



EFFECT OF RHIZOBIUM INOCULATION ON SEED VIGOUR IN CHICK PEA (*CICER ARIETIUM* L.)

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ABSTRACT

Effect of Rhizobium inoculation on seed vigour was studied. Rhizobium inoculation could not increase seed vigour in most of the seed vigour evaluations but protein content of grain increased in the raya of 16-18% with Rhizobium inoculation. As far as seed vigour is concerned only in brick gravel test it was observed that inoculation with Rhizobium resulted in significant increase in seed vigour.

Key words : Rhizobium inoculation, Seed vigour, Brick gravel test, Protein.

INTRODUCTION

The importance of pulses in the economy, particularly under dry land agriculture, cannot be over emphasised. Pulses occupy an important place in the diet and have a permanent place in the agricultural economy of India. Chick pea is the very important pulse crop in India. Production of any crop primarily depends on the seed quality and vigorous seeds always have better potential for good germination and over all on yield. Legumes are able to utilise the unlimited resources of air nitrogen; this presents a unique opportunity to devise agricultural system without large investment in fertilizer nitrogen. Usually chemical fertilizers prohibitively expensive in developing countries and their substitution by Biological Nitrogen Fixation should be given high priority. In view of the above consideration, the present study was conducted during Rabi season of 2001-02 with the objective to examine the effect of Rhizobium inoculation on seed quality.

MATERIALS AND METHODS

Procurement of Rhizobium strains

Two different strains of chick pea Rhizobium namely

G-3 and TAL-620 were obtained from Soil Microbiology Laboratory, G.B. Pant University of Agricultural Technology, Pant Nagar, Purity of Culture, was examined microscopically by streaking on congo red yeast extract Manitol Agar medium (CRYEMA).

Screening for Streptomycin resistance

Both the strains were screened against different concentration of Streptomycin to examine antibiotic resistance. Introduction of each isolate was prepared by suspending cells of mother culture into saline solutions. One ml of the suspension of each strain was inoculated into test tube in duplicate containing 5ml yeast extract Manitol broth and antibiotic concentrations ranging from 0 to 1000ug/ml. tubes were shaken on a rotatory shaker at room temperature for 7 days. At the end, cells from each tube were streaked on CRYMA Plates to confirm whether the organism growing in tubes is Rhizobium or any other contaminants.

Experiment and experimental design

Randomised block design having four treatments and five replications was followed. The details are given

below:

1. No inoculation (control)(I₀)
2. Inoculation with strain G-3 (I₁)
3. Inoculation with strain TAL-620 (I₂)
4. Inoculation with mixture of G-3+TAL-620 in 50:50 ratio w/w (I₃)

Inoculation preparation- Carrier based inoculum was used in the study. It was prepared by growing each strain for 72 hours in separate conical flasks containing 20ml YEM broth on rotatory shaker. Cells in the end were collected by centrifugation and suspended in sterilized water. The cell suspension then was mixed in charcoal.

Seed sowing and post planting operations

Un-inoculated seeds (control) were sown first followed by inoculated treatments. Seeds were treated with carrier based culture in polythene bags by thorough mixing of the cultures with moist seeds. Sowing was done in 3-4 cm. deep furrow. To maintain equal population and appropriate plant to plant distance either thinning or gap filling was done. Three hand weeding were done. Dithane M45 and thidone were sprayed to protect the crop from Ascochyta blight and Heliothis attack.

Observations- observations on nodulation and plant growth were recorded. Grain and straw yield were recorded from the four central rows. The net area harvested was 3.6 sq. meters. Grain yield was recorded at 11% moisture level. The weight of 100 grains was also recorded.

The seeds harvested from different plots (treatments) were tested for seed quality in terms of vigour.

Evolutions were based on laboratory tests.

Standard germination test

The standard germination test was made between towel papers. After moistening the towel papers in tap water, excess water from these was drained out by pressing the towel papers.

150 seeds from each treatment were placed between two layers of three germination towels each having 45cm × 27.5 cm size and it was placed on sheet of butter paper.

The towel paper was rolled in a manner that some top portion remained uncovered. The rolls were placed in a seed germinator at 25°C. Watering was done with a sprinkler as and when required. The germination was evaluated as per ISTA rules(1966) and was expressed as percent germination.

Accelerated aging test

150 seeds of each treatment were taken at

45°C. temperature and 98±1% relative humidity for 72 hours (Delouche *et al.*, 1967) . After 72 hours seeds were put to germination test as described above.

Hot water treatment

150 seed from each treatment were subjected to hot water treatment test. For this the seeds were placed in a wire mesh strainer. The strainer was dipped in hot water (60°C.) for 60 seconds (Bryd, 1970; Delouche and Agarwal, 1971; Tripathi, 1975) and then cooled immediately in the running tap water and blotted dry. Later, these seeds were subjected to germination test, similar to method mentioned above.

Seedling vigour test

30 seeds from each treatment were planted on moist towel paper similar to method described for standard germination test in 3 replication of 10 seeds each, with radicle end of each seed oriented in the same direction. The towel paper were then rolled and put into germinator, vertically at 25°C. The seedling length (hypocotyle + radicle), in centimeter was measured on 5th day.

Brick gravel test

20 seeds from each treatment were planted in soil in small plastic pots. A 30 mm layer of moist gravel (2 to 3 mm size) were placed above soil layer covering seeds. Seedling coming out of brick layer on 7th day were counted. Their percentage was calculated.

RESULTS AND DISCUSSION

Effect of Rhizobium inoculated on seed vigour was examined by treating the grains obtained with different inoculation treatments as seed, therefore seed inoculation was made through different laboratory tests.

Seed quality evaluation

The analysis of variance for seed quality evaluations has been presented in table 1. The mean sum of squares due to germination percentage (Brick gravel test) were found to be significant at 5% level indicating thereby significant difference in regard to control and inoculation treatments. The mean sum of squares due to other evaluation namely, standard germination test, hot water treatment, accelerated aging and seedling g length were found to be non-significant, indicating thereby their inability to reveal any differences between control and inoculations treatments. Thus, amongst the seed quality evaluations. The brick gravel test appeared to be most satisfactory to bring out differences, if any in the inoculation treatments.

The mean values regarding seed quality evaluations are given in table 2 as has been described earlier. Only

Table 1 : Analysis of variance.

Source of variation	d.f.	Mean sum of square Germination percentage				
		Standard germination test	Brick gravel test	Hot water test	Accelerated aging	Seedling length
Replication	3	0.7031	70.0000	0.8125	6.2031	0.1056
Treatment	4	2.6667	151.6666*	2.4167	7.1250	0.2292
Error	12	1.5000	26.6666	1.7292	3.1354	0.5515
Total	19					

*significant at 5% level

CV=	0.5477	5.6437	1.3446	2.8062	8.9047
CD=	NS	7.12	NS	NS	NS

Table 2 : Table of means of different laboratory vigour test.

Test	Un-inoculated control	Inoculated			SEM±
		G-3	TAL-620	G-3+TAL-620	
Standard germination	97.6 ^a	98.8 ^a	99.2 ^a	98.0 ^a	0.55
Germination %(accelerated aging)	61.6 ^a	62.8 ^a	66.4 ^a	63.6 ^a	0.79
Germination %(hot water treatment)	96.8 ^a	98.0 ^a	98.4 ^a	98.0 ^a	0.59
% of seedling coming out of brick gravel layer(Brick gravel test)	86.0	98.0	94.0	88.0	0.23
Seedling height(cm)	8.1	8.5	8.2	8.6	0.33

the brick gravel test revealed the significant differences in regard to seed quality as affected by various inoculation treatments. The seed inoculated with Rhizobium strain G-3 gave the highest number of vigorous seedling (98%) which were significantly superior to control (86%) and their inoculated with Rhizobium mixture (G-3+TAL-620) (88%) but at par with those inoculated with Rhizobium strain TAL-620 indicating thereby that Rhizobium inoculation does improve the seed vigour, also the effect of individual Rhizobium strains when used individually gave better results as compared to when used as a mixture.

The other tests namely, standard germination, germination percentage hot water treatment and germination percentage accelerated aging though did not reveal any significant difference in old quality. A cursory review of the mean values in table 2 indicated that the values (seed vigour evaluation) were higher in the seed inoculated with either of the Rhizobium strain as compare to control indicating thereby that inoculation did have some beneficial effect on the seed quality.

The results revealed that under stress condition (Brick gravel test) the seeds obtained with inoculated treatments performed significantly better. The number of seedlings

emerged were significantly higher than control. The strain G-3 gave better performance than strain TAL-620. The other vigour test, though significantly not significant, also revealed that the inoculated seeds had higher seed vigour than control. The reasons for enhanced seed vigour when seeds were inoculated with Rhizobium strains is due to increased protein content in the seeds harvested from inoculated treatments. The protein content has been reported to be affecting the seed vigour significantly (Grabe, 1967). These results are also supported by Bishwas *et al.* (2000), who observed enhanced seedling vigour and yield by Rhizobial inoculation in rice. However, the results obtained in present study open up the possibilities of study this aspect in greater detail.

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