EFFECT OF NITROGEN FIXING BACTERIA ON TILLERING, PHOTOSYNTHETIC RATE AND CHLOROPHYLL CONTENT IN PLANT GROWTH REGULATOR INDUCED NODULATED WHEAT

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Abstract: Under laboratory conditions the seedlings were raised to induce the nodule-like out growths using different growth regulators 2, 4-D (0.5 ppm), IBA (8 ppm) and NAA (8 ppm) in nitrogen free Hoagland solution. The seedlings were inoculated with bacterial culture such as Azorhizobium caulinodans (ORS 571) and Nostoc (mixed strains) in wheat variety C-306. Another set was raised with Azorhizobium caulinodans and Nostoc without plant growth regulators as uninoculated control. The treated paranodulated wheat seedlings were transferred to pots. The data collected on 60 days after transplanting revealed that the plants treated with Nostoc either alone or with different growth regulators had higher tiller number, chlorophyll content and leaf area per plant. The maximum effect was observed with IBA + Nostoc followed by 2, 4-D and NAA as compare to Nostoc treated plants. The Azorhizobium caulinodans treated plants were also better than control; it was also observed that the photosynthetic rate was maximum with 2, 4-D treated plants followed by Azorhizobium caulinodans and Azorhizobium caulinodans with NAA and IBA. The biomass production was maximum in Azorhizobium caulinodans treated plants followed by Nostoc along with growth regulators; however IBA and Azorhizobium caulinodans gives the maximum biomass production.

Keywords: Plant growth regulators, Azorhizobium caulinodans, Nostoc, Nodulation, Photosynthesis, Chlorophyll content, Tiller number

INTRODUCTION

Wheat is an important food crop of India. The modern wheat varieties respond well to inorganic nitrogen fertilization and other inputs. With the increasing demand of nitrogen fertilizers has created a problem in meeting out the required amount of nitrogen. The nitrogen use efficiency has also declined as well as nitrogen is creating health problems due to the nitrate depletion and also polluting the usable water to human beings. Hence the use of bio-fertilizer for nitrogen supply may be an alternative idea which is cheap and eco-friendly. The plant growth regulators such as 2,4-D, IBA and NAA has been used to create nodulation in cereal crops by different workers (Tchan and Kennedy, 1989 in wheat, Ladha et al., 1987 in rice and Luo et al., 1987 in maize).

The use of different bacteria such as Azorhizobium caulinodans has also been reported to fix nitrogen and its effect on growth and yield (Ridge et al., 1993, Christiansen Weniger and Vanderleyden, 1994). Nostoc has also been reported to enhance the growth and yield of plant through enhancing the nitrogenase activity (Gantar and Elhai, 1991, Spiller and Gunasekaran, 1993). However the use of plant growth regulators, Azorhizobium caulinodans and Nostoc affects the tillering, leaf area, photosynthetic rate and its biological yield was the object of the present study in the paranodulated and uninoculated control wheat plant.

MATERIAL AND METHOD

Sterilized seeds (dipped in HgCl₂ 5% for three minutes and rinsed thoroughly with water) were sown in sand gravel. After seven days seedlings were transferred to test tubes covered with black paper, filled with Nitrogen free Hoagland solution with different treatments to induce para-nodules with 2, 4-D (0.5 ppm), IBA (8 ppm) and NAA (8 ppm). These para-nodulated seedlings were introduced to liquid cyanobacterial culture (Nostoc mixed strain) and Azorhizobium caulinodans (ORS 571). After 7-8 days induced para-nodules can be seen with naked eyes. The plants were then transferred in cemented pots (50 x 50 x 50 cm³) and 12 plants were maintained in each pot. The tillers were counted at different stage of growth and the leaf area was measured using the leaf area meter (LICOR 3000 model USA) and was expressed as cm²/plant. The
chlorophyll content in leaf was determined at different stages using the method described by Hiscox and Israelstam (1979). Photosynthetic rate, stomatal conductance and resistance were measured by IRGA (Infra Red Gas Analyzer) LICOR 6250. At harvest the total biomass production was measured including straw and grain yield in different treatments. The data were statistically analyzed (Table 1).

RESULT AND DISCUSSIONS

The addition of the synthetic auxin 2,4-D to wheat seedlings resulting in the formation of nodule like outgrowths that can best be described as modified lateral roots. It was found that concentration (0.5 ppm) formed 100% of plants these outgrowths, addition of 2,4-D also significantly reduced shoot and root length but dry weight was unaffected. Synthetic auxin NAA (8 ppm) induces some root thickenings, but at lower concentration had no effect on root development. NAA does not play a very significant role in bio-mass, yield, root shoot length and on other factors. IBA (8 ppm) also shows some outgrowth on roots of wheat seedlings and cause proliferation, high leaf area, high tiller number, good bio-mass and better grain yield.

Cytological investigations revealed that there were more *Azorhizobium caulinodans* present within the para-nodule than in the root system. This indicates that the major part of ethylene production come from the *Azorhizobium caulinodans* activities within the para-nodules. While *Nostoc* enters in the form of aseriate packages in epidermis. *Nostoc* (Cyanobacteria) were found either as hormogonia filaments or aseriate packages. The aseriate packages which develop from filaments often formed a thick layer surrounding the root surface (Gantar et al., 2006). The total chlorophyll contents at 60 DAS revealed that it was more with *Nostoc* followed by *Azorhizobium caulinodans* which was better than control. The plants inoculated with *Nostoc* or *Azorhizobium caulinodans* have more chlorophyll content than the plants grown with 2, 4-D IBA and NAA alone. However the plants treated with IBA + *Nostoc* and IBA + *Azorhizobium caulinodans* had higher total chlorophyll content followed by 2,4-D < *Nostoc* < *Azorhizobium caulinodans* and the lowest chlorophyll content was noted in NAA treated either with *Nostoc* or *Azorhizobium caulinodans*. The results obtained has been supported by the earlier work done by division of Microbiology I.A.R.I. (Kaushik et. al., 2008) where the wheat seedlings treated with *Nostoc* (sp. K,3) had higher nitrogenase activity, enhanced higher biomass used as an index of nitrogen fixation.

The photosynthetic rates measured at 60 days after transplantation in the pots revealed that it was highest in *Azorhizobium caulinodans* treated plants followed by *Nostoc* and significantly lower in untreated plants. The plants treated with 2, 4-D along with *Azorhizobium caulinodans* or *Nostoc* had shown the higher photosynthetic rates with better stomatal conductance and least stomatal resistance. The plants treated with IBA or NAA either alone or with *Azorhizobium caulinodans* or *Nostoc* had poor photosynthetic rate consequent upon the relatively low stomatal conductance and higher stomatal resistance.

The results are in confirmatory with the earlier reports (Tchan and Kennedy 1991, Panwar 1993 in wheat and rice) and (Saikia et al., 2006 in maize). It is evident from Table 1 that IBA treated plants either alone or with *Azorhizobium caulinodans* and *Nostoc* have higher seed and straw yield (biological yield) followed by 2,4-D treated plants and NAA treated plants, however, it was better than control.

IBA inoculated with *Azorhizobium caulinodans* had produced maximum biological yield followed by IBA with *Nostoc* or alone. The NPK content in seed and straw was relatively more with 2, 4-D treated *Azorhizobium caulinodans* inoculated plants followed by 2, 4-D inoculated with *Nostoc* followed by IBA and NAA treated plants either alone or with different bio fertilizers used in this study. These results are in confirmatory with the earlier report by (Elanchezian and Panwar, 1997 in wheat) and (Naidu, 2001 in rice) through non significant differences were found in potash content in seed and straw. The protein content was maximum in *Nostoc* inoculated plants followed by *Azorhizobium caulinodans*, either alone or with auxins.
Table 1: showing the effect of plant growth regulators and *Azorhizobium caulinodans* and *Nostoc* on tillering leaf area chlorophyll content and photosynthetic rate at 60 DAS.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Tiller no. 60 DAS</th>
<th>Leaf Area 60 DAS</th>
<th>Chlorophyll content 60 DAS</th>
<th>Photosynthetic Rate 60 DAS</th>
<th>Biological Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.74</td>
<td>66.52</td>
<td>2.956</td>
<td>11.383</td>
<td>10.24</td>
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<tr>
<td><em>A. caulinodans</em></td>
<td>4.50</td>
<td>94.25</td>
<td>3.082</td>
<td>21.787</td>
<td>12.38</td>
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<tr>
<td><em>Nostoc</em></td>
<td>5.12</td>
<td>100.25</td>
<td>3.229</td>
<td>17.357</td>
<td>13.27</td>
</tr>
<tr>
<td>2,4-D</td>
<td>4.13</td>
<td>70.25</td>
<td>3.058</td>
<td>23.290</td>
<td>12.21</td>
</tr>
<tr>
<td>2,4-D + Azo.</td>
<td>5.64</td>
<td>114.52</td>
<td>3.538</td>
<td>31.930</td>
<td>13.56</td>
</tr>
<tr>
<td>2,4-D + Nostoc</td>
<td>6.13</td>
<td>120.42</td>
<td>3.580</td>
<td>26.540</td>
<td>13.12</td>
</tr>
<tr>
<td>IBA</td>
<td>4.26</td>
<td>75.25</td>
<td>2.954</td>
<td>13.480</td>
<td>14.29</td>
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<tr>
<td>IBA + Azo</td>
<td>5.75</td>
<td>118.62</td>
<td>3.615</td>
<td>17.040</td>
<td>15.42</td>
</tr>
<tr>
<td>IBA + Nostoc</td>
<td>6.36</td>
<td>127.54</td>
<td>3.698</td>
<td>15.900</td>
<td>14.82</td>
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<td>NAA</td>
<td>4.03</td>
<td>67.52</td>
<td>2.931</td>
<td>14.940</td>
<td>12.08</td>
</tr>
<tr>
<td>NAA + Azo.</td>
<td>4.26</td>
<td>108.42</td>
<td>3.106</td>
<td>18.730</td>
<td>13.11</td>
</tr>
<tr>
<td>NAA + Nostoc</td>
<td>5.20</td>
<td>112.65</td>
<td>3.201</td>
<td>16.220</td>
<td>12.78</td>
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<tr>
<td>CD at 5%</td>
<td>0.296</td>
<td>5.64</td>
<td>0.177</td>
<td>1.238</td>
<td>0.912</td>
</tr>
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</table>

REFERENCES


