International Journal of Advanced Research in Biological Sciences

www.ijarbs.com

Research Article

Effect of *Agrobacterium rhizogenes* and *Rhizobium* on growth, yield and biochemical parameters of groundnut plants

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Abstract

The present study was carried out to know the effect of *Agrobacterium rhizogenes* and *Rhizobium* on growth, yield and some biochemical parameters of groundnut plants. The groundnut seeds were treated with *Agrobacterium rhizogenes* and grown on pots containing sterilized soil, normal soil, plus *Rhizobium* introduced soils. The parameters like plant weight, root length, number of lateral roots and length, number of nodules and nodule weight, fresh and dry weight production, pod yield were significantly influenced by *Agrobacterium rhizogenes* and *Rhizobium.* The biochemical parameters like soluble protein; total free amino acids and total Chlorophyll content was also influenced by *Agrobacterium rhizogenes* and *Rhizobium*. In legume crops, the increased lateral branching of hairy roots and nodulation is useful for increased nitrogen fixation.

Keywords: *Agrobacterium rhizogenes* ; *Rhizobium;* plant weight; root length; number of lateral roots and length; number of nodules; soluble protein; total free amino acids; total Chlorophyll content.

Introduction

Groundnut (*Arachis hypogaea L.*) is one of the main oil yielding croups. The cotyledons are rich in fatty edible oil. The oil extracted and used in cooking is known as groundnut oil. The seeds are also edible. The oil cake after the extraction of the oil is a good cattle feed. The cotyledons are also rich in proteins. The plant is extensively cultivated for its underground pods. The plant (*Arachis hypogaea L. – Papilionaceae*) is a native of Brazil, but now cultivated extensively all over the world. The seeds have 40-50% oil. The oil is also used in the manufacture of vanaspathi. Poorer grades of oil find use in the manufacture of soaps. Oil cake is a good fertilizer.

Agrobacterium rhizogenes, a soil-borne bacterium, is well known to induce hairy roots when Ri T-DNA is integrated into the plant genome. Plants regenerated from the hairy roots in several plant species exhibit characteristic phenotypes such as shortened internodes, wrinkled leaves and abundant

root mass with intensive lateral branching (Tepter, 1984). Moreover, the transgenic plants produced from hairy roots are usually non-chimeric because the hairy roots originated from single cells and each hairy root consists of uniformly transformed cells.

The morphological alterations caused by integration of Ri T-DNA might be of interest for breeding some chops such as sweet potato (Otani *et al*., 1996) and ornamental plants (Otani *et al*., 1996; Godo *et al*., 1997). In legumes, proliferous root growth and abundant lateral branching of hairy roots are considered to be useful for improving nitrogen fixation. However, only a few studies have reported on regeneration from having roots in leguminous species such as Lotus and Medicago (Spano *et al.,* 1997). Two types of beneficial association between microbes and higher plants are most important in agriculture i.e. crop production. One is the symbiotic association between the microbe *Rhizobium* and leguminous plants. These diazotrophic (Nitrogen fixing) organisms found in the root nodules of the leguminous plants such as Alfalfa, Clover, Pea and Soybean can convert molecular N_2 into a form that the host plant can use to make amino acids. *Rhizobium* reduces molecular nitrogen to ammonia. In this process, the enzyme nitrogenase plays a key role. The ammonia may the be interconnected by host cell enzymes to other usable from such as nitrate and nitrate ions, amino acids and nitrogenous bases. Leguminous plants with *Rhizobium* containing root nodules are able to fix ten times more nitrogen than. It improves crop yield and enriches the surrounding soil.

In plants such as Clover, Peanuts, Beans and Alfalfa, nitrogen-fixing bacteria such as *Rhizobium* live inside the root nodules. The microbes take in N_2 and provide nitrogen in an instable form for these plants. Farmers may grow both Clover and Alfalfa to increase the nitrogen content of agricultural soils, especially when they want to plant crops that need large amounts of nitrogen. Many farmers alternate planting and nitrogen such crops such as Alfalfa and nitrogen poor crops such as Corn. Alternate planting is called crop rotation. This plant maintains the nutrient quality of the soil.

Objectives

The present study was carried out with the following objectives. To know the effect of *Agrobacterium rhizogenes* on root induction in field conditions. To analyse the combining effect of *Agrobacterium rhizogenes* and *Rhizobium* on various growth parameters of groundnut cultivars. To evaluate the effect of *Rhizobium* on root nodulation. To estimate the protein, amino acid and chlorophyll content in groundnut with respect to seed treatment with *Agrobacterium rhizogenes* and *Rhizobium* introduced in pot culture.

Materials and Methods

Seed material

The groundnut seed was used for present study.

Rhizobium culture

The pure culture of *Rhizobium* sp. was obtained from Research Center Tamil Nadu Agricultural University, Vamban, pudukkottai – district.

Agrobacterium rhizogenes *culture*

The pure cultures are *Agrobacterium rhizogenes* was obtained from Microbial Type Culture Collection Center, Chandigarh, Assam-India. The experiments were carried out using pot culture with five different soil samples along seed treatment with *Agrobacterium rhizogenes.*

Treatment – I

Autoclaved soil plus seed.

Treatment –II

Autoclaved soil plus seed plus *Agrobacterium rhizogens.*

Treatment – III

Normal soil plus seed.

Treatment - IV

Normal soil plus seed plus *Agrobacterium rhizogenes* plus *Rhizobium.*

Treatment - V

Normal soil plus pricked seeds plus *Agrobacterium rhizogenes* plus *Rhizobium*

Seed treatment with *Agrobacterium rhizogenes*

Seed inoculation of *Agrobacterium rhizogenes* was done by 24 hrs old cultures on *Agrobacterium rhizogenes* with 10% sucrose and 40% gum arabic to from slurry to which seeds wee added with the result the uniforms coat of the *Rhizogenes* was formed around the needs. The inoculated seeds were dried in shade and sown immediately in the pots containing autoclaved soil and normal soil. Among the five different treatments of *Rhizogenes* in one treatment. The seeds were uniformly pricked the help of sterile needle to facilitate the infection process.

Procedure

Broth culture of *Agrobacterium rhizogenes* was prepared by using nutrient broth. The overnight grown culture was centrifuged and the supernatant was discarded and the pellet was taken. Fifty grams of Gum Arabic and 5 gms of sucrose was weighted and mixed in 50 ml of distilled water. After that the mixture boiled for 15 minutes. The mixture was cooled for 30 minutes. In a semisolid state the pellet was mixed thoroughly with the gum arabic sugar slurry. Then the seeds mixed with inoculums slurry, by hand so as to uniformly cool the seeds with the inoculums. After that the seeds were sown in the pots.

Preparation of *Rhizobium* culture

The *Rhizobium* culture was inoculated with Yeast Extract Manitol Agar medium. Then incubate the plates for 10 days. With the help of scalpel or needle scrap the growth of the bacterium from the plates and suspended in water. This inoculum was mixed with the soil (only in treatment 4 and 5) before sowing of seeds.

The following growth, yields and biochemical parameters were studied

Plant height, Number of branches, Root length, Number of lateral roots, Lateral root length, Number of nodules and individual nodule weight, Fresh and Dry weight of the whole plant and Pod yield.

Biochemical estimation.

Extraction and estimation of buffer soluble proteins and Extraction and estimation of free amino acids.
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Extraction and estimation of buffer soluble proteins-

The buffer soluble protein was estimated by the biurette method (Jayaraman, 1981). Two grams of fresh plant material was extracted using 10 ml of ice cold, Trisu-Hel buffer pH 7.2 (Tris hydroxy methyl amino acid) using ice cold morter and pestle. The extract was centrifuged at 10,000 rpm for 20 minutes. The supernatant was taken for the estimation of soluble protein. The volume of supernatant was measured.

To a known quantity of the supernatant, an equal volume of ice cold 20 percent Trichloro acetic acid (TCA) was add and left in the ice bath for 30 minutes. A white precipitate was formed. The precipitate was separated by centrifugation. Then the precipitate was dissolved completely using 2 ml of 5% sodium hydroxide. The sodium hydroxide dissolved precipitate was taken for the estimation of protein by the biurette method using ERMA photoelectric colorimeter read at 620 nm. 5 ml of extract and 3.5 ml of distilled water and 6 ml of biurette reagent was added, a purple color formed. Bovine serum albumin (BSA) (Sigma Chemical Company, U.S.A) was used as standard.

Extraction and estimation of Free Amino Acids

Two grams of pestle using 80% ethanol with a small amount of acid washed sand. The homogenate was then filtered through a filter paper, and the residue was further extracted twice with the same solvent. The homogenate was centrifuged at 10,000 rpm for 20 minutes. The supernatant was used for quantitative estimation total free aminoacid using ninhydrin reagent.

Two grams of ninhydrin was dissolved in 25ml of acetone. To this solution 25ml of 0.2 m acetone buffer pH 5.5 was added. 0.5ml of extract was taken in a test tube and it was made up to 4ml with distilled water. Then 1ml of ninhydrin reagent was added and test tubes were kept in boiling water bath for 15 minutes. A pink color was developed. Then the tubes were cooled to the room temperature and 1ml of 50% ethanol was added. The absorbance of pink color solution was measured at 530nm [green filter] using Erma photoelectric colorimeter. The amount of amino acid was expressed in mg per gram fresh weight by using a standard curve. The amount of free amino acid content was estimated in the control and treated seeds and seedlings at 24, 72,120, 160 and 268 hours, after germination.

Extraction and estimation of chlerophyll content

The chlorophyll content was estimated according to the method of Arnom (1949). One gram of fresh leaves was homogenate in 5ml of 80% acetone and 1ml of 0.5% $CaCO_3$ solution using a mortar and pestle. The extract was filtered using a cotton cloth. The debris was washed with fresh acetone until it become colorless. Then the extract was centrifuged at 5000 rpm for 20 minutes. The supernatant was made up to a known volume. It was used for the estimation of total chlorophyll by measuring the optical density at 645nm and 663nm (red filter) using the Erma photoelectric colorimeter, Japan.

Results

The seeds of groundnut plants were treated with *Agrobacterium rhizogenes* and inoculated on normal soil, autoclaved soil and *Rhizobium* introduced soils in pot culture. After 90 days, various growth and yield parameters and some biochemical parameters were studied. All the parameters were highly influenced by *Agrobacterium rhizogenes* and also by *Rhizobium.*

Plant height

The plant height was observed on 90^{th} day. The plant height was varied from 12.36 cm to 26.36 cm in groundnut plants, The height was highly increased in Treatment 5 (Table.1).

Number of branches

In autoclaved soil (Treatment 1) branching was very poor (2.01 in groundnut plants) (Table.2) where as it was recorded 5.33 and 5.02 in *Rhizobium* introduced soil in groundnut plants respectively. But seeds subjected to pricking for *Agrobacterium rhizogenes* treatment has more branchesin normal soill.

Root length

The root length was also measured on 90^{th} day (Table. 3). In autoclaved soil (Treatment 1) the root length was 7.04 in groundnut plants and 6.03 in groundnut plants but the plants derived from pricked seeds these was and almost double fold increase in root length was observed (in groundnut plants 13.16 cm and groundnut plants in 4.12 cm) followed by plants derived from normal soil with *Agrobacterium rhizogenes* and *Rhizobium.*

Lateral roots

The plants cultivated in autoclaved soil have no lateral roots (Table. 4). Seeds containing *Agrobacterium rhizogenes* having lateral roots. The highest number of lateral roots was observed in Treatment 5. It was 16.85 cm in groundnut plants and 15.28 cm in groundnut plants. There was a

steady increase in number of lateral roots in Treatments 3, 4 and 5 in both the cultivars.

Lateral root length

The length of the lateral roots was recorded from 5.88cm to 12.72cm in groundnut plants it was differed from 5.0cm to 11.52cm (Table. 5).

Root nodule production

In Treatment 2 the nodule number was very low but in Treatment 4 and 5. The plants having more number of lateral roots (Table. 6). Due to *Rhizobium* inoculation the nodule percentage was highly increased. The maximum number 45.42 in groundnut plants.

Nodule weight

The weight of the nodule was highly increased in *Agrobacterium rhizogenes* treated and *Rhizobium* introduced pots (Table7). The increasing number of lateral roots may be responsible for increased number of nodules due to infection with *Rhizobium.*

Fresh and Dry weight of the plants

The mean fresh and dry weight of the plants was observed in all the five treatments on 90^{th} day (Table 8). Both fresh and dry weight was significantly increased in Treatment 3,4 and 5. The plants cultivated in Treatment 1 the fresh weight was 7.37 gms in groundnut plants were as it was 46.23 in Treatment 5. While it was 6.85gms in groundnut plants 4 where the plants derived from Treatment 1. The similar trend was also observed in dry weight also.

Pod number

The increasing number of root length and lateral roots needed to increase the number of nodules. It was responsible for increasing yield. Based on treatments the pod number was varied from 3.0 to 26.2 in groundnut plants and 2.5 to 23.7 in groundnut plants . Like that of growth and yield parameters the biochemical contest also varied based on the treatment.

Protein

The buffer soluble protein was estimated on 90^{th} day from leaves. In treatment 3,4 and 5, there was a linear increase in protein contest in treatment 1 and 2, there was a significant decrease in protein content was recorded.

Amino acid

The free amino acid content was estimated on 90^{th} day. Like that of proteins the same trend was also observed in amino acid content in Treatment 3,4 and 5. There was a steadily increased in amino acid content. But in treatment 1 and 2, it was highly decreased. It was ranged from 40 to 169mg in groundnut plants .

Chlorophyll

The chlorophyll content was estimated on 60^{th} day. We have observed variations in all the five treatments. Among the five different treatments, in treatments 3 to 5 the chlorophyll contest was highly increased. But in treatments 1 and 2 it was drastically reduced.

Discussion

Agrobacterium rhizogenes were mainly used for hairy root induction in *in vitro* condition. *Agrobacterium tumificians* and *Agrobacterium rhizogenes* were commonly used in genetic engineering studies of higher plants. The fast growing hairy roots or unique in the genetic and biosynthetic stability and the fast growth, offers an additional advantage. These fast growing hairy roots can be used as a continuous source for the production of secondary metabolites. More ever, transformed roots are able to regenerate whole viable plants and maintain the genetic stability during further sub culturing and plant regeneration.

In our present investigations, numbers of lateral roots were increased in *Agrobacterium rhizogenes* treated plants. Akasaka, *et al*., (1998), reported

Table.1 Effect of *Agrobacterium Rhizogenes* and *Rhizobium* on plant height in groundnut cultivars (Mean ± SD)

S.No	Treatments	Plant height (cm)	
		Groundnut Variety 1	Groundnut Variety 2
1	T1	12.36 ± 1.14	10.65 ± 1.30
2	T2	17.13 ± 1.48	14.11 ± 1.67
3	T3	21.76 ± 1.64	19.62 ± 1.17
4	T4	22.26 ± 1.30	20.53 ± 1.02
5	T5	26.26± 1.51	24.55 ± 1.16

T1 – Autoclaved soil plus seed. T2 – Autoclaved soil plus seed plus *Agrobacterium rhizogenes.* T3 – Normal soil plus seed. T4 – Normal soil plus seed plus *Agrobacterium rhizogenes plus Rhizobium* introduced soil. T5 – Normal soil plus pricked seed plus *Agrobacterium rhizogenes plus rhizogenes plus Rhizobium* introduced soil.

Table.2 Effect of *Agrobacterium rhizogenes* and *Rhizobium* on number of branches in groundnut cultivars (Mean ± SD)

S.No	Treatments	Numbers of branches	
		Groundnut Variety 1	Groundnut Variety 2
1	T1	2.01 ± 1.14	2.01 ± 0.04
2	T2	3.51 ± 0.22	3.22 ± 0.33
3	T3	4.33 ± 0.28	4.10 ± 0.29
4	T4	5.33 ± 0.47	5.02 ± 0.42
5	T5	7.01± 0.62	6.52 ± 0.69

T1 – Autoclaved soil plus seed. T2 – Autoclaved soil plus seed plus *Agrobacterium rhizogenes.* T3 – Normal soil plus seed. T4 – Normal soil plus seed plus *Agrobacterium rhizogenes plus Rhizobium* introduced soil. T5 – Normal soil plus pricked seed plus *Agrobacterium rhizogenes plus rhizogenes plus Rhizobium* introduced soil.

Table.3 Effect of *Agrobacterium rhizogenes* on root formation in groundnut cultivars (Mean ± SD)

S.No	Treatments	Root length (cm)	
		Groundnut Variety 1	**Groundnut Variety 2**
1	T1	7.04 ± 0.63	6.83 ± 0.49
2	T2	8.82 ± 0.74	7.22 ± 0.68
3	T3	10.66 ±1.02	9.62 ± 0.75
4	T4	12.28 ±0.89	11.50 ±0.48
5	T5	13.16 ±0.75	12.60 ± 0.17

T1 – Autoclaved soil plus seed. T2 – Autoclaved soil plus seed plus *Agrobacterium rhizogenes.* T3 – Normal soil plus seed. T4 – Normal soil plus seed plus *Agrobacterium rhizogenes plus Rhizobium* introduced soil. T5 – Normal soil plus pricked seed plus *Agrobacterium rhizogenes plus rhizogenes plus Rhizobium* introduced soil.

Table. 4 Effect of *Agrobacterium rhizogenes* on laterual root formation in groundnut cultivars (Mean ± SD)

S.no	Treatments	Number of laterul root(cm)	
		Groundnut Variety 1	**Groundnut Variety 2**
1	T1	0.00 ± 0.00	0.00 ± 0.00
2	T2	5.00 ± 0.49	4.50 ± 0.40
3	T3	7.21 ± 0.74	6.84 ± 0.65
4	T4	15.26 ± 1.02	14.05 ± 0.99
5	T5	16.85± 1.10	15.28 ± 1.10

T1 – Autoclaved soil plus seed. T2 – Autoclaved soil plus seed plus *Agrobacterium rhizogenes.* T3 – Normal soil plus seed. T4 – Normal soil plus seed plus *Agrobacterium rhizogenes plus Rhizobium* introduced soil. T5 – Normal soil plus pricked seed plus *Agrobacterium rhizogenes plus rhizogenes plus Rhizobium* introduced soil.

Table. 5 Effect of *Agrobacterium rhizogenes* on laterual root lenth in groundnut cultivars (Mean ± SD)

S.No	Treatments	Laterul root length (cm)	
		Groundnut Variety 1	Groundnut Variety 2
1	T1	0.00 ± 0.00	0.00 ± 0.00
2	T2	5.88 ± 0.44	5.00 ± 0.38
3	T3	9.03 ± 0.52	8.80 ± 0.47
4	T4	10.48 ± 0.67	9.54 ± 0.63
5	T5	12.72± 0.78	11.52 ± 0.72

T1 – Autoclaved soil plus seed. T2 – Autoclaved soil plus seed plus *Agrobacterium rhizogenes.* T3 – Normal soil plus seed. T4 – Normal soil plus seed plus *Agrobacterium rhizogenes plus Rhizobium* introduced soil. T5 – Normal soil plus pricked seed plus *Agrobacterium rhizogenes plus rhizogenes plus Rhizobium* introduced soil.

Table. 6 Effect of *Agrobacterium rhizogenes* and *Rhizobium* on root nodule production in groundnut cultivars (Mean ± SD)

S.No	Treatments	Number of nodules per plant	
		Groundnut Variety 1	Groundnut Variety 2
1	T1	0.00 ± 0.00	0.00 ± 0.00
2	T2	3.45 ± 0.35	3.00 ± 0.28
3	T3	17.61 ± 0.54	16.10 ± 0.44
4	T4	37.01 ± 1.41	35.25 ± 1.21
5	T5	45.42± 1.58	42.1 ± 1.43

T1 – Autoclaved soil plus seed. T2 – Autoclaved soil plus seed plus *Agrobacterium rhizogenes.* T3 – Normal soil plus seed. T4 – Normal soil plus seed plus *Agrobacterium rhizogenes plus Rhizobium* introduced soil. T5 – Normal soil plus pricked seed plus *Agrobacterium rhizogenes plus rhizogenes plus Rhizobium* introduced soil.

Table. Effect of *Agrobacterium rhizogenes* and *Rhizobium* on root nodule weight in groundnut cultivars (Mean ± SD)

S.No	Treatments	Root nodule weight (mg)	
		Groundnut Variety 1	Groundnut Variety 2
1	T1	0.000 ± 0.000	0.000 ± 0.000
2	T2	0.020 ± 0.003	0.017 ± 0.004
3	T3	0.092 ± 0.010	0.035 ± 0.001
4	T4	0.092 ± 0.020	0.081 ± 0.002
5	T5	0.123 ± 0.021	0.110 ± 0.006

T1 – Autoclaved soil plus seed. T2 – Autoclaved soil plus seed plus *Agrobacterium rhizogenes.* T3 – Normal soil plus seed. T4 – Normal soil plus seed plus *Agrobacterium rhizogenes plus Rhizobium* introduced soil. T5 – Normal soil plus pricked seed plus *Agrobacterium rhizogenes plus rhizogenes plus Rhizobium* introduced soil.

Table. 8 Effect of *Agrobacterium rhizogenes* and *Rhizobium* on whole plant weight in groundnut cultivars (Mean ± SD)

S.No	Treatments	Whole plant Weight (gms)	
		Groundnut Variety 1	Groundnut Variety 2
1	T1	7.37 ± 1.30	6.85 ± 1.58
2	T2	15.68 ± 1.52	13.52 ± 1.35
3	T3	32.55 ± 1.82	30.25 ± 1.48
4	T4	36.27 ± 1.93	35.72 ± 1.16
5	T5	46.23 ± 2.07	44.23 ± 1.99

T1 – Autoclaved soil plus seed. T2 – Autoclaved soil plus seed plus *Agrobacterium rhizogenes.* T3 – Normal soil plus seed. T4 – Normal soil plus seed plus *Agrobacterium rhizogenes plus Rhizobium* introduced soil. T5 – Normal soil plus pricked seed plus *Agrobacterium rhizogenes plus rhizogenes plus Rhizobium* introduced soil.

Table. 9 Effect of *Agrobacterium rhizogenes* and *Rhizobium* on dry weight in groundnut cultivars (Mean ± SD)

S.No	Treatments	Dry weight (gms)	
		Groundnut Variety 1	Groundnut Variety 2
1	T1	2.21±0.33	2.00±0.27
2	T2	8.08±0.68	7.20±0.52
3	T3	11.40±1.00	10.12±0.98
4	T4	14.85±1.48	13.00±1.22
5	T5	17.01±1.77	16.05±1.60

T1 – Autoclaved soil plus seed. T2 – Autoclaved soil plus seed plus *Agrobacterium rhizogenes.* T3 – Normal soil plus seed. T4 – Normal soil plus seed plus *Agrobacterium rhizogenes plus Rhizobium* introduced soil. T5 – Normal soil plus pricked seed plus *Agrobacterium rhizogenes plus rhizogenes plus Rhizobium* introduced soil.

Table. 10 Effect of Agrobacterium rhizogenes and Rhizobium on pod yield in groundnut cultivars (Mean ± SD)

S.No	Treatments	Numbers of pods per plant	
		Groundnut Variety 1	Groundnut Variety 2
1	T1	3.0 ± 0.85	2.5 ± 0.63
2	T2	9.0 ± 1.10	7.0 ± 0.98
3	T3	18.8 ± 1.25	15.3 ± 1.12
4	T4	23.2 ± 1.77	20.2 ± 1.25
5	T5	26.2 ± 1.95	23.7 ± 1.76

T1 – Autoclaved soil plus seed. T2 – Autoclaved soil plus seed plus *Agrobacterium rhizogenes.* T3 – Normal soil plus seed. T4 – Normal soil plus seed plus *Agrobacterium rhizogenes plus Rhizobium* introduced soil. T5 – Normal soil plus pricked seed plus *Agrobacterium rhizogenes plus rhizogenes plus Rhizobium* introduced soil.

Table. 11 Effect of *Agrobacterium rhizogenes* and *Rhizobium* on amount of protein (mg/gm/fw) in groundnut cultivars (Mean ± SD)

S.No	Treatments	Amount of protein (mg/gm/FW)	
		Groundnut Variety 1	Groundnut Variety 2
1	1	55	51
2	2	75	70
3	3	210	206
4	4	222	218
5	5	228	223

T1 – Autoclaved soil plus seed. T2 – Autoclaved soil plus seed plus *Agrobacterium rhizogenes.* T3 – Normal soil plus seed. T4 – Normal soil plus seed plus *Agrobacterium rhizogenes* plus *Rhizobium* introduced soil. T5 – Normal soil plus pricked seed plus *Agrobacterium rhizogenes* plus *rhizogenes* plus *Rhizobium* introduced soil.

Table.12 Effect of *Agrobacterium rhizogenes* and *Rhizobium* on amount of amino acid (mg/gm/FW) in groundnut cultivars

S.No	Treatments	Amount of amino acid (mg/gm/FW)	
		Groundnut Variety 1	Groundnut Variety 2
1	T1	40	37
2	T2	52	50
3	T3	125	121
4	T4	150	147
5	T5	169	164

T1 – Autoclaved soil plus seed. T2 – Autoclaved soil plus seed plus *Agrobacterium rhizogenes.* T3 – Normal soil plus seed. T4 – Normal soil plus seed plus *Agrobacterium rhizogenes plus Rhizobium* introduced soil. T5 – Normal soil plus pricked seed plus *Agrobacterium rhizogenes plus rhizogenes plus Rhizobium* introduced soil.

Table. 13 Effect of *Agrobacterium rhizogenes* and *Rhizobium* on amount of chlorohyll (mg/gm/FW) in groundnut cultivars

S.no	Treatments	Amount of chlorophyll (mg/gm/FW)	
		Groundnut Variety 1	Groundnut Variety 2
1	T1	0.0110	0.0106
2	T2	0.0184	0.0180
3	T3	0.0395	0.0391
4	T4	0.0412	0.0409
5	T5	0.0492	0.0488

T1 – Autoclaved soil plus seed. T2 – Autoclaved soil plus seed plus *Agrobacterium rhizogenes.* T3 – Normal soil plus seed. T4 – Normal soil plus seed plus *Agrobacterium rhizogenes* plus *Rhizobium* introduced soil. T5 – Normal soil plus pricked seed plus *Agrobacterium rhizogenes* plus *rhizogenes* plus *Rhizobium* introduced soil.

hairy root induction from matured embryo axes of groundnut in invitro condition.

In our study we have observed the increasing tendency of several biochemical parameters. The same was supported by Dubey, *et al*., (1982). They reported that the seeds inoculated with *Rhizobium* strains has increased the nitrogen content in the root, shoot, grain and straw.over uninoculated seeds in *Vigna radiata*. The same trend was also observed by Jarak, *et al., (*1989).

In our present research work, we noticed the increasing trend in nitrogenase compounds like proteins and amino acids. In *Rhizobium* infected plants, increased nodulation was also increased. The similar study was already carried out. The inoculation of *Rhizobium* increased root at the time of flowering stage over control. (uninoculated in Pea).

In *Vigna radiata* Dubey, *et al.*, (1992), reported increased grain yield, straw yield, increased nodule formation in *Vigna radiata*. The similar trend was also observed in our study. Patel and Saxena (1994). Found that the growth and yield of black gram was increased by seed soacking treatment with different plant growth regulators.

We found that, in *Rhizobium* inoculated seeds; nitrogenase compounds were increased due to seed treatment with *Rhizobium*. The nitrogen gaining by baterizetion in chickpea was already reported by Sonaria and Maurya. (1985).

In groundnut, the biochemical parameters like chlorophyll, soluble protein and nitrogenase activity were increased by Brassinosteroids, Brassinosteroids have unique growth promoting activity, when applied exogenously at submicro molar concentrations. The same trend was also observed in *Rhizobium* treated groundnut. Logesh Kumar Jain and Pushpendra Singh. (2000), Observed increased chlorophyll content in the leaves for seed inoculation with *Rhizobium* and in combination with *phosphobacteria* in black gram. *Rhizobium* has the ability for the growth and nutrition of cowpea.

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