Enhancement of polygalacturonase activity during auxin induced para nodulation and endorhizosphere colonization of _Azospirillum_ in rice roots

C Sekar, N N Prasad & M D Sundaram
Department of Agricultural Microbiology, Annamalai University, Annamalai Nagar 608 002, India

Received 9 October 1998; revised 10 August 1999

Effect of different auxins, namely, 2,4-dichlorophenoxyacetic acid (2,4-D), naphthalene acetic acid (NAA) and indole acetic acid (IAA) and _Azospirillum brasilense_ bioinoculation on the enhancement of polygalacturonase (PG) activity in rice roots during _para_ nodulation and endorhizosphere colonization of _Azospirillum_ was studied under _in vitro_ condition. It was observed that _Azospirillum_ bioinoculation could augment PG activity of rice roots to a lesser extent without any root morphogenesis whereas auxin application together with _Azospirillum_ bioinoculation enhanced PG activity of rice roots to a higher level which resulted in better root morphogenesis (para nodule) and endorhizosphere colonisation of _A. brasilense_. Among the three auxins tested, 2,4-D, even at lower concentration (0.5 ppm) enhanced the rice root PG activity, root morphogenesis and endorhizosphere colonization of _Azospirillum_ while it was 2.0 ppm with NAA and variable with IAA. It is concluded that there is a positive correlation existing among PG activity, degree of root morphogenesis and endorhizosphere colonization of _Azospirillum brasilense_ in rice roots and the degree of correlation is determined by the chemical composition, concentration and mode of action of the auxin utilised.

The rhizobiocoenosis of _Azospirillum_ with rice plant plays a significant role in crop productivity. The degree of _Azospirillum_ rhizobiocoenosis is greatly influenced by the entry of diazotroph into the macropartner and its subsequent colonization therein\(^1\). Hence, cellular manipulation of introducing a complete nitrogen fixing system into rice root is of much importance in the present context\(^2\). Different methods have been employed to achieve the same including the use of auxins\(^4\). Tehan and Kennedy\(^6\) demonstrated the auxin induced nodulation in wheat and termed the same as "Para nodules", since it differs from the naturally occurring legume nodules. Also, the auxin treatment augmented the endorhizosphere colonization of _Azospirillum brasilense_ cells along with their nitrogen fixing ability in wheat roots.

Exogenous application of auxins to plant roots alters the physiology of the same and result in root morphogenesis through induction of hydrolytic enzymes\(^2\)\(^-\)\(^4\). Host root polygalacturonase activity (PG) plays a significant role in root morphogenesis and in the endorhizosphere colonisation of microbes, as a defense response in plants\(^5\). However, there have been no report, so far, on the involvement of host root polygalacturonases during auxin induced _Para_ nodulation process and subsequent endorhizosphere colonisation of microbial partner therein.

Hence, the present study was undertaken to explore the possible involvement of auxin induced polygalacturonases activity during the _para_ nodulation process and subsequent endorhizosphere colonisation of _Azospirillum brasilense_ in rice roots.

Rice (_Oryza sativa_ L.) cv. IR-50 seeds were surface sterilised and germinated according to Sriskandarajah _et al_.\(^6\). Uncontaminated rice seedlings, aged about 2-3 days, were transferred aseptically to growth chambers as detailed by Gitte _et al_.\(^1\) for hydroponic growth in weaver's medium\(^5\). Growth chambers @ 25 plants/chamber were maintained at 20° and 30°C, as minimum and maximum temperature and with continuous light.

_Azospirillum brasilense_ CA-10 (an isolate from IR-50 rice rhizosphere grown under lowland condition at Mannargudi, Tamilnadu, India), characterised according to Tarrand _et al_.\(^1\) and maintained in semisolid malate medium\(^7\) at 30°C, was used.

The auxins, namely, 2,4-dichlorophenoxyacetic acid (2,4-D), naphthalene acetic acid (NAA) and indole acetic acid (IAA), (CDH, India) were tested for their _para_ nodulation efficiency in rice plant. The auxins were filter sterilised and a stock solution of each auxin with different concentrations, namely, 2,4-D (0.1-3.0 mg L\(^-1\)), NAA (1-10 mg L\(^-1\)) and IAA (10-100 mgL\(^-1\)), prepared. After 1 week growth of rice plant in growth chamber whereas the roots of rice seedlings developed one or two main roots without lateral root development, 25 ml of 24 hr old culture.
of *Azospirillum* isolate was added (ca. $10^7$ cells per plant) to each growth chamber, as bacterial inoculation. The plants were also treated with different concentrations of auxins, maintained in stock solutions. Three replications were maintained for each treatment.

After 5 days of bioinoculation and auxin application, 5 plants from each replication were selected randomly for PG activity assay. The entire root system of rice plants from each concentration were excised, ground in a mortar with 10 ml of deionised water per gram of fresh weight of the material. The homogenate was expressed through cheese cloth and the collected liquid was centrifuged at 7000 g for 20 min, clear supernatants decanted, dialysed overnight against distilled water and used as enzyme source. The exopolypgalacturonase (Exo-PG) was assayed in 0.1 M sodium citrate buffer at pH 4.5 and that of endopolypgalacturonase (Endo-PG) in 0.1 M acetate buffer at pH 5.2 employing 0.5% sodium polypectate as substrate. To 4 ml of respective substrate, 2 ml of enzyme source and 1 drop of sodium fluoride were added and the reaction mixture incubated at 30°C for 22 hr. After the incubation period, 0.5 ml of 0.5 M HCl and 10 ml of 0.01 M thiobarbituric acid (TBA) were added to reaction mixture, heated in water bath for 1 hr, cooled and centrifuged at 2000 rpm. The absorbance of the clear solution was measured at 510 nm in spectronic-20 colorimeter. Five other rice plants were selected from each replication and used for the enumeration of the number of *para* nodules per rice plant and the colony forming units (CFU) of *Azospirillum brasilense* from the endorhizosphere of rice according to Sriskandarajah et al.\(^{13}\).

Inoculation of *Azospirillum brasilense*, without any auxin application, unable either to stunt the main root or to arrest the lateral root development of one week old rice plant. The result clearly implicates the positive role of auxins on induction of *para* - nodules in rice roots and confirms the earlier findings on the subject.\(^{4,8,13}\). Interestingly, the PG activity could be induced and endorhizosphere colonisation could be increased to a lesser extent in rice roots by inoculated *Azospirillum brasilense* while the uninoculated control failed to do so. Induction of host root polygalacturonase is an indirect measure of the host defense response to the invading microbes and necessary for the accumulation of host cell wall like material to form a new layer or sheath at the entry point of microorganisms\(^{12}\). This is the first report of its kind to show the induction of PG activity in rice roots due to *Azospirillum* inoculation.

Exogenous application of auxins, namely, 2,4-D, NAA and IAA to rice yielded root morphogenesis and could able to produce *para*-nodules in rice root system. Application of 2,4-D to rice roots could able to produce *para*-nodules even at lower concentration, namely, 0.5 ppm, while it was observed as 2 and 50 ppm, with NAA and IAA, respectively. Moreover, 2,4-D at the highest concentration tested, namely, 2 ppm, invariably altered the *para*-nodule morphology towards roundness and the same was found to be distributed at regular intervals along the primary roots (Fig.1). But, NAA and IAA at their maximum concentration, namely 8 and 50 ppm respectively might shorten the lateral roots strongly but failed to cause the rounded appearance of *para* nodule as induced by 2,4-D. The result clearly revealed the importance of the
chemical composition, concentration and the mode of action of auxins in plant root system for the determination of para-nodule morphology. Sriskandarajah et al.13 studied the auxin induced para-nodulation in wheat and reported that the biological or chemical instability of NAA and IAA might be the reason for their poor performance. Andrads18 reported that NAA and IAA taken up by pea roots were metabolically conjugated to other molecules while 2,4-D was not metabolised. Penny and Penny19 reported that the effect of 2,4-D in a particular location was much stronger than any other auxin. IAA, applied at 50 ppm concentration yielded para-nodule morphology similar to NAA (8 ppm) but the effect could not be reproduced and variable due to biological or chemical instability of the same. Hence, the effect of IAA on the subject is not discussed in the present context. Though, increasing levels of 2,4-D and NAA up to 2.0 and 8.0 ppm, respectively, inducted more para-nodes in rice, 1.5 and 4.0 ppm concentration of the same were found to be optimum for significant induction of para-nodes in rice. Rice plants maintained at 2.5 and 10.0 ppm of 2,4-D and NAA, respectively, were found to be withered and died without any para-nodule induction.

Exogenous application of auxins to rice roots was also found to enhance the rice root PG activity and subsequent endorhizosphere colonisation of *Azospirillum brasilense*. Between the two auxins tested, 2,4-D was found to induct the rice root PG activity and endorhizosphere colonisation of *Azospirillum brasilense* to a maximum level compared to NAA. A positive correlation was recorded between the increasing levels of auxins and the rice root PG activity and subsequent endorhizosphere colonisation of *Azospirillum*. 1.5 and 4.0 ppm of 2,4-D and NAA, respectively, were found to be optimum concentration in the mechanism because there was a sharp decline in the rate of increase of these parameters beyond this concentration. Regarding the mode of cleavage of pectic substrates, Endo-PG cleavage was found to be dominant over Exo-PG cleavage (Table 1).

*Azospirillum*, as a potent producer of IAA in plant rhizosphere, causes 'tuning fork' formation in root hairs3,10 and alternation in cell arrangement of the cortex region12. The positive role of auxin-induced host root PG activity is to be suggested for these mechanisms. Burstrom1 observed a decrease in growth and increase in cell wall plasticity of wheat due to IAA application and also reported that this might be achieved through the modification of middle lamella. Sriskandarajah et al.13 also reported the enlarged basal cells of the auxin induced para-nodes. These results suggesting the occurrence of host root PG activity during exogenous auxin application. Moreover, Christiansen-Weniger and Vanderleyden25 reported the efficiency of host to encapsulate the intracellularly colonising *Azospirillum*

---

### Table 1 — Enhancement of polygalacturonase (PG) activity and endorhizosphere colonisation of *Azospirillum brasilense* in rice roots

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of para-nodes/plant</th>
<th>PG activity</th>
<th>Endorhizosphere colonization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>Azospirillum</em> alone</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>Azospirillum</em> plus</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2,4-D, 0.5 ppm</td>
<td>8.2</td>
<td>0.208</td>
<td>0.482</td>
</tr>
<tr>
<td>2,4-D, 1.0 ppm</td>
<td>12.8</td>
<td>0.260</td>
<td>0.552</td>
</tr>
<tr>
<td>2,4-D, 1.5 ppm</td>
<td>25.0</td>
<td>0.319</td>
<td>0.699</td>
</tr>
<tr>
<td>2,4-D, 2.0 ppm</td>
<td>26.2</td>
<td>0.347</td>
<td>0.721</td>
</tr>
<tr>
<td>2,4-D, 2.5 ppm</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>Azospirillum</em> plus</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>NAA, 2.0 ppm</td>
<td>6.4</td>
<td>0.168</td>
<td>0.367</td>
</tr>
<tr>
<td>NAA, 4.0 ppm</td>
<td>15.2</td>
<td>0.268</td>
<td>0.532</td>
</tr>
<tr>
<td>NAA, 6.0 ppm</td>
<td>16.8</td>
<td>0.284</td>
<td>0.552</td>
</tr>
<tr>
<td>NAA, 8.0 ppm</td>
<td>18.0</td>
<td>0.292</td>
<td>0.585</td>
</tr>
<tr>
<td>NAA, 10.0 ppm</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

a — Rice plant withered and died
b — Value beyond detection limit (log 3.0 CFU g⁻¹ dry root)
c — IAA effect could not be reproduced and variable, omitted
d — log CFU g⁻¹ dry root
e — average of 5 plants.
cells with a membrane layer, resembled to the peribacteriod membrane of nodule tissue during 2,4-D induced para-nodulation in maize. The formation of membrane layer around the invading cells indicated the involvement of host root PG activity, as a defence response. The results of the present study also clearly confirmed the role of auxins on enhancement of PG activity, a process essential for root morphogenesis and endorhizosphere colonisation of Azospirillum, in rice roots.

However, studies on the rice root PG activity at succession intervals of incubation time with auxin, duration of incubation for achieving maximum endorhizosphere colonisation and ultrastructure studies on the entry and establishment of Azospirillum into the para nodules will be further explored.

The authors are thankful to ICAR, New Delhi for financial assistance through All India Co-ordinated Research Project on Biological Nitrogen Fixation.

References