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GROWTH BEHAVIOUR AND INDOLE ACETIC ACID (IAA) PRODUCTION BY A *RHIZOBIUM* SP. ISOLATED FROM *CAJANUS CAJAN* PLANT

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ABSTRACT

The Root nodules of *Cajanus Cajan* plant produced high amount of (35.56 ug/gm) of Indole acetic acid from Hg plants as compared to the N plant. The IAA was measured maximum from *Rhizobium spp*. of *C.cajan* plant at 24h, when the bacteria reached the stationary phase of growth. Mannitol & KNO3 preferred to be a good carbon and nitrogen source for IAA production in all N, Ng, H & Hg *Rhizobium* strains. The possible relationship between the rhizobial IAA production & legume rhizobia symbioses in relation to stress is discussed.

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KEY WORDS

Cajanus Cajan, indole acetic acid, Rhizobium, Nodules and stress

INTRODUCTION

Soil salinity is a major abiotic stress in plant agriculture. This has led research into the physiological and biochemical mechanisms under spinning salt tolerance, with the aim of improving crop plants. Plants have a centralized system of response, enabling them to respond to any adverse conditions, regardless of the nature of stress. Plants have also been observed to respond to environmental extremes by changing the hormonal balance (1). In a number of plant species, often-rapid changes in hormonal levels are commonly observed in response to stress.

Auxins indole acetic acid (IAA) plant hormone is wide spread among plant associated bacteria. IAA is produced from tryptophan via. the intermediate indole acetamide and has been implicated in the induction of plant tumors (2). Rhizobial IAA production created great interest in the formation and development of root nodules, but most of the *Rhizobium* sp. known for IAA production were isolated from leguminous herbs (3) first reported the production of IAA by the *Rhizobium* sp. isolated from a leguminous tree, *pongamia pinnata*. The production of IAA by nodule-bacteria have important physiological implications, alone or in conjunction with other plant hormones, might be involved in several stages of the symbiotic relationship. It is suggested that the hormone produced by *Rhizobium* sp. could trigger the division of the cortical cells in the roots and thus helps in initiating the development of the nodules (4). It has been suggested that in osmotic conditions the level of IAA hormone is increased, which controls the changes in cell wall extensibility. since this hormone has been known to increase the growth behaviour in osmotic stresses.

Cajanus cajan linn. sp., which is a good source of proteins, vitamins and minerals, is basic food stuffs, particularly in tropical and subtropical areas. However production and availability is not enough to fulfill the requirements of human consumption because of the increase in the area of arid zones and adverse climatic conditions and agronomic conditions such as drought and salinity (5). Stansell and smittle (6) reported that salts stressed during any stage of growth seriously hamper the productivity of plants and the large use of weedicide, which make the soil more saline and affects the growth of plants. Hence in saline environment and under adverse environmental conditions. the need for the selection of more tolerant *Cajanus cajan* plant can be recommended for cultivation

MATERIALS AND METHODS

The microorganisms, growth medium and growth conditions: The *Rhizobium* was isolated aseptically from the root nodules of *Cajanus cajan* (L.) Millsp. Plant of AKPH 2022 variety. The strains were identified and isolated according to (Shende & Patil, 2005 AJMBES in press).

The Skerman medium having 1% (w/v) mannitol (YEM) with slight modification having 0.01% w/v CaCl2 & H2O instead of NaCl and CaCO3 at pH 6.9. It was supplemented with different isomers of tryptophan (L or DL or D tryptophan). The *Rhizobium* was incubated in 100ml conical flask, each containing 25ml of medium, in three replicates at 30º± 2ºC on a rotatory shaker (150 rpm). The bacterial growth was measured turbidimetrically by a spectrophotometer at 540 nm (4).

Estimation of IAA production from Rhizobium spp.:

The medium was centrifuged after growth and the SN was used for IAA extraction following the method of Sinha & Basu (3) and estimated

calorimetrically according to Gordan and Weber (7).

Estimation of IAA and Tryptophan from the nodules of C.cajan plant

1 g nodule from *C.cajan* plant grown in different stress conditions was taken and ground in 10ml methanol to a fine suspension using pestle and mortar. The homogenate was G4 glass filtered by adding 25ml methanol. The filtrate was evaporated at 30ºC to an aqueous residue; cold 0.5M K2HPO4 was added to adjust the PH 8.5. It was then transferred in separating funnel and shaken with 10ml petroleum ether. The step was repeated again and shaken with 10ml diethyl ether, and each time the lipid fraction was discarded. The aqueous layer was adjusted to PH 3 by adding 2.8M phosphoric acid. The IAA was extracted with 10ml diethyl ether, which is then extracted with 10ml 50mM K2HPO4. Again the PH of the solution was adjusted to PH 3 with 0.28M phosphoric acid and the IAA was passed into a final 10ml diethyl ether. The ether was evaporated and the residue was dissolved in 1ml of cold redistilled methanol. IAA was estimated from methanol spectrophotometrically according to Stoessl (1970) (8).

100mg of nodules were taken and ground in 5ml papain solution, and then it was incubated at 65°C overnight. Cooled and centrifuged, the clear supernatant was collected and tryptophan was estimated at 545 nm according to Sadasivam & Manickam (9).

Estimation of IAA oxidase and peroxidase:

To check the level of IAA oxidase in the bacterial cells, the bacteria were grown in L-tryptophan supplemented Yeast extract mineral broth containing the preferred carbon source. The cells were collected at stationary phase of growth by centrifugation at 5000g for 10min at 4ºC. The cells were washed with sterile D.W, centrifuged again and used for the extraction of enzymes. IAA oxidase was extracted to 10ml 0.05M phosphate buffer pH 5.3, after crushing the bacteria and the oxidase activity was assayed following Sinha & Basu (3). To check the level of Peroxidase activity 50mM phosphate buffer pH6.8, 1ml 50mM hydrogen peroxide, 1ml enzyme extract and 1ml 2mM IAA solution were taken and incubated for 1 h at room temperature (25°± 3°C) *Rhizobium* cells were extracted following Kar and Mishra (10) and it was estimated following Kokkinakis and Brooks (11).

Culture of the symbiont:

In initial studies, different carbon sources (each at 1% w/v were added in Yeast extract medium at 30º±2ºC for 24 h supplemented with L tryptophan (2.5mg/ml). The *Rhizobium* was grown in yeast extract medium with 0.1% of nitrogen source, growth and IAA production was checked.

RESULTS AND DISCUSSION

The symbiont *Rhizobium* N, Ng, H & Hg strains were isolated from the healthy root nodules of *Cajanus cajan* plant. The identification of the bacteria was done according to the Manual of Microbiological methods following Bergey's Manual (12). The plants of different strains (N) were grown in normal conditions; (NG) in normal conditions containing 4% glyphosate, H in halophillic conditions with 300mM NaCl and HG with 300mM NaCl 6% containing glyphosate. The *Cajanus cajan* plants were grown in above aseptical conditions and the nodulation was rechecked.

The *Rhizobium* strains reached its stationary phase of growth at 24 h (Fig 1) much faster than the *Rhizobium* spp. isolated from *sesbania grandiflora* (96 hrs) (13) and D. Sissoo (120 hrs) (14). The bacterial growth and IAA production started immediately after inoculation. Maximum IAA was produced at 24 hr in Hg *Rhizobium* strain. Veselov etal (15) reported that osmotic shock due to PEG treatment resulted in accumulation of IAA. Yurekli.F (16) also stated that IAA level increased in leaves of P. acutifolius under salt stress. After 24 hrs the IAA production started to decline sharply (Fig 1). The fall in the level of

IAA in the medium was due to the release of IAA degrading enzymes, IAA oxidase and peroxidase, by the *Rhizobium.* IAA oxidase and peroxidase activity were 1.58, 1.64, 1.72 &1.76 and 1.63,1.58,1.75 & 185 resp in *Rhizobium* cells(ug of IAA converted/mg enzyme/h). A similar decline in IAA level was also observed in cultures of *Rhizobium* spp. (17 &18). The decline was also observed in *leguminosarum* bv. *Phaseoli* and *R.leguminosarum* (19). The *Rhizobium* preferred L-trytophan for growth and IAA production, and it was reported earlier by Dullaart (20) & Chattopadhyay(14). Here IAA production increased with an increase in concentration of L-tryptophan up to 25 mg/dl. IAA is produced from tryptophan via the intermediate indole acetamide and has been implicated in the induction of plant tumor. Beneficial bacteria synthesize IAA predominantly by an alternate try-dependent pathway, through indole pyruvic acid. In stress conditions this hormone actively participates in adaptive responses of plants (21 & 2). Hence the IAA was maximum in Hg *Rhizobium* grown in salt & glyphosate condition.

The root nodules of *Cajanus cajan* plant contained high amount of IAA in halophilic plants as compared to normal plants. In stress conditions of NaCl & glyphosate the concentration of IAA was 36.56 ug/ml and in presence of NaCl 30.04 ug/ml; whereas in normal conditions the IAA production was 16.98 ug/ml; whereas in glyphosate condition it was 21.33 ug/ml and Tryptophan content was 4mg in N, 4.2mg in Ng, 5.1mg in H & 5.8mg in Hg/100mg of nodules taken. This type of research was reported by Hunter (22) that *Bradyrhizobia Japonica* clones produced large amount of IAA in culture, which also formed nodules, which accumulated large amount of IAA in culture.

These nodules contained bacteriods with an increase in IAA producing capacity. These results suggested that the bacteriods produced IAA from tryptophan, which accumulated in the nodules and in stress conditions it enhanced as the salinity and weedicidal stress were given to plants. However there are much evidence to the suggestion that hormonal signals control growth in saline soils (Munn,s 2002)23. In a number of plant species often-rapid changes in hormonal levels are commonly observed in response to stress. The root nodules of D. *lanceolaria* contained high amount of IAA (7.42ug/g fresh tissues). The *Rhizobium* sp. isolated from the root nodules of the plant produced a high amount of IAA in culture that might be responsible for the high level of IAA in the root nodules (24).

All the 10 carbon sources (each at 1 % w/v final concentration was utilized by the *Rhizobium* spp. for growth & IAA production to different extents (Table). The most suitable promoter was Mannitol for broth growth & IAA production. The next suitable carbon source was Glucose. Earlier reports showed the role of carbon sources for IAA production in Rhizobium species by --------(25 & 26). Among the tested 10 organic & inorganic nitrogen sources, L-asparagine was most effective for IAA production. The optimum concentration of L-asparagine was 0.02% w/v (27) which showed that the *Rhizobium* species isolated from root nodules of a leguminious herb *Alysicarpus vaginalis* also preferred most L-asparagine for IAA production. Vincent J M (28) suggested that *Rhizobium* sp. could utilize several nitrogen sources for growth, which might be responsible for increased IAA production. Some amino acids like alanine & aspartic acid inhibit the conversion of trytophan to IAA by *Sinorhizobium meliloti* (29). The production of IAA by nodule-bacteria might have important physiological implications as it seemed reasonable to suggest that IAA, alone or in conjugation with other plant hormones, might be involved in several stages of the symbiotic relationship (30). Genes induced by IAA are probably involved in the execution of vital cellular functions and development processes. In addition nodular IAA has been reported to be transported to other parts of plants. Thus the nodules of *D. lanceolaria* might also serve as a rich source of IAA for other plants when required. Jacobi *et al* (31) have suggested that 23 KDA IAA binding protein of the symbiosome could be a part of an auxin efflux carrier system required to control auxin concentration in

infected soybean nodule cells. This also indirectly evidenced the transport of nodular IAA. Our studies in vivo & in vitro of the growth of IAA was to investigate the level of hormones & its effects in different stress conditions of salinity and weedicide, as auxin promoter cell elongation of the plant. Thus in different osmotic shocks the IAA level were checked. Amzallag G N (32) reported that salinity triggered an imbalance in phytohormones & to understand the physiology & biochemical mechanism, conferring salinity & glyphosate (weedicide) to this species is very important for the development of selection & breeding strategies.

Table 1
IAA production by the Rhizobium sp. affected with different carbon sources.

	IAA production (ug/ml)			
Carbon sources	**N strain**	**Ng**	**H**	**Hg**
Control	49.62	49.62	51.8	53.97
Galactose	51.8	56.15	52.27	49.62
Xylose	43.09	49.62	44.44	49.62
Sorbitol	43.09	44.44	55.06	58.32
Ribose	45.27	45.27	52.88	61.59
Sucrose	51.8	60.50	53.97	62.68
Fructose	51.8	59.41	55.06	65.94
Arabinose	61.59	62.68	53.97	67.03
Maltose	52.88	62.68	62.68	65.94
Lactose	52.88	64.85	62.68	65.94
Glucose	56.15	64.85	61.59	68.12
Mannitol	57.24	65.94	62.68	71.38

The *Rhizobium* were grown in L-tryptophan (2.5mg/ml) supplemented Yeast extract mineral medium at 30º± 2º C for 20 hr. In control, the medium was devoid of any supplemented carbon source. The carbon sources were supplemented at 1% (w/v) level individually.

Table 2
IAA productions by the Rhizobium sp. affected with different Nitrogen sources.

	IAA production (ug/ml)			
Nitrogen	**N strain**	**Ng**	**H**	**Hg**
Control	44.44	48.53	48.53	51.8
Glycine	44.18	52.88	53.97	59.41
NH4Cl	45.7	53.97	56.15	59.41
KNO3	53.97	60.50	57.24	61.59
NaNo3	67.03	72.47	71.38	75.73
Asparagine	65.94	72.47	65.94	76.84
NH_3SO_4	62.68	64.85	65.94	73.56
Sodium azide	61.59	61.59	62.68	72.47

Alanine	60.50	61.59	62.68	67.03
Cysteine	56.15	62.68	64.85	70.29
Amygadlin	32.21	44.18	49.62	56.15
Creatinine	45.27	53.97	51.8	64.85

The *Rhizobium* were grown in L-tryptophan (2.5mg/ml) supplemented Yeast extractmineral medium at 30º ± 2º C for 20 hr. In control, the medium was devoid of anysupplemented nitrogen source. The Nitrogen sources were supplemented at 0.1%(w/v) level individually.

Table 3
IAA estimated from the nodules of *C.cajan* plant in different stress conditions.

N	Ng	H	Hg
16.98 ug/ml	21.33 ug/ml	30.04 ug/ml	36.56 ug/ml

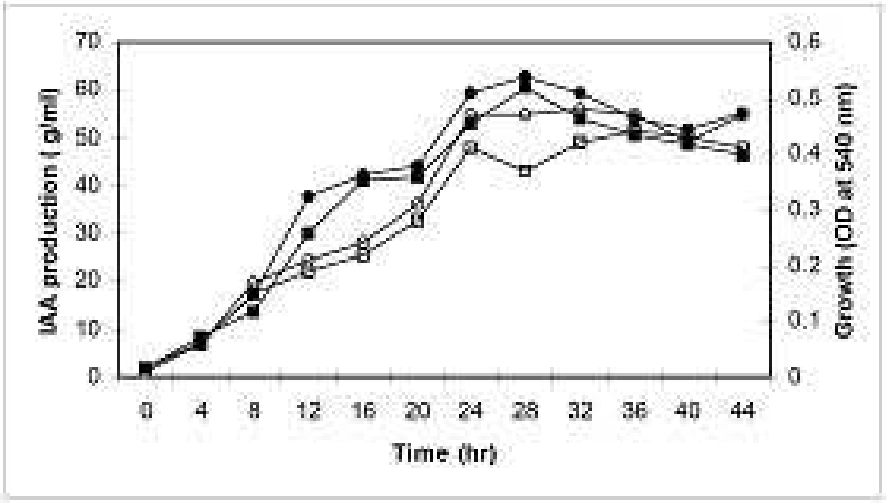

Fig.1- Growth (N, N_G) and IAA Production (N, N_G) by the *Rhizobium* spp. culture.

● – H_GGrowth
■ – N Growth
◈ – N_G IAA Production
□ - N IAA Production

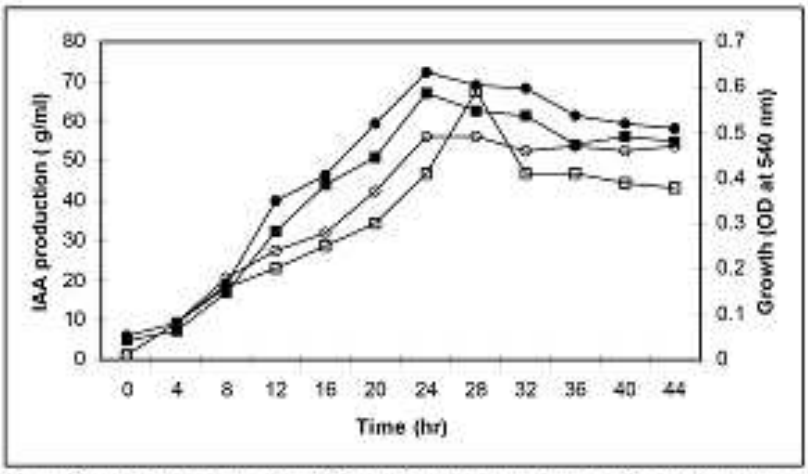

Fig.1- Growth (H, H_G) and IAA Production (H, H_G) by the ***Rhizobium*** spp. culture.

● – H_G Growth
■ – H Growth
o – H_G IAA Production
□ - H IAA Production

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