-32 contain five plasmids each and race-30 contains four plasmids. The diffused band (Chro) equal to 20 kb represents sheared host chromosome. Lane m, 1 kb DNA ladder.

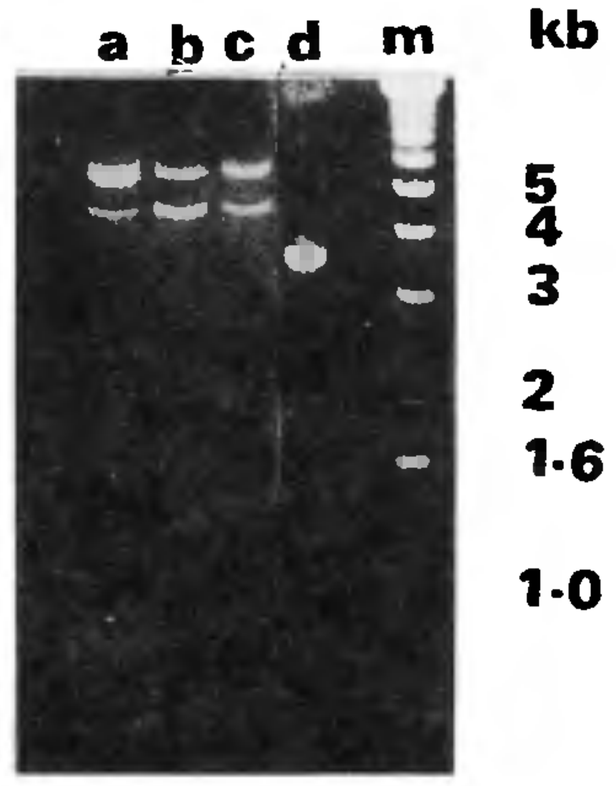

Figure 3. *Eco*RI restriction pattern of the 3.7 kb plasmid of different races of *Xcm*. Lane a, race-26; lane b, race-30; lane c, race-31 and lane d, undigested plasmid of 3.7 kb (control). Lane m, 1 kb DNA ladder.

strains, the 3.7 kb plasmid was similarly restricted producing two restriction fragments (4.5 kb and 5.5 kb) (Figure 3) in each indicating two *Eco*RI sites. This widely present miniplasmid (3.7 kb), if further characterized, might be helpful in developing genetic tools for plasmid-based detection[10,11] of this pathogen.

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ACKNOWLEDGEMENTS. We thank Prof. Anupam Varma and Dr V. G. Malathi for critical discussion and their kind help.

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Efficient regeneration of *Taxus baccata* by a non-hormonal chemical treatment

The Himalayan yew, *Taxus baccata*, is the only source of taxol, the anticancer drug in India. A very small number of this tree are found in the forests in Jageshwar area of Almora district situated at an altitude of about 1800 m. The *Taxus* plants are growing in association with dominant *Cedrus deodara* forest without interfering with each other. Large scale cutting, looping and stamping has brought the *Taxus* at the verge of extinction in this area of Kumaun Himalaya. The growth of the tree is very slow and has a long seed dormancy period. Propagation of the plant occurs through seeds and not through vegetative means in natural conditions. Formation of taxol in cell suspension cultures requires a long incubation period and which, therefore, cannot compete with commercial field propagation of *Taxus* sp.[1]. An embryo culture method to develop seedlings has been described with very little success[2]. Several factors have been studied to induce rooting in cuttings of *Taxus* sp. and it was observed that a rooting hormone IBA was usually only effective in increasing the speed of rooting[3]. Here we describe a non-hormonal chemical treatment to induce rooting in cuttings of the Himalayan yew.

Apical cuttings of 10–15 cm length of *T. baccata* were brought in sterile polythene bags from the Jageshwar forest area in December 1994. The cuttings were dipped in 2% non-hormonal chemical for 24 h and planted in polythene bags containing soil with 50% decomposed oak leaves, 25% cow-dung manure and 25% garden soil. These planted bags were kept in shade for 10 days and then were placed in the open. The atmospheric temperature of the place ranged between -4° and 25°C during the period of experiment. The *Taxus* cuttings experienced a heavy snow-fall in the second week of January 1995 and remained covered completely for 10 days under the snow. For three months till March, the cuttings continued to survive but remained dormant. In April, however, the apical dormant buds started enlarging and gradually grew into a branch of about 10 cm during the subsequent three months. The base of the cuttings under soil exhibited callus formation which increased in size with time. When the size of the callus reached about 9 mm, root (5 cm long) formation was observed. The rate of survival and regeneration of the cuttings was 70%.

These findings emphasize the importance of propagating *Taxus* through cuttings by non-hormonal chemical treatment easily. This investigation may help increase *Taxus* population for periodic harvesting of twigs and needles to meet the taxol requirements as well as in saving the Himalayan yew from exploitation and extinction.

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ACKNOWLEDGEMENTS. We thank Mr Vijendra Paul, Secretary, Hill Development, Govt of UP and Dr S. S. Mishra, Director, Herbal Research and Development Institute, Chamoli, for continuous encouragement.

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