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RESEARCH ARTICLE

EFFECT OF AGROBACTERIUM ON GROWTH AND DEVELOPMENT OF *VIGNA MUNGO* L.

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ABSTRACT

Totally six strains of *Agrobacterium* species was isolated from the soil samples of six different leguminous crop areas namely Kudikkadu, Thandangorai, Nadium, Athirampattinum, Buthalur, and Kattuthottam in Thanjavur Dist by using yeast extract mannitol agar (YEMA) medium. The isolated bacteria were characterized and identified as *Agrobacterium rhizogenes* based on the morphological, biochemical, cultural and pathogenicity tests. Biochemical characteristics such as total carbohydrate, protein, free amino acids, chlorophyll content and cholesterol were estimated from the agrobacterial isolates.

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INTRODUCTION

Bacteria within the genera *Agrobacterium* and *Rhizobium* have the unique capacity to induce prolific root formation, nitrogen fixing root nodules and autonomous crown-gall tumors on many higher plants including most dicots, some monocots and some gymnosperms (Allen and Allen, 1958, Matthysse, 2006, Bouzar, *et al*., 1993 and Bouzar and Moore, 1987). *Agrobacterium* are Alphaproteobacteria is common in most soils that closely interact with plants in two respects. This bacterium is a soil borne microbes has worldwide distribution (Furuya *et al*., 2004). *A. tumefaciens* is a member of Rhizobiaceae family. These are Gram negative, rod-shaped and motile bacteria that grow aerobically without forming endospores (Collins, 2001). The great deal of dispute over the classification and nomenclature of *Agrobacterium* and *Rhizobium* because of a number of characteristics they share in common (Farrand, *et al*., 2003; Young, *et al*., 2001 and Young, *et al*., 2003). Two major lineages were distinguished: One included *Agrobacterium rhizogenes* along with most of the *Rhizobium* sp. and the other included *A. tumefaciens* (Young, *et al*., 2001). The genus *Agrobacterium* is polyphyletic (Holmes and Roberts, 1981). In this study the *Agrobacterium* species are effectively act as a biofertilizers, to penetrate the hairy root system and to improve the growth regulation of leguminous plants.

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MATERIALS AND METHODS

Isolation and culturing of strains (Vincent, 1970)

To collect the six different strains of *V.mungo* crop cultivated soil samples of Kudikkadu, Nadium, Athirampattinum, Buthalur, Thandangorai and Kattuthottam of Thanjavur District. The soil samples are serially diluted from 10^{-1} to 10^{-6}. The suspension (10^{-5}) was plated over yeast extract mannitol agar (YEMA) medium commonly used for selective isolation of rhizobium.

Characterization of *Agrobacterium* Isolates (Vincent, 1970)

The *Agrobacterium* isolates was characterized based on the cultural, biochemical and physiological characteristics with Congo red test, Hofer's alkaline broth test. Growth in glucose peptone agar, reaction of litmus milk, staining of poly â-hydroxybutyrate (PHB) and 3-ketolactose test and citrate utilization test were also performed.

Pathogenicity test

Agrobacterium isolates was confirmed by the pathogenicity test i.e., hairy root formation. The pathogenicity studies, with seeds were impregnated to *Agrobacterium* cultures and then sowing in different pot individually and maintained control pot. The plant growth parameters for every 15 day of intervals, with measured morphometric growth, yields and biochemical parameters were analysed.

The morphometric analysis with initials of 15th day and 30th day and finally weighted yield parameters also measured. Estimation of biochemical was carried out by the standard methods of proteins (Lowry *et al*., 1957), carbohydrate (Dubios *et al*., (1956), Chlorophyll (Arnom, 1949) cholesterol (Sperry and Brand, 1943) and amino acids (Jayaraman, 1981).

RESULTS AND DISCUSSION

The soil samples were collected from six different localities namely Kudikkadu, Nadium, Athirampattinum, Buthalur, Thandangorai and Kattuthottam of Thanjavur District. Six different strains of *Agrobacterium* species were isolated and separated from colony morphology in Table 1. All these tests already has been conducted and reported by Klecz-Kowska, *et al*. (1968), Murugesan, *et al.* (2011) and Kumar *et al*. (2013).

The strain were confirmed with the help of Bergey's Manual of Determinative of Bacteriology in 9th edition. *Agrobacterium* from *Rhizobium* by 3-ketolactose test, whereas the *Agrobacterium* produced yellow ring of precipitate of CuO around the colonies of the bacterium, when plates were flooded with Benedict's reagent by Moore *et al*. (1988) and Gaur *et al*. (1973). The six *Agrobacterium* species, three species are entirely different from other species. So, the three species was taken and the perform pathogenicity test for the growth of *V.mumgo*. The plant growth was measured in regular interval of 15th day of treatment. The morphometric analysis such as shoot length, root length, plant height, shoot breadth, leaf length, leaf breadth, number of lateral roots, number of nodules and individual nodule weight and some primary metabolites, such as carbohydrate, protein, chlorophyll, amino acids and cholesterol content was measured and results were positive representation (Table 3 & 4 and Fig 1).

Table 1. Morphological characterization of *Agrobacterium rhizogenes* isolated from *V.mungo* soil samples

S .No	Area	Colour	Appearance	Size (mm)	No. of colonies
1	Kudikkadu	Pink	Circular, elevation	2	49
2	Kattuthottam	Pink	Smooth, opaque	2	7
3	Thandangorai	Pink	Circular, transculent	2	48
4	Budalur	Pink	Convex, irregular	2	41
5	Nadiyam	Pink	Circular, irregular	2	26
6	Adirampatinam	Pink	Smooth, margin	3	26

Table 2. Identification of *Agrobacterium* sp by various biochemical test

Name of the test	Strain I	Strain II	Strain III	Strain IV	Strain V	Strain VI
Gram staining	-	-	-	-	-	-
Motility	+	+	+	+	+	+
Indole	-	-	-	-	-	-
Methyl red	-	-	+	-	-	-
Voges proskauer	-	-	-	-	-	-
Citrate utilization	+	-	+	+	+	-
Catalase	+	+	+	+	+	+
CHO fermentation test						
Glucose	+	+	+	+	+	+
Lactose	-	+	-	-	-	-
Mannitol	-	-	-	-	-	-
Congo red	+	+	+	+	+	+
Hoffer's alkaline test	+	+	+	+	+	+
∞-ketolactose agar test	+	+	+	+	+	+
Glucose peptone agar	+	+	+	+	+	+
Litmus milk	+	+	+	+	+	+

(+) positive, (-) negative

Table 3. Morphometeric analysis of *V.mungo* treated with *Agrobacterium rhizogenes* after15th day growth and development

S.No	Parameters	Control	Ar 1	Ar 2	Ar 3
1	Shoot length (cm)	13.4	16.2	15.3	14.5
2	Root length (cm)	3.3	7.0	5.3	5.0
3	Plant height (cm)	16.7	23.2	20.6	19.5
4	Shoot breadth (mm)	1.2	1.0	1.3	2.0
5	Lateral root (Nos)	5.0	9.0	10	6.0
6	Leaf length (cm)	4.0	3.2	6.2	4.5
7	Leaf width (cm)	1.2	2.2	3.7	2.1
8	Leaf area (cm^2)	2.4	3.52	11.47	4.73
9	No. of leaf (Nos)	2.0	5.0	5.0	5.0
10	No.of nodules (Nos)	-	5.0	16.0	10.0

Ar- *Agrobacterium rhizogenes*

The confirmation of *Agrobacterium* was made by the specific tests viz, 3-ketolactase test, carbohydrate fermentation test, citrate utilization test, Hofer's alkaline test, glucose peptone agar test, and Benedict's test were conducted for all the strains (Table 2).

The morphometric effect compared to 15th and 30th day, the growth and development was highly generated. The treatment (T1) are highly response the plant growth promoting *Agrobacterium rhizogenes*.

Table 4. Morphometeric analysis of *V.mungo* treated with *Agrobacterium rhizogene* after 30th day growth and development

S.No	Parameters	Control	Ar 1	Ar 2	Ar 3
1	Shoot length (cm)	16.2	24	14.0	24.0
2	Root length (cm)	5.8	5.5	7.0	5.0
3	Plant height (cm)	22.0	29.5	21.0	29.0
4	Shoot breadth (mm)	2.0	2.0	3.0	2.0
5	Lateral root (Nos)	7.0	14.0	15.0	10.0
6	Leaf length (cm)	5.0	5.5	4.0	7.0
7	Leaf width (cm)	2.0	3.0	2.2	4.0
8	Leaf area (cm^2)	5.0	8.25	4.4	14.0
9	No. of leaf (Nos)	6.0	11.0	14.0	14.0
10	No.of nodules (Nos)	-	10.0	6.0	19.0

Ar- *Agrobacterium rhizogenes*

Table 5. Effect of different strains of *Agrobacterium rhizogenes* to growth and yield parameters of *V.mungo*

S.No	Treatment	No. of pods (nos)	Pod weight (mg)	No. of seed
1	Control	3.0	1.7	18
2	Ar 1	4.0	1.6	21
3	Ar 2	3.0	1.1	19
4	Ar 3	4.0	2.1	23

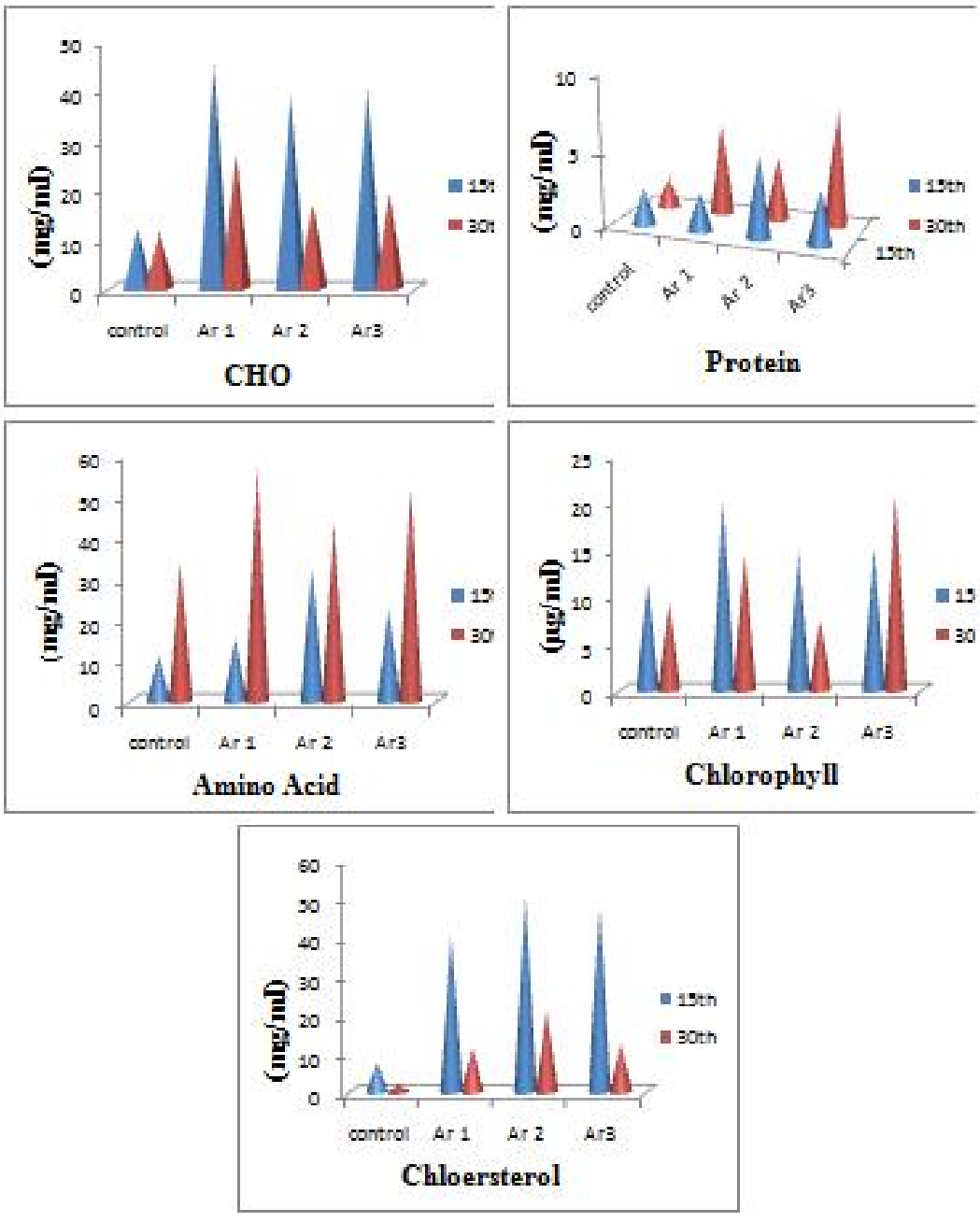

Fig. 1. Estimation of primary metaolities of leaves of *Vigna mungo*

Agrobacterium rhizogenes were mainly used for hairy root induction in *in vitro* condition. In this present study also yield parameters and growth of the plant were observed and tabulated in Table 5. T1 treatment were measured 2.1 mg pod weight and count in 23 seed, whereas others the pod weight ranges from 1.1 to 1.8 and number of seed ranges from 18 to 21, when as compared to control. The similar work was investigated and observed in *Vigna radiata* by Dubey, *et al.* (1992), reported increased grain yield, straw yield, increased nodule formation and Patel and Saxena (1994). In black gram. *A. rhizogenes* when *Rhizobium* present along with *Agrobacterium*, it could inhibit the formation of hairy roots in the host species, but *Agrobacterium* would induce the formation of nodules by Rhizobium. Similarly, hairy root production test has already been reported by several workers (Kersters, *et al*., 1973; Kersters and De Ley, 1984; Moore *et al*., 1988; Mougel, *et al*., 2001; Jarak, *et al.,* 1989; Logesh Kumar Jain and Pushpendra Singh 2000 and Rajesh Singh, *et al*., 2014). In our study concluded that, the *Agrobacterium rhizogenes* were effective for the growth and development including yield parameters of *Vigna mungo* plant and they may be promote the growth of plant. *Agrobacterium rhizogenes* aren't induce the crown gall disease in leguminous plant and they may be support the nitrogen fixation in root osystem.

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