rooting of shoots on kanamycin-containing media, indicated that stably transformed plantlets were regenerated. In addition, since regeneration from these zones occurs with minimal hormonal manipulations, the possibility of regenerating somaclonal variants is also minimized.

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Detection and role of chlorotic toxin and phytohormones in the pathogenesis of Alternaria blight in *Brassica napus*

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Destruxins are known to play an important role in the pathogenesis of the black spot disease of several *Brassica* species caused by *Alternaria brassicae*. Vivotoxicity of destruxin B was shown by the isolation and characterization from the infected leaves of *Brassica napus*. HPLC purified compound showed chlorotic activity during foliar bioassay on detached *B. napus* leaves. FTIR spectra showed depsipeptide nature of this toxic compound. An interesting compound antagonistic to the activity of chlorotic toxin was also observed which was removed during purification. This compound was suspected to be a phytohormone due to its ability to induce rooting at the surface of the *B. napus* leaves during foliar bioassay.

DESTRUXINS, a group of cyclodepsipeptides are known to play an important part in the pathogenesis of the entomopathogenic fungus *Oospora destructor*[1] and plant pathogenic fungus *Alternaria brassicae* causing black spot disease on several *Brassica* species[2]. Destruxin B isolated from *A. brassicae* was shown to cause similar symptoms on the leaves of *Brassica* sp. as that of the pathogen. The use of reversed-phase HPLC for preparative isolation of the two major toxins, destruxin B[3] and homodestruxin B[4] from the culture broth of *A. brassicae* was demonstrated by Bains and Tewari[2] and Tyagi[5]. Buchwaldt and Jensen[6] reported destruxin B in the leaves of *B. napus* infected by *A. brassicae*. In the present study an attempt was made to look for the presence of destruxin B like compounds in naturally infected leaves of *B. napus*.

Naturally infected leaves (500 g) of *B. napus* cv. BNCN-3 were used to purify the toxin using the method of Tyagi[5]. The leaves were macerated in distilled water (2 l) and filtered through 4 layers of muslin cloth. The filtrate after centrifugation at 5990 g for 15 min was extracted with equal volume of ethyl acetate and flash-evaporated to dryness at 45°C. Dried sample was

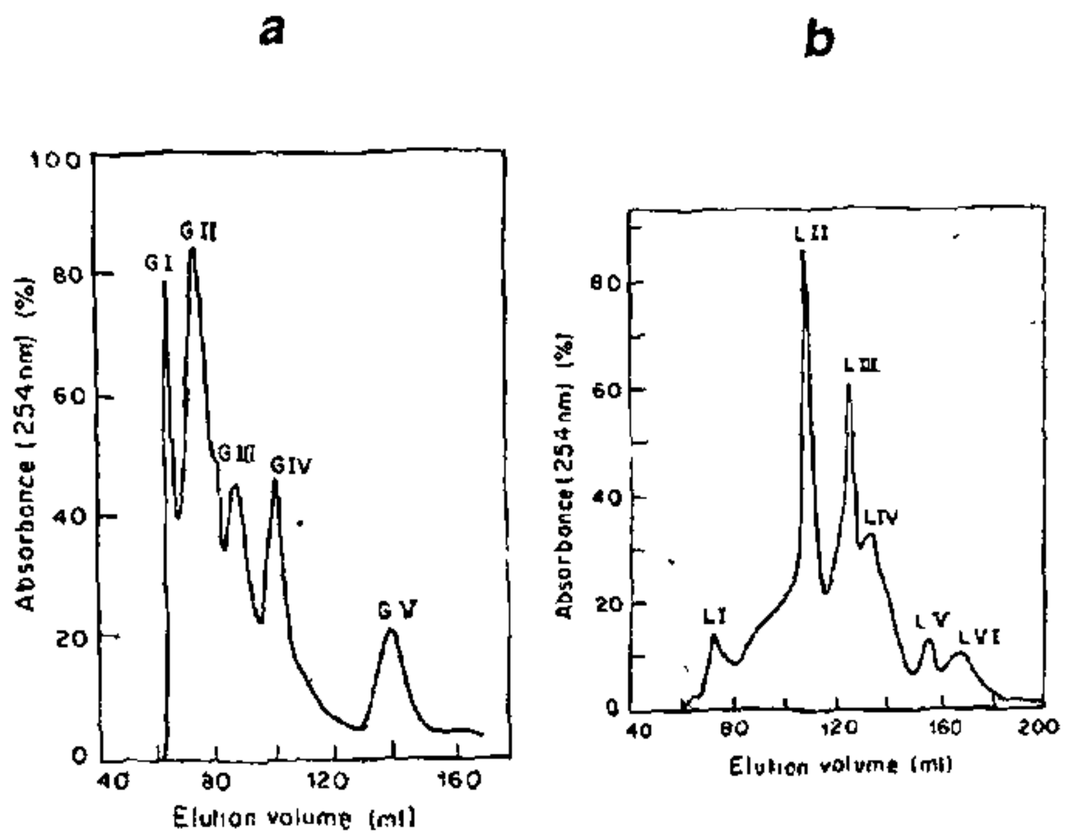

Figure 1. *a*, Chromatography of crude extract from Alternaria blight affected leaves of *Brassica napus* on Sephadex G-10 column (80 × 1 6 cm), *b*, Rechromatography of fraction G II from 1 a on Sephadex LH-20 column (80 × 1 6 cm). L II – Chlorotic activity. L III – Root induction activity.

Figure 2. *a*, Chlorotic symptoms observed on foliar bioassay 1 Toxin fraction L II 2 Control (distilled water); *b*, Root induction activity of L III fraction on *B napus* leaf a L III fraction b Control (distilled water)

dissolved in 5 ml of distilled water and bioassayed on detached *B. napus* leaves. No necrotic or chlorotic symptoms were observed after 72 h of incubation. This crude preparation was further purified on the Sephadex G-10 gel column (80 × 1.6 cm) with a void volume of 65 ml. The column was developed and eluted with water. The eluate from the column was monitored at 254 nm. None of the 5 major peaks obtained after G-10 (Figure 1 *a*) demonstrated any chlorotic activity on foliar bioassay on *B napus* leaves. However, when each of these fractions was further purified by LII-20 chromatography, fractions at peak no. 2 (L II, Figure 1 *b*) showed chlorotic activity (Figure 2 *a*). Interestingly, peak L III in the LII-20 chromatography fractions was found to induce rooting at the base of the *B. napus* leaves during foliar bioassay (Figure 2 *b*). Initiation of thin filamentary roots with positive geotropism start was observed after 72 h. This kind of root induction activity has been observed in fraction from culture filtrate of *A brassicae* by Tewari's group at the University of Alberta, Canada (J. P. Tewari, personal communication). It is interesting to note that the chlorotic activity from the leaf extract could be detected only after the root-inducing activity has been separated. In addition, both Suri and Mandahar[7] and Tyagi[5] observed a green island during foliar bioassay which has been correlated to the presence of cytokinin. Thus the presence of these two hormone-like activities in culture filtrate as well as in infected leaves indicates some unidentified role for phytohormones in the pathogenesis of *A. brassicae*. Incidentally *A. brassicae* sporulates poorly in culture but profusely on the infected leaves and dual culture[8].

Chlorotic toxin partially purified by gel filtration and adsorption chromatography was further purified on HPLC using C_{18} Micro Bonda Pak column (3.9 × 300 mm). A solvent system containing 50% water and 50% acetonitrile with 0.05% trifluoroacetic acid at a flow rate of 1 ml/min was used for elution. Absorbance was monitored at 235 nm. The chlorotic toxin activity was noticed at a retention time (10.68 min) which compares well with the retention time of 10.64 minutes observed for destruxin B by Tyagi[5]. The infrared spectrum of the chlorotic toxin purified from the infected leaves matched well with that from the standard toxin. Both the toxins showed strong peaks at 3389 cm^{-1} and 2959 cm^{-1} which represented N-methyl peptide bond and 1272 cm^{-1}, 1072 cm^{-1} representing depsi-peptide bond which are the characteristic features of destruxin B.

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