Effect of gibberellins and the growth retardant CCC on the nodulation of soya

P. M. WILLIAMS and MARGARITA SICARDI DE MALLORCA
Instituto Venezolano de Investigaciones Cientificas, Centro de Microbiologia y Biologia Celular, Apartado 1827, Caracas 1010–A, Venezuela

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Summary  The effect of exogenous applications of gibberellins (GAs) or the growth retardant β-chloroethyltrimethylammonium chloride (CCC) on root nodule formation and activity (C2H2-reduction) in soya was studied. Daily foliar application of GA3 (2.89×10^-6 M) delayed the formation of nodule initials and reduced the numbers, mass nodule and specific activity of nodules by 43%, 31% and 47% respectively, without affecting plant growth. Similar effects on nodulation were produced by foliar application of GA4 (3.01×10^-5 M) or GA7 (3.03×10^-5 M), or by the addition of GA3 (2.89×10^-6 M) to the rooting medium. GA effectiveness in reducing nodule numbers was decreased by delaying its application until after the initial infection process had occurred, but the nodules formed were smaller and less active than those of the untreated control plants. The GA effect on nodulation and nodule activity was not associated with alterations in root exudate or due to a direct inhibitory effect of the hormone on the nitrogenase system. When the endogenous root content of GA-like substances was reduced (86% decrease) by foliar application of CCC (6.30×10^-5 M), nodule numbers were increased by 56%, but nodule size and total nodule activity were similar to those of control plants. The GA and CCC treatments had no effect on rhizobial growth in liquid culture nor on root colonisation by rhizobia.

The results suggest that the endogenous content of root GA may have a regulatory role in both the infection process and in subsequent nodule morphogenesis, thus controlling both the number and effectiveness of the root nodules formed.

Introduction

Establishment of the legume/Rhizobium symbiotic association requires a number of physiological and morphological stages involving both the micro- and macrosymbiont. Rhizobia proliferate in the rhizosphere of potential host plants and attach to root surfaces. Infection thread development may occur, depending on the host, in developing epidermal cells or in zones of the root containing mature root hairs, developing root hairs or in areas where no root hairs are present at the time of inoculation. In general, infections induce curling and branching of root hairs and rhizobia are carried within the infection thread as it follows the nucleus towards the base of the hair cell. After thread penetration of the root hair cell wall and entrance into adjacent cortical cells, it branches into many newly divided cortical cells where the rhizobia are released.
into the host cell cytoplasm and transformed into bacteroids contained in envelopes of host plasmamembrane. The nitrogenase enzyme converts atmospheric dinotrogen to ammonia at the expense of host photosynthate.

The regulation of the above process has become an important area of study and considerable evidence indicates that nodulation is controlled by the host. For example, ineffective nodules are normally formed in greater numbers than effective nodules\textsuperscript{13}, suggesting that nodule development is regulated by feedback mechanisms by substances present in effective nodules. Similarly, the fact that nodules are often formed in groups\textsuperscript{13} and that removal of the first formed nodules stimulates further nodulation\textsuperscript{14}, provide further evidence for the operation of such feedback mechanisms of control. In soya, evidence has been obtained for a form of regulation of nodulation that is effective within a few hours after inoculation; infections occurring soon after inoculation, reducing subsequent infections in slightly younger cells\textsuperscript{3}. Such a rapid mechanism also appears to be operative in cowpea\textsuperscript{2}.

Evidence implicating the involvement of GAs in the regulation mechanisms of nodulation is provided by the fact that this hormone is particularly concentrated in nodular tissue\textsuperscript{5,18,22} and its exogenous application to a number of legumes has been shown to inhibit nodule formation\textsuperscript{7,12,20}. Furthermore, GA production by cultures of Rhizobium has been established\textsuperscript{9,22}. The demonstration of GA excretion by root nodules and root apices of \textit{Phaseolus vulgaris}\textsuperscript{18}, along with previous results showing that the excision of the first formed nodules or root tips of clover stimulated further nodulation\textsuperscript{14}, and that in plants grown in association\textsuperscript{15} or in succession\textsuperscript{16} on a limited volume of medium, nodulation was inhibited, led to the suggestion that nodule GAs may regulate further nodulation of the root\textsuperscript{18}.

In an attempt to gain a better understanding of the role of GAs in the nodulation process of legumes, the effect of exogenous application of GAs and CCC, an inhibitor of GA biosynthesis\textsuperscript{1,10,11,23}, on the nodulation of soya was studied. Since the preliminary experiments showed that nodule numbers were reduced and increased by the GA and CCC treatments respectively, further studies were performed to locate the stage(s) of nodule formation affected by the hormone.

\textbf{Materials and methods}

\textit{Bacterial strain}

\textit{Rhizobium japonicum} strain IVIC-1, a native isolate of Zimbabwe (MAR 492), forms effective nodules on soya (\textit{Glycine max} (L.) Merr.).

\textit{Media}

All media were prepared with distilled water and, when required, were solidified with 1.5\% Difco Bacto-Agar. YM medium was a yeast-mannitol broth\textsuperscript{21} and the soya nutrient solution (NS) was a nitrogen-free mineral salt solution\textsuperscript{9}. 