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SHORT COMMUNICATION

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Effect of temperature on flavonoid production in pigeonpea [*Cajanus cajan* (L) Millsp] in relation to nodulation

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Abstract To ascertain the reasons for poor nodulation of pigeonpea, we studied the effect of high temperature on the production of flavonoids by the pigeonpea host. A high temperature affected flavonoid production by pigeonpea and mungbean. At 37 °C pigeonpea root exudates contained four flavonoids and the root extract contained five. The proportion of the second flavonoid in the pigeonpea and the mungbean was higher and the proportion of the third flavonoid was lower at 37 °C compared to 30 °C. At the higher temperature the flavonoids exuded from pigeonpea roots were same those in the root homogenate.

Key words *Rhizobium* spp. · *Bradyrhizobium* spp. · Temperature · Pigeonpea · Flavonoids · Nodulation · N_2 fixation

Introduction

Nodule formation in legumes involves a series of interacting steps (Verma 1992). During an early exchange of signals host roots secrete highly specific factors called flavonoids, which induce nodulation (*nod*) genes in rhizobia (Redmond et al. 1986; Zaat et al. 1987; Maxwell et al. 1989) and thereby nod signals are synthesized (de Bruijn and Downie 1991; Kondorosi 1992). The extent of nodulation in a host legume (Kapulnik et al. 1987) is determined by nod signals that regulate nodule formation by controlling growth and differentiation in the host plant. Pigeonpea nodulation in northern regions of India is poor (Khurana and Dudeja 1981), and different reasons for this have been investigated (Dudeja and Khurana 1988a, b, c; 1989a, b, c; 1994). One possible reason is that the higher temperatures prevailing in this region might adversely affect the production of flavonoids by pigeonpea. Therefore, we studied the effects of high temperatures on flavonoid production by pigeonpea in comparison with that in mungbean, a better nodulation crop.

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Materials and methods

Root exudates of pigeonpea (cv UPAS 120) and mungbean (cv K851) were collected at temperatures of 30 and 37 °C after 10 days of growth. These temperatures were permissive and non-permissive for nodulation of pigeonpea, respectively (Dudeja and Khurana 1989a). Root exudates were collected in root exudate collection tubes (Dudeja and Khurana 1988a), and root extract was prepared by macerating roots in methanol followed by filtration and concentration under a vacuum below 40 °C. Both root exudates and root extracts were sterilized by membrane filter assembly using 0.45-μm membrane filters.

To detect flavonoids, root exudates/extracts were analysed by chromatography. Thin-layer chromatographic plates were prepared by homogenously spreading a slurry of 25 g silica gel-G in 50 ml distilled water, using a spreader 1 mm thick. The plates were air-dried and then placed in an oven at 110 °C for 30 min. Root exudates and extracts of pigeonpea and mungbean were spotted (150 μl) on the plates, and then the plates were run using methanol: chloroform (70:30 vol:vol) as a solvent system in a tank presaturated with the same solvent. After 1 h the plates were removed, dried, and visualized under an ultraviolet transilluminator (Harborne 1983). The root exudates were also analysed by high-performance liquid chromatography (Bioanalytic Systems, USA). The analysis was carried out by a reverse-phase 18 octadecylsilica column with an ultraviolet detector set at 254 nm. The solvent system used was methanol: water (1:1 vol:vol) acidified with glacial acetic acid (2.5 ml litre^{-1}) and the solvent was degassed before use. The flow rate was adjusted to 0.5 ml min^{-1} and the temperature of the column was set at 40 °C. Since standard flavonoids were not available, the numbers and proportions of different peaks were recorded.

Results and discussion

Thin-layer chromatographic analysis of root exudates and extracts of pigeonpea showed the presence of three

flavonoids in the root exudates and four flavonoids in the root extracts of the pigeonpea (Fig. 1). In the mungbean, there were four flavonoids in the root exudates and five in the root extracts. The total number of flavonoids, the colour of flavonoid spots and their rates of flow showed that the flavonoids present in the root exudates were different from those of the root extracts of pigeonpea and mungbean. The spectra of the flavonoids exuded by the roots of the pigeonpea and the mungbean were not the same as those inside the root. Similarly, in *Vicia sativa*, 7 flavonoids have been reported in root exudates compared to 10 in the root homogenate, only 4 of which were the same and the remaining different (Zaat et al. 1988).

High-performance liquid chromatography of the root exudates showed that temperature affected the flavonoid production by both hosts, both qualitatively and quantitatively. In root exudates of the pigeonpea five peaks were detected at 30 °C with retention times of 5.0, 6.0, 10.0, 11.0, and 12.5 min, whereas at 37 °C, an additional peak corresponding to a retention time of 9.0 min was also detected (Fig. 2). The relative proportion of flavonoids corresponding to the second peak was considerably higher at 37 °C compared to 30 °C. In the root exudates of the mungbean, the number of flavonoids detected at both temperatures was same, being four, but the retention time was different (Fig. 3). Like the pigeonpea, the relative proportion of the second flavonoid increased whereas the relative proportion of the third flavonoid decreased at the higher temperature. In the pigeonpea, previous work has revealed the presence of five or six flavonoids (Dahiya et al. 1984; Dahiya 1991). Thus the higher temperature changed the spectrum of flavonoids exuded by the pigeonpea but had no affect on the production of

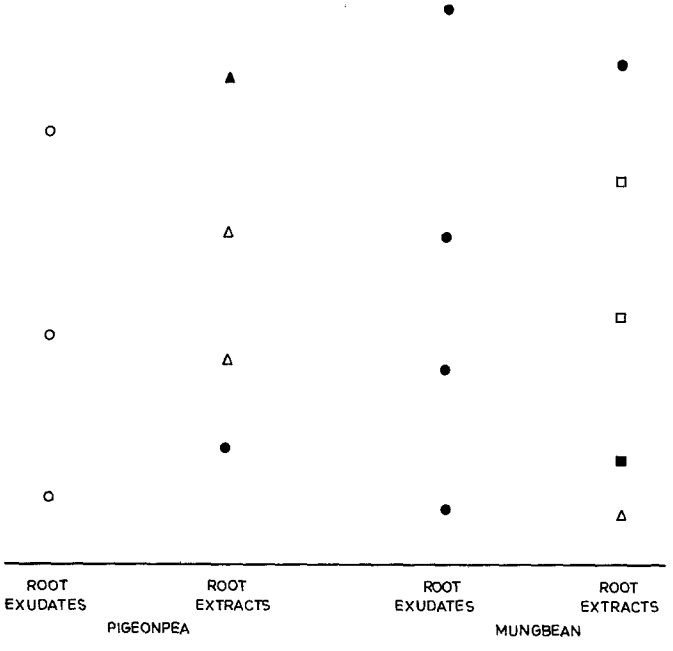

Fig. 1 Analysis by thin-layer chromatography of root exudates and extracts of pigeonpea and mungbean. ○, whitish blue; ●, blue; ▲, bluish yellow; △, yellow; □, light blue; ■, intensified blue

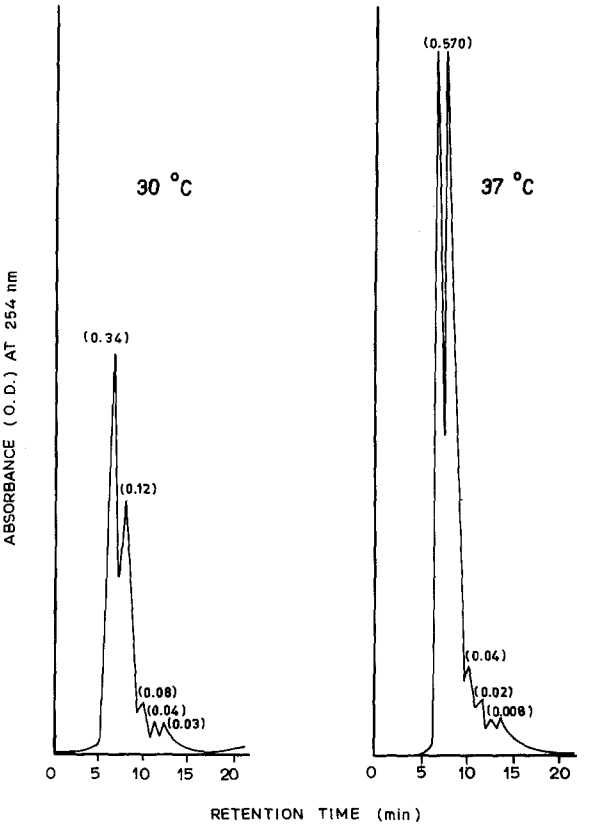

Fig. 2 High-performance liquid chromatography of root exudates of pigeonpea collected at 30° and 37 °C (*O.D.* optical density)

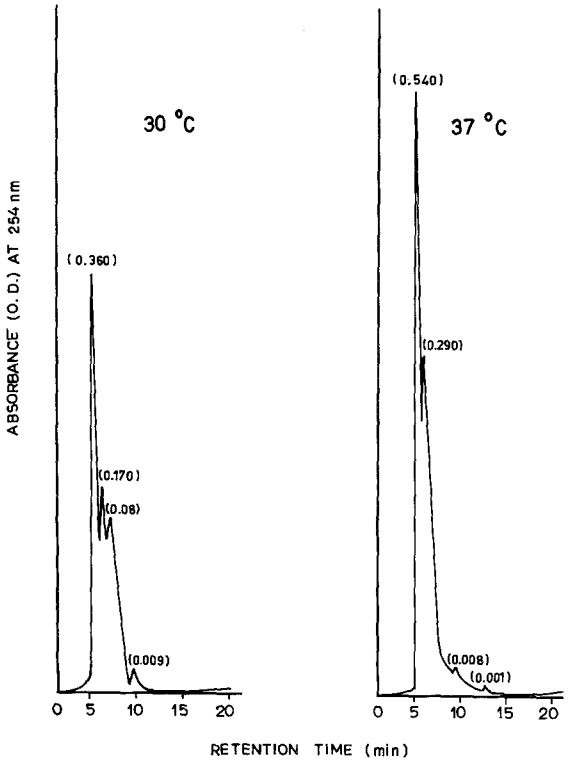

Fig. 3 High-performance liquid chromatography of root exudates of mungbean collected at 30° and 37 °C (*O.D.* optical density)

flavonoids in the mungbean. The type of flavonoid present in the mungbean root exudates at the higher temperature was similar to that inside the roots, indicating that the flavonoids from the roots were exuded without modification or processing, as reported in alfalfa, in which unmodified flavonoid was exuded from seeds (Hartwig and Phillips 1991).

Since these flavonoids are necessary for the induction of *nod* genes, we conclude that higher temperature prevalent in this region might change the relative proportion and type of flavonoids produced under field conditions and thus restrict the number of nodules formed by the pigeonpea host. Further studies on the identification of inducers and anti-inducers of *nod* genes in the pigeonpea will provide a better understanding of the nodulation problem in the pigeonpea.

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