# The contribution of binucleate *Rhizoctonia* AG - V on Phosphorus uptake by plant*

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*Rhizoctonia* is a soil-borne fungal pathogen which widely exists in the soil. This study, by comparing the two isolates of the AG-V which are new an stemsons group (AG)(Bs-J-06-7-1 and DL-YT-06-4-9), we find P absorption differences as well as their hyphae in the system P absorption rate interacting with three plants, and clear its specificity with plant interactions. Screening for the effect of hypo toxicity binucleate *Rhizoctonias* on promoting P absorption of three plants, the result is that there were significant differences on contribution rate of P absorption of fungus when two hypo-toxicity *Rhizoctonias* interacted with corns in 40days, and the comparative results would be Bs-J-06-7-1>DL-YT-06-4-9, that when two hypo toxicity *Rhizoctonias* interacted with tomatoes in 40days, and the comparative results would be DL-YT-06-4-9> Bs-J-06-7-1, but when two hypo toxicity *Rhizoctonias* interacted with rapes in 45 days, and the comparative results would be Bs-J-06-7-1>DL-YT-06-4-9. Through comparative analysis of quantity and contribution rate of P absorption of fungus in this study, we found that the interaction of two hypotoxicity binucleate *Rhizoctonias* with corn, tomato and rape had some positive effect. But effect on P absorption tomatoes is not very obvious.

*Keyword:* Hypo Toxicity Binucleate *Rhizoctonia*; P absorption; Interaction.

## 1. Introduction

*Rhizoctonia* spp. is a soil-borne fungal pathogen which widely exists in the soil. Since De Carolle established *Rhizoctonia*, people found that the fungi can infect 260 plants of 43 families, such as rice, corn, soy bean, barley, wheat. *Rhizoctonia* cause the symptom such as plant grain dry and withered and is one

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of the important pathogen of plant soil borne disease [1-8]. According to the number of nuclei, *Rhizoctonia* is divided into multinuclia and bionuclia[6, 7, 9]. At present, teleomorph *Ceratobasidium spp.* was divided into 16 different an astomosis group (AG) A - V by experts [10]. I although most parasitic in the roots of plants, but the pathogen city of the ginger and taro was significantly lower than pathogenic *Rhizoctonia solani*, and also to promote the absorption of N, P and K and other nutrients , especially for the absorption of P prime [4, 11] for ginger tissue culture. AG-D cause wheat sheath blight, and AG-R, P cause *Betula spp.* damping off. AG-A mild can infect leguminous plants including soybeans, and other fusion group generally does not cause plant [4, 6, 12]. There are only a few AGs of *Ceratobasidium spp.* are pathogenic and most of *Rhizoctonia* species are weak pathogenicity or no pathogenicity. These AGs weaking pathogenicity or no pathogenic parasite on the roots of plants. The correlations between these AGs and other flora is often neglected by people, such as *Ceratobasidium spp.*, most of the AGs belongs to the endophytic fungi, host is widespread, their relationship with the plant is not clear. Project through studing for *Rhizoctonia* population genetic diversity in the southwest region of LRGR found that a variety of health plant roots have a large number of *Ceratobasidium spp*. in addition to the orchidaceae, such as strawberries, colocasia, ginger, cuckoo, leguminosae, cruciferae, etc[11].

Phosphorus is a non-renewable resource. Our country is the world's largest consumer. Phosphorus utilization rate is very low in season which resulted in the accumulation of soil phosphorus, and wasting phosphorus resources and seriously affecting the environment quality [13]. Thus process improving soil phosphorus efficient absorption and utilization of rhizosphere become one of the hot spot of plant nutrition. project group found in addition to the separatio AG - P subgroup from *betula alnoides*, the others all species isolated from ginger can promote ginger somaclone to absorpting for mineral elements N, P, K , especially obviously promote the absorption of P and increased by 30-200%, when project group cultured together these new species isolated from ginger with ginger soma clone .

This experiment analysis and comparison group of two isolates of AG - V fusion by finding new non-pathogenic *Rhizoctonia solani* which boost P absorption of plants in the prophase work, through comparing the new population AG - V in pure culture as well as their interactions with plants in the system of hyphae P absorption rate, then find AG-V non-pathogenic *Rhizoctonia solani* promoting the phosphorus absorption. At the same time study, we study AG-V with plants and plant absorption of the relationship between the effective phosphorus efficiency, clearing its specificity, with plant interactions under different P supply level to promote the growth of the plant growth and its effect

and mechanism of strains beneficial for *Rhizoctonia solani* of the development and effective utilization of lay the foundation.

## 2. Materials and Methods

### 2.1. *The cultivation of AG-V*

The strains cultured in PDA culture medium included two strains in new anastomosis groups of AG-V which were offered by Prof. Genhua Yang form his preliminary study(which meant AG-V(Bs-J-06-7-1), AG-V (DL-YT-06 -4-9)).

#### 2.1.1. *Preparation of culture medium*

The synthetic fluid nutrient medium was adopted in this study, the ormulation of Hoagland nutrient solution was used for microelement while the formulation of Amon nutrient solution was used for microelement. After inoculating *Rhizoctonias* of different population with equal quality into the culture mediums with different concentration, the difference in efficiency of P absorption could be analyzed in terms of concentration changes of P measured in different culture time.

Table 1. Formulation of Hoagland nutrient solution.

| macroelement | (# ml added in per 1 mol/L solution) |
|---|---|
| $KH_2PO_4$ | 1 |
| $KNO_3$ | 5 |
| $Ca(NO_3)_2$ | 5 |
| $MgSO_4$ | 2 |
| microelement | (# ml added in per 1 mol/L solution) |
| $H_3BO_3$ | 2.86 |
| $MnCl_2.4H_2O$ | 1.81 |
| $ZnSO_4.7H_2O$ | 0.22 |
| $CuSO_4.5H_2O$ | 0.08 |
| $H_2MoO_4.H_2O$ | 0.02 |

1 ml FeEDTA (Ferrum Ethylene Diamine Tetraacetic Acid) solution was added in per 1 L culture medium

#### 2.1.2. *Determination of P content in solution (vanadium molybdate yellow colormetric method)*

The sample solution of 5.00 to 10.00 mL (P content was 0.05 to 1.0 mg ) was transferred to a 50 ml-volumetric flask, then 2 drops of 2,6-DNP (or 2,4-DNP) was added into the sample solution, NaOH of 6 mol-$L^{-1}$ was added for PH adjustment until the colour of solution just became yellow. Added vanadium

ammonium molybdate reagent of 10.00 ml precisely, diluted with water and shaked well. Blank experiment was performed simultaneously. After 15 min, colorimetric method was performed using a spectrophotometer at 450 nm, blank solution was used to adjusting the zero position of light absorption value.

Preparation of standard curves: 0, 1.0, 2.5, 5, 7.5, 10.0, 15.0 mL of P standard solution of which the concentration was 50 $ug.mL^{-1}$ was sucked up and transferred to a 50 ml-volumetric flask, equal parts of heating digestion solution was added to the volumetric flask respectively, color developing and colorimetric assays were performed the same way as above. The standard series of P concentration should be 0, 1.0, 2.5, 5, 7 .5, 10.0, 15.0 $ug.mL^{-1}$ of P.

## 2.2. *Selection of effects of hypotoxicity Rhizoctonias on promoting P absorption of plants*

### 2.2.2. *Experimental soil*

Soils included sifted river sands and perlites were mixed with equivalent volume and autoclaved for sterilization, and tested all kinds of physicochemical indexes such as PH value, available nitrogen, total P, available P and available K.

### 2.2.3. *Experimental plants*

Graminaceous crops (corn), Solanacae crops (tomato), Cruciferae crops (rape).

### 2.2.4. *Interaction with two experimental strains and three crops*

Two strain samples were cultured by inoculating with barleys in soil samples as above-mentioned. Samples without inoculation were as control. The relative water content in soil was kept in 75% to 80% (normal water) using weight method. The samples were irrigated 2 ml of Hoagland nutrient solution (0 mol/L solution as control) with different P concentration (0mol/L, 0.25 mol/L, 0.5 mol/L, 0.75 mol/L, 1 mol/L) every two days and replenished water every day. With barleys, for example, the number of plants needed in this experiment should be: two disposals (two strains in AG-V anastomosis groups), every disposal was repeated for 3 times, every two plants were seemed as one repetition, so the number of plants needed should be 60 of strains. The determination of corresponding indexes was performed after 1 to 3 month.

#### 2.2.5. *Determination of biomass of three crops after inoculation*

Determination of plant height, leaf number, top dry weight and root dry weight by routine methods and determination of content values of total P by $H_2SO_4$-$H_2O_2$ heating digestion method and phosphorus vanadium molybdate yellow colorimetric method.

### 2.3. *Determination of content values of total P in plants*

#### 2.3.1. *Preparation of sample solution ( $H_2SO_4$-$H_2O_2$ heating digestion method)*

0.3000g to 0.5000g of plant sample (shifted through 0.25 mm mesh sieve) which had been dried and grinded was transferred to a 50 ml-open bottle (or a heating digestion tube) (the neck of the bottle should not be contaminated with samples), a little water was dripped to wet samples at first, then added into 8 ml concentrated sulfuric acid, shaken well (it would be better to be placed over night) and covered bottleneck with a bending-neck funnel. The open bottle was heated slowly on a hotplate initially, when the concentrated sulfuric acid was decomposed with lots of smoke, the temperature should be raised then. When the colour of solution was heated to be brownish black, taken down the open bottle and bending-neck funnel after it became cooler and dripped 10 drop of 30 % $H_2O_2$ with continuous shaking the bottle, then reheated the open bottle for 5mins (kept faint boiling).Taken down the open bottle and bending-neck funnel after it became cooler and dripped 5 to 10 drop of 30 % $H_2O_2$ repeatedly. Carry on again and again thus three to five times. The volume of $H_2O_2$ added should be successive decreased. When the solution became colourless or clear, reheated it again for 5 to 10 min (to get rid of remainder $H_2O_2$), cooled the open bottle and watered the funnel with a small amount of water which flowed into the bottle. The heating digestion solution was transferred to a 100 ml- volumetric flask and diluted with water, shaken well. The solution was filtrated or placed until it became clear for t P absorption.

#### 2.3.2. *Determination of content values of total P in plants*

The sample solution of 5.00 to 10.00 mL (P content was 0.05 to 1.0 mg )was transferred to a 50 ml-volumetric flask, then 2 drops of 2,6-DNP (or 2,4-DNP) was added into the sample solution, NaOH of 6mol.$L^{-1}$ was added for PH adjustment until the colour of solution just became yellow. Added vanadium ammonium molybdate reagent of 10.00 ml precisely, diluted with water and shaked well. Blank experiment was performed simultaneously. After 15 min,

colorimetric method was performed using a spectrophotometer at 450 nm, blank solution was used to adjusting the zero position of light absorption value.

Preparation of standard curves: 0, 1.0, 2.5, 5, 7.5, 10.0, 15.0 mL of P standard solution of which the concentration was 50ug.mL$^{-1}$ was sucked up and transferred to a 50 ml-volumetric flask, equal parts of heating digestion solution was added to the volumetric flask respectively, color developing and colorimetric assays were performed the same way as above. The standard series of P concen tration should be 0, 1.0, 2.5, 5, 7.5, 10.0, 15.0 ug.mL$^{-1}$ of P.

### 2.4. *Calculation method of relevant indexes*

Contribution rates of strains (%) = (dry weight of plants within inoculation- dry weight of plants without inoculation)/ dry weight of plants within inoculation.

Contents of P absorbed by fungus= contents of total P absorbed by plants within inoculation-contents of total P absorbed by plants without inoculation.

Contribution rates of P absorption (%) = (total content of P absorption of plants with inoculation-total content of P absorption of plants with inoculation)/ total content of P absorption of plants with inoculation *100.

Strain effect (%) = (one of biomass of a plant with inoculation - one of biomass of the plant without inoculation) / one of biomass of the plant without inoculation*100.

Total P of a plant (%)=ρ.V* divide ratio*10-4 /m (ρ-P concentration of Color-substrate solution acquired from standard curves, V-volume of color-substrate solution, m- quality of dried samples (g), 10-4-the conversion factor for converting ug/L of unit concentration into percentage composition ).

### 2.5. *Statistical analysis*

All biomass statistical analyses were carried out using Excel 2003 software. Significance of difference analysis was carried out using ANOVA procedure in SAS (9.1) software. Multiple comparison analysis was carried out using LSD method.

## 3. Results and Analysis

### 3.1. *Selection of binucleate Rhizoctonias characterized by hypotoxicity and high P absorption*

After all biomass statistical analyses were carried out using Excel 2003 software. significance of difference analysis was carried out using ANOVA procedure in SAS (9.1) software and multiple comparison analysis was carried out using LSD method, as shown in Table 2, there were significant differences ($P=0.0001<0.01$)

in two hypotoxicity *Rhizoctonias* cultured in Hoagland nutrient solution for 15 to 30 days, which meant the comparative results of P concentration in solution would be AG-V (DL-YT-06-4-9)>AG-V(Bs-J-06-7-1). The results showed that, two hypotoxicity binucleate *Rhizoctonias* had the effect of absorbing P which performed as the tender of absorbing P and then releasing P in the nutrient solution.

Table 2. P contents and variance analysis of two hypotoxicity Rhizoctonias in Hoagland nutrient solution for 15 to 30 days

| Treatment | 15days | 2days | 25days | 30days | ANOVA |
|---|---|---|---|---|---|
| 1 | 0.5606 | 0.535 | 0.4364 | 0.4547 | 0.4967b |
| 2 | 0.7141 | 0.5277 | 0.4145 | 0.462 | 0.5296a |

Note: 1-AG-V (Bs-J-06-7-1), 2-AG-V (DL-YT-06-4-9). Lower case letters in the same lines represents significant difference ($P<0.05$), and the ANOVA value is the mean value analyzed by SAS software, similarly hereinafter

After all biomass statistical analyses were carried out using Excel 2003 software. significance of difference analysis was carried out using ANOVA procedure in SAS (9.1) software and multiple comparison analysis was carried out using LSD method, as shown in Table 3, there were significantly differences ($P=0.0001<0.01$) in two hypotoxicity *Rhizoctonias* cultured in Hoagland nutrient solution for 5 to 25 days, which meant the comparative results of concentration of P in solution would be AG-V(DL-YT-06-4-9)>AG-V(Bs-J-06-7-1)>CK. The results showed that, two strains would to be binucleate *Rhizoctonias* characterized by hypotoxicity and high P absorption resulting from the concentration of P in two strains were higher than concentration in CK (sterile), which meant these two stains had little significant effect of absorbing P in Hoagland nutrient solution.

Table 3. Contents of P and variance analysis of two hypotoxicity *Rhizoctonias* in Hoagland nutrient solution for 5 to 25 days.

| Treatment | 5days | 10days | 15days | 20days | 25days | ANOVA |
|---|---|---|---|---|---|---|
| CK | 0.4108 | 0.4035 | 0.5424 | 0.3816 | 0.4693 | 0.4415c |
| 1 | 0.4839 | 0.3377 | 0.5972 | 0.4254 | 0.4949 | 0.4678b |
| 2 | 0.535 | 0.2866 | 0.5899 | 0.462 | 0.5277 | 0.4802a |

### 3.2. *Screening for the effect of hypotoxicity binucleate Rhizoctonias on promoting P absorption of three plants*

As shown in Table 4, two hypotoxicity binucleate *Rhizoctonia spp*. had effects on promoting P absorption under the concentration of 0mol/L, 0.25mol/L, 0.5mol/L, 0.75mol/L and 1mol/L when these *Rhizoctonias* interacted with corns within 40 days. Among these data, Interaction with corns under the concentration of 0mol/L had the biggest impact on P absorption. There were

significant differences on contribution rate of P absorption of fungus when two hypotoxicity *Rhizoctonias* interacted with corns in 40days, and the comparative results would be AG-V(Bs-J-06-7-1)>AG-V(DL-YT-06-4-9). AG-V(Bs-J-06-7-1), AG-V (DL-YT-06-4-9) could enhance the effect of corns on P absorption under any concentration.

Two hypotoxicity binucleate *Rhizoctonias* had effects on promoting P absorption under the concentration of 0.25mol/L, 0.75 mol/L and 1 mol/L when these *Rhizoctonias* interacted with tomatoes within 50 days. There were significant differences on contribution rate of P absorption of fungus when two hypotoxicity *Rhizoctonias* interacted with tomatoes in 40days, and the comparative results would be AG-V(DL-YT-06-4-9) > AG-V(Bs-J-06-7-1). AG-V (DL-YT-06-4-9) strain could provide an effect on promoting P absorption under any concentration.

Two hypotoxicity binucleate *Rhizoctonias* had effects on promoting P absorption under tap water condition and the connection of 0 mol/L, 0.5mol/L, 0.75mol/L and 1mol/L when these *Rhizoctonias* interacted with rapes within 45 days, and when interacted with rapes under the concentration of 1mol/L, the strains had the biggest impact on promoting P absorption. There were significant differences on contribution rate of P absorption of fungus when two hypotoxicity *Rhizoctonias* interacted with rapes in 45 days, and the comparative results would be AG-V(Bs-J-06-7-1)>AG-V(DL-YT-06-4-9). The strains of AG-V (DL-YT-06-4-9) could promote P absorption of rapes under all concentrations.

Table 4. Fungal phosphorus uptake contribution rate between two kinds of AG-V Binucleate Rhizoctonia spp. and plants

| Plants | Treat-ment | Tap-water | 0mol/L | 0.25mol/L | 0.5mol/L | 0.75mol/L | 1mol/L |
|---|---|---|---|---|---|---|---|
| corns within 40 days | 1 | 19.3232 | 63.2563 | 26.2393 | 30.1464 | 32.5667 | 14.6104 |
| | 2 | 18.3956 | 67.5523 | 39.2012 | -10.8749 | 34.8403 | 18.284 |
| tomatoes within 50 days | 1 | -17.564 | -62.2828 | 30.0113 | 10.1295 | 30.5336 | 6.3173 |
| | 2 | 20.2986 | 3.2735 | 25.0102 | 30.3989 | 25.8493 | 28.231 |
| rapes within 40 days | 1 | 291.5923 | 43.4506 | -40.0302 | 17.1936 | 28.7581 | 52.4535 |
| | 2 | 122.839 | 48.9919 | 4.5478 | 22.0662 | 28.7581 | 53.0265 |

Note: 1-AG-V (Bs-J-06-7-1), 2-AG-V (DL-YT-06-4-9)

## 4. Discussions

Found in the test wire weak poison binucleate *Rhizoctonias* in Hoagland nutrient solution in the hypha growth is not very good, in shaking table is not very good grow hyphae that explain Hoagland nutrient solution does not promote weak

poison dbinucleate *Rhizoctonias* growth advantage. It may be associated with Hoagland nutrient solution concentration of choice, at the same time, it may be the growth of hypha is restrained in Hoagland nutrient solution, and for the absorption of P has certain effect. Studies have shown that, under the certain condition of nutrition (applying strength Hoagland nutrient solution of 5% ~ 50%), growth of mycorrhizal fungi and host plants underground part of phosphorus concentration was significantly positively related to growth and development of mycorrhizal fungi with the increase of concentration of phosphorus, but More than a critical concentration, with the increase of phosphorus concentration will be reduced [14]. so we need to screen Hoagland nutrient solution concentration, can we come to promote two hypotoxicity binucleate *Rhizoctonias* growth and f optimal concentration of phosphorus .

Through comparative analysis of quantity and contribution rate of P absorption of fungus in this study, we found that the interaction of two Hypotoxicity binucleate *Rhizoctonias* with corn, tomato and rape had some positive effect. But effect on P absorption tomatoes is not very obvious.

The experiments showed that, there were different degrees of effect on Promoting P absorption when two hypotoxicity binucleate *Rhizoctonias* interacted with corns, meanwhile the low P stress of two hypotoxicity binucleate *Rhizoctonias* enhancing the effect on P absorption was from 0 mol/L to 0.25 mol/L. Similarly, two hypotoxicity binucleate *Rhizoctonias* also had different degrees of effect on promoting P absorption when interacted with rapes and the low P stress of two hypotoxicity binucleate *Rhizoctonias* enhancing the effect on P absorption was 1 mol/L.

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