RESEARCH PAPER

Effect of *Rhizobium japonicum* in relation to nodulation and chlorophyll content of soybean

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The soybean(*Glycine max*), commonly called 'Cindrella crop' or 'king of legumes' *R. japonicum* is the symbiotic microorganism which forms root nodules on the roots of soybean to fix atmospheric nitrogen.The seeds of soybean were coated with *R. japonicum* to prepare legume inoculants.These seeds were referred as treated seeds.The treated seeds and untreated seeds were sown in the field.After 15 days of sowing of treated seeds and untreated seeds,the nodulation was checked.It was noted that treated seeds shown more nodules as compaired to untreated seeds. The investigation was again carried out to check the effect of *R.japonicum* in relation to chlorophyll content.The plants arrised from treated seeds shown high chlorophyll content as compaired to the plants arrised from untreated seeds.

Key words **:** *Glycine max,Rhizobium japonicum,*Legume inoculants,Nodulation, Chlorophyll content.

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INTRODUCTION

The *Rhizobium* culture strains are antigenically very selective and require particular host for nodulation. The surface antigen on the rhizobial cells recognizes the binding sites (specific root exudates) on the roots of the leguminous plants. This characteristic makes them host-specific. Specific rhizobial cell can penetrate the roots of the specific leguminous plants only and form nodules. They multiply within the nodule using the carbon source from the plant and in turn fix part of the atmospheric nitrogen to the plant.

Leguminous plants such as soybean (*Glycine max*) form root nodules that contains population of *Rhizobium* a nitrogen fixing bacterium.The symbiotic association between the plant and the *Rhizobium* is initiated when bacteria in the soil attach to root hairs.This highly specific attachment process is mediated by plant proteins, the lectins, that bind the bacteria to the surface of root hairs;that is then penetrated by the microbes. The bacteria from the root hairs travel to inside and infect the root cells.The infected root cells divide and form a nitrogen fixing root nodule.The nodule provides the anaerobic environment necessary for nitrogen fixation (Aneja, 1996).

There is increase in the shoot length,root length,shoot dry weight, root dry weight, nodule number, chlorophyll-a, chlorophyll-b and total chlorophyll content of soybean. plants arrised due to *R. japonicum* treated seeds as compaired to untreated seeds.The *R. japonicum* strains could significantly improve the plant growth. This is due to that *R. japonicum* produces various growth promoting substances,fix atmospheric nitrogen symbiotically with the help of soyabean plant.The *Rhizobium* spp.are being used as biofertilizers to reduce the need for expensive chemical fertilizers. About 10 to 15 per cent increase of crop yield can be achieved with the use of this culture.

RESEARCH METHODOLOGY

Isolation of *Rhizobium japonicum*:

R japonicum was isolated from fresh,healthy,unbroken and pink nodules from soybean (*Glycine max*) roots on sterile yeast extract mannitol agar (YEMA) medium using pour plate technique.The incubation was at 26^0C for four days.After incubation,large gummy colonies appear on sterile YEMA plates (Kamthane, 2010).

After isolation,the seeds were inoculated with *R japonicum.*

Seeds treatment with *R japonicum*:

This was done by mixing isolated rhizobia culture in 10

per cent sugar and 40 per cent gum arabic to form a slurry.In this seeds were added. With the result, a uniform coat of the *R japonicum* was formed around the seeds.The treated seeds were dried in shade and sown immediately (Aneja, 1996).

The roots of plants arrised from the treated seeds (TRE)and untreated seeds(UNT-control) were observed for the production of nodules after 15 days of sowing of seeds in the field.The seedlings were removed from the inoculated plot for counting the nodules.The comparision was done between nodules produced from the roots of plants arrised from the treated seeds and untreated seeds.

Estimation of chlorophyll content:

The leaves of the plants of treated and untreated seeds were taken. 2 g leaf tissue homogenized with few ml of pure acetone separately. It was filtered through whatman filter paper No.1 with the addition of 80 per cent acetone.The filtration was repeated with 80 per cent acetone up to the pulp and filter paper lost green colour.100 ml of volume of the filtrate was done by adding 80 per cent acetone(Arnon,1949).This process was made on alternate days of sowing *i.e.*10^{th}, 12^{th}, 14^{th}, 16^{th}, 18^{th}, 20^{th} days. The chlorophyll extract optical density was read with spectrophotometer set at 645nm and 663nm against 80 per cent acetone solvent as blank. The quantity of chlorophyll present (mg/g of tissue) in the extract was calculated according to the following formulae:

Chlorophyll-a=(12.7xD663)-(2.69xD645)xV/1000x1/W

Chlorophyll-b=(22.9xD645)-(4.68xD663)xV/1000x1/W

Total chlorophyll=(20.2xD645)+(8.02xD663)xV/1000x1/W

where-

D=Optical density at 645 and 663 nm

V=The final volume of 80 per cent acetone chlorophyll extract

W=Fresh weight in grams of the tissue extracted

RESEARCH FINDINGS AND ANALYSIS

The seedlings of the treated and untreated(UNT)seeds were removed from the inoculated plots after 15 days of sowing. The mean of nodules developed was taken. The seedlings of the treated seeds showed the development of 7 nodules on the roots.The seedlings of the untreated seeds showed development of 2 nodules on the roots.The treatment of the seeds with *R . japonicum* increases the nodulation (Annapurna *et al.,* 1998). The rhizobia fix more nitrogen.This shows that *Rhizobium japonicum* inoculation with the seeds is benifitial to increase the crop yield.

The chlorophyll content was also estimated.The chlorophyll content of the leaves of the plants from treated leaves was increased over untreated seeds plant leaves (Table 1, 2 and 3). This increase in the chlorophyll content of leaves increases the photosynthetic activity of the plants (Zitco *et al.*, 1985).This increase in the photosynthetic activity of the plants ultimately increases the crop yield.There was increase in the chlorophyll-a,chlorophyll-b and total chlorophyll content of the plants up to 18 days of sowing.After this,there was no large increase in the chlorophyll. It was near about constant one.

Table 1 : Influence of *R.japonicum* on the chlorophyll-a content (mg/g fresh weight) of soybean leaves

Plant	Chlorophyll-a content(mg/g) Incubation period(Days)					
	10	12	14	16	18	20
Glycine max UNT	2.75	2.78	2.97	3.10	3.33	3.33
Glycine max TRE	4.05	4.21	4.33	4.38	4.51	4.51

Table 2 : Influence of *R.japonicum* on the chlorophyll-b content (mg/g fresh weight) of soybean leaves

Plant	Chlorophyll-b content(mg/g) Incubation period(Days)					
	10	12	14	16	18	20
Glycine max UNT	0.95	0.99	1.08	1.13	1.27	1.28
Glycine max TRE	1.12	1.20	1.59	2.22	2.45	2.46

Table 3 : Influence of *R.japonicum* on the total chlorophyll content (mg/g fresh weight) of soybean leaves

Plants	Total chlorophyll content(mg/g) Incubation period(Days)					
	10	12	14	16	18	20
Glycine max UNT	3.80	3.91	3.98	4.33	4.48	4.48
Glycine max TRE	5.41	5.45	5.68	5.88	6.53	6.62

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