



REVIEW ARTICLE

Investigation on microfloral association in the roots of *Macrotyloma uniflorum* (Lam.) Verdc., a medicinally important tropical pulse-crop and their possible applications for crop improvement: A review

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Abstract

Macrotyloma uniflorum (Lam.) Verdc., an economically important medicinal plant belongs to the *Leguminosae* family. Being Afro-Asian origin, the plant has long tradition of uses. It is primarily used for its antiurolithiatic property although it has other medicinal uses. Being *Leguminosae* member, this plant can form rhizobial nodules and mycorrhizal associations. The rhizobia obtained from this plant are mostly belonged to *Bradyrhizobium* sp. Although, *Rhizobium pusence* has also been reported. Microbes as biofertilizers can be used to increase yield of this plant, as well as there is great potential for utilizing the microbes derived from this plant. In this review we aim to describe the plant *M. uniflorum* - its taxonomic characteristics, economic uses, putative active constituents, and beneficial microflora along with their applications.

Keywords

Antiurolithiatic property, beneficial microflora, *Macrotyloma uniflorum*, putative active constituents

Introduction

Plant Material

Kidney stone is one of the common diseases that affect humankind from immemorable past, as early as 4800 BCE Egyptian mummies (1) and it can affect the renal system by various extent. It is known to affect about 10% to 12% of the population (1, 2). Calcium oxalate is the most common type of kidney stone (1). Calcium oxalate or Calcium-oxalate-phosphate crystals account for about 80% of cases, while Calcium phosphate crystals account for less than 5%, Uric acid accounts for about 10%, Struvite accounts for about 10% and Cysteine accounts for less than 1% (1, 3). There have been arguments about some disadvantage in use of synthetic drugs and other treatment methods that may be overcome with help of herbal medicine or natural products (1). A broad range of plants or plant – based chemical extracts including flavonoids, phenolics, steroidal saponins etc (1) are known to have some degree of antiurolithic activities (2). However, *Macrotyloma uniflorum* (Lam.) Verdc. is traditionally well known for antiurolithic activity of its seed infusions (1, 3, 4-10). Additionally, the plant *M. uniflorum* (Lam.) Verdc. has many other medicinal activities including anti-hypercholesterolemic, antioxidative, anti-hyperglycemic, anti-diabetic, anti-obesity, anti-hypersensitive (8, 10) which are discussed in Fig. 1.

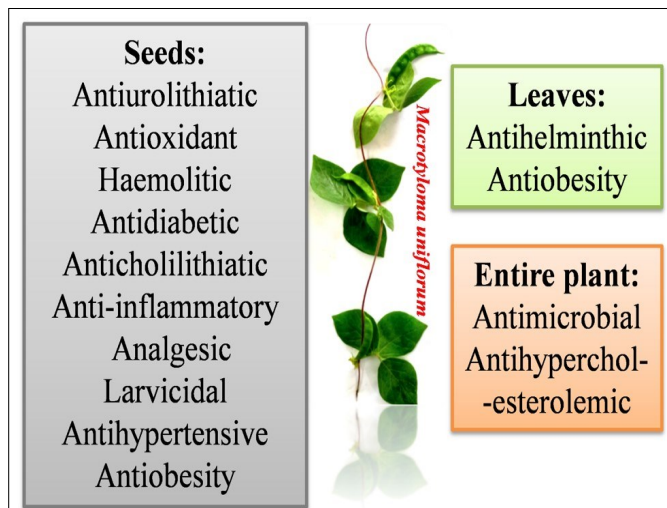


Fig. 1. Medicinal properties of *Macrotyloma uniflorum*.

This plant is rich with protein and seeds have high nutritional value containing vitamins, minerals, soluble fibers, fat and carbohydrate (10). *M. uniflorum* adds important amount of nitrogen to the soil (11). However, this tropical plant is remained heavily underutilized (6), as well as the association of microflora with this economically important plant, or application of microflora to increase its agricultural production, remains very poorly studied, with a very scanty amount of literature available. The aim of this review is to discuss about *M. uniflorum* including its medicinal properties, economic uses and microflora associated with this plant. To date various investigations have been carried out on *M. uniflorum* but wide gaps remain in its microflora. In this review, we have also tried to address some future prospects to unveil microflora associated with *M. uniflorum*.

Taxonomic identity of *Macrotyloma uniflorum* (Lam.) Verdc.

M. uniflorum (Lam.) Verdc., or its taxonomic synonym, *Dolichos uniflorus* Lam. and *Dolichos biflorus* auct. non L. is a tropical pulse crop plant with medicinal importance. It belongs to the family Leguminosae or Papilionaceae (4, 8, 10). It is native to the old world tropics (4). Earliest archaeological specimen of *M. uniflorum* has been obtained from the Indian Neolithic and Chalcolithic sites of Vindhyan plateau (12). During domestication, a gradual thinning of the seed coat has been found. Among these, the oldest specimens date from 2000 - 1700 BC (12). In vernacular language the plant is also known as horse gram or Madras gram (En), Kulthi (Hindi), Grain de Cheval (French), Feijoeiro de lagartixa, Favalinha, Culita (Polish) (4) Kulattha (Sanskrit), Kurti Kalai (Bengali), Gahot (Kumaon and Gahrwal), Kollu (Tamil), Ullavallu (Telugu) etc. (8, 10). Some authors have been considered this plant to be tolerant to drought, salinity and heavy metal stress and it can grow under low soil fertility condition, wide range of climatic temperature. This gives the plant a wide range of habitat, and may supply foods to people living in adverse situations (4, 6).

M. uniflorum includes different varieties which are listed in Table 1.

Morphology of the plant (13-15) is represented in Fig. 2.

Table 1. Different known varieties of *Macrotyloma uniflorum* (4)

Different varieties of <i>M. uniflorum</i>	Description
<i>M. uniflorum</i> var. <i>uniflorum</i>	It is probably a native to Indian subcontinent, found wild in southern Asia and Namibia. Cultivated widely in tropics. Pods 6-8 mm wide.
<i>M. uniflorum</i> var. <i>stenocarpum</i> (Brenan) Verdc.	It is a little bit highland variety; generally occurs at 800-1200 m altitude in the Central, East, Southern Africa and in India. A pod (4 - 5 mm wide) with the stipe rarely exceeds the calyx tube. Cultivated in Australia and California US.
<i>M. uniflorum</i> var. <i>verrucosum</i> Verdc.	Occurs in East and Southern Africa up to 550 m altitude in grassland and thicket. Pods 4 - 5.5 mm wide, distinctly stiped, margin warted.
<i>M. uniflorum</i> var. <i>benadirianum</i> (Chiov.) Verdc.	Occur in East Africa (Somalia, Kenya) at sea level altitude, on sand dunes and thin soils on coral rag.

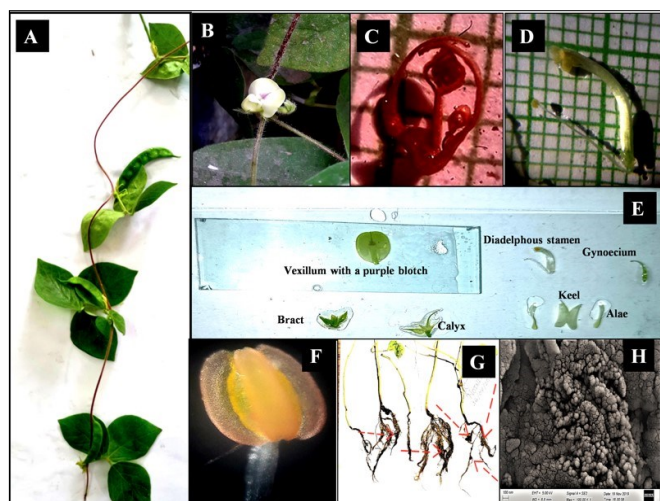


Fig. 2. Morphological characters of *Macrotyloma uniflorum* (where, A, B, C, D, E, G, H represent plant body, flower, flower transverse section, diadelphous stamen, floral parts, anther, root nodule and SEM of root nodule respectively). Photographs C, D, F by Rajarshi Rit, distributed under a CC BY 4.0 license.

Taxonomic description of *M. uniflorum* (10, 16, 17) is as follows:

Habit

Herbaceous, sometimes woody root; twining sub - erect annual; 60 to 90 cm tall; stem cylindrical with slightly hairy to tomentose stems.

Leaves

About 3.5 - 7.5 cm long, pinnate trifoliolate, stipules 7 - 10 mm long, petiolate, stipulate, stipule on attachment of petiole base. Stipule: lanceolate, 4 - 10 mm long, striate. Stipel: filiform, lanceolate or obsolete. Trifoliolate, terminal leaflet symmetrical and lateral leaflets asymmetrical, 2 - 4 cm broad. Softly tomentose on both surfaces, lower (abaxial) surface paler. Petioles: about 1 - 7 cm long. Rachis: 2.5 - 10 mm long. Petiolules: 1 - 2 mm long. Leaflets: 1 - 7 cm × 1 - 4 cm., Acute or slightly acuminate, ovate or ovate - rhombic; rounded at the base.

Flowers

Axillary, clustered, pseudoracemose, pedicels 0.3 - 0.5 cm long. Calyx tube bell - shaped, lobes 4 or 5, deltoid, corolla: glabrous, creamy - yellowish, whitish or greenish; papilionaceous, vexillum roundish to oval, often with a purple blotch, wings narrow, the keel is not twisted. Stamens: 10 in number, diadelphous (9 fused and 1 free). Anther: uniform, ovary linear - oblong to linear, style filiform, thin, glabrous or shortly pubescent, not bearded. Stigma: terminal, subcapitate, often brush.

Pods

Straight or curved, linear or linear - oblong, compressed, dehiscent, and not septate. The seeds are attached by a short central hilum surrounded with an obsolete or scarcely developed aril.

Seeds

Ovoid in shape, 4 - 6 mm long, 3 - 5 mm broad, color varying from pale brown through dark brown to black. Decorated with faint mottles or small scattered black spots with central hilum. Each pod contains about 5 - 8 seeds. Seeds albuminous (Endospermous).

Pollen Grains

Pollen Grains tuberculate or spinulose, zonitriplicate.

Economic uses of *Macrotyloma uniflorum* (Lam.) Verdc.

Food

The seeds are used as pulse or culinary (4, 6).

Fodder

The stem and leaves are used as cattle food. The boiled seeds are usually for horse food, from which it gets the name horse gram (4).

Nutritional Properties of the Seeds

M. uniflorum seeds are rich in protein and serve as great source of protein among poor people. However, Phytic acid, an antinutritional factor, is higher in *M. uniflorum* than many other legumes (10) which is being focused as a beneficial chemical compound for its antioxidant, anticarcinogenic and anti-ulcer properties. Seemingly, Phytic acid is an important constituent that play role in antiurolithic activity. Nutritional profile of *M. uniflorum* seeds is mentioned (Table 2).

Table 2. Nutritional profile of *Macrotyloma uniflorum* seeds (4, 6, 10)

Seed contents	Amount	
Carbohydrate	60.5% (w/w)	
Protein	22.5% (w/w)	
Fat	1.0% (w/w)	
Amino acids (% dw)	Arginine	8.80
	Cysteine	1.96
	Histidine	3.15
	Isoleucine	6.14
	Leucine	8.96
	Lysine	8.63

Amino acids (% dw)	Methionine	1.16
	Phenylalanine	6.31
	Threonine	3.82
	Tryptophan	1.16
Total ash value	Valine	6.47
	Calcium	4.68 % (w/w)
Essential minerals (mg/g)	Magnesium	1.01
	Phosphorus	0.4-1.90
	Vit B1 (Thiamin)	0.13-4.20
Vitamins (µg/g)	Vit B2 (Riboflavin)	4.2
	Vit B3 (Niacin)	0.9
	Oleic acid	15
Fatty acids (%)	Linoleic acid	8.9 - 16.8
	Linolenic acid	40.3 - 45.6
		11.6 - 14.3

Medicinal properties

Ancient Indian medical (Ayurveda) and religious literature (including Charaka Samhita and Susruta Samhita) has been thoroughly discussed the uses of this plant (18). In modern times, in folk and Ayurvedic system of medicine, the seed infusion of this plant is predominantly used as Antiurolithic (helps in removal and prevention of kidney stone). Traditionally *M. uniflorum* seeds were used to cure diarrhoea, dysuria, hepatomegaly, hiccup, obesity, asthma etc (8). Medicinal properties of entire plant, leaves and seeds (10) are presented in Fig.1.

Putative active constituents that impart into medicinal activities

Phytic Acid

M. uniflorum seeds have been seen to contain quite a high amount of Phytic acid (6) and it has been suggested that Phytic acid may be one of the constituents responsible for antiurolithic activity of *M. uniflorum* (9). A significant quantity of Phytic acid in horse gram has been revealed in embryonic axe fraction and cotyledon (19). Phytic acid content in cotyledons of *M. uniflorum* has been estimated as 8.42 ± 0.41 mg g⁻¹ (19). Phytic acid has been considered as an antinutrient, since it reduces bioavailability of certain minerals; however, it has been considered as potent antioxidant, anticarcinogenic agent that reduces the rate of cell proliferation and augments the immune system (20). Phytic acid has hypoglycemic or hypolipidemic effects (20). The metal chelating ability of phytate gives it a strong free radical scavenging ability that reduces the bioavailability of most pro-oxidant metallic iron unavailable to participate in the Fenton reaction and catalyze hydroxyl radical formation *in vitro*. Thus, Phytate may check oxidative damage, such as lipid peroxidation and may thereby lessen atherosclerotic lesions (6). Phytic acid, or myo-inositol hexaphosphate is a modification of the sugar alcohol, myo-inositol, each of its six hydroxyl groups esterified with phosphate. The molecule acts as a powerful chelating

agent, locking in cations like Ca^{2+} , Fe^{3+} etc. Phytic acid is nowadays highly valued for its beneficial properties such as repair of intestinal epithelium (21) and suppression of hepatic lipogenesis (22). It has been reported that Phytic acid helps to prevent calcium oxalate formation *in vitro* (23) and *in vivo* (24). Phytate can be excreted through urinary route (25). Therefore, we can suspect that Phytic acid works by directly interfering with renal calculi, although further studies are needed to determine whether Phytic acid can act as an adjunctive therapeutic agent for the treatment of renal calculi. Conversely, it has been suggested that Phytic acid may increase the risk of renal calculi formation (26). A three-fold mechanism has been suggested by which Phytic acid inhibits renal stone formation, viz., direct interference with Ca^{2+} , dissolves calcium oxalate and antioxidant activity (9) which is mentioned in Fig. 3.

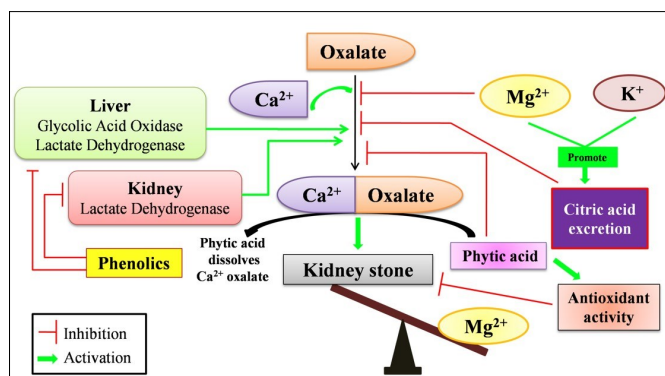


Fig. 3. Magnesium depletion provokes kidney stone formation. Mg^{2+} competes with Ca^{2+} to bind with oxalate, thus does not let to form. Magnesium, along with potassium, promotes excretion of citric acid that interfere calcium oxalate crystallisation. Phenolics interfere the certain enzymes responsible for Calcium oxalate formation. Phytic acid inhibits renal stone formation interfering with Ca^{2+} and also dissolves calcium oxalate.

Magnesium

Magnesium depletion and the incidence of kidney stones are correlated (2). It has been suggested that high magnesium (Mg^{2+}) content in *M. uniflorum* interferes with the

formation of calcium oxalate crystals and prevents renal stones formation. Application of aqueous extract of *M. uniflorum* (AEMU) seeds at 400 mg kg^{-1} and 800 mg kg^{-1} for 14 days on ethylene glycol (EG) induced urolithiasis in rats has been found to induce the levels of urinary and serum Mg^{2+} in rat. After application of AEMU seeds at 400 mg kg^{-1} , urinary and serum Mg^{2+} levels have been found to increase by 1.97 and 1.53 times respectively, compared to EG induced-non AEMU treated experimental set. Whereas, application of AEMU seeds at 800 mg kg^{-1} have been shown to promote the level of urinary Mg^{2+} 2.48 times and serum Mg^{2+} 1.64 times compared to EG induced-non AEMU treated experimental set (27). The mechanism by which Mg^{2+} inhibits kidney stone formation (9) has been demonstrated in Fig.3. Along with Mg^{2+} , Citrate and Phytate have also been reported to be effective against renal calcium oxalate crystallization (28). Furthermore, it has been examined that the combination of Mg^{2+} and Phytate showed a synergistic effect on the inhibition of calcium oxalate crystallization but the Mg^{2+} - citrate combination was not true for that purpose (24). Mg^{2+} in a quantity of 125 mg L^{-1} has been shown to be slightly more effective than 800 mg L^{-1} citrate in delaying the induction time of calcium oxalate crystallization (24).

Phenolic compounds and flavonoids

M. uniflorum contains a broad range of phenolics and Quercetin, a flavonoid (6). Tannins (phenolics) and flavonoids work as smooth muscle relaxants that help in expulsion of kidney stones (5). Phenolics decrease the formation of calcium oxalate by interfering with certain enzymes involved in oxalate production, such as Glycolic Acid Oxidase in liver and Lactate Dehydrogenase in liver and kidney (5) which is demonstrated in Fig. 3. Different phenolic compounds and flavonoids, obtained from *M. uniflorum* (6, 19) are listed (Table 3).

Table 3. Different phenolics and flavonoids, derived from *Macrotyloma uniflorum* (6) (where, different letters stand for statistically significant difference)

Compounds		Cotyledon	Embryonic Axe	Seed Coat	
Flavonoids	Quercetin	9.7±0.55c	113.4±6.0b	129.5±11.3a	
	Kaempferol	6.0±0.25c	67.4±3.7b	117.2±10.5a	
	Myricetin	2.4±0.07b	32.9±3.3a	35.5±5.2a	
	Daidzein	4.1±0.08b	22.2±1.3a	0.94±0.03c	
	Genistein	Not detected	44.7±3.22	Not detected	
	Vanillic acid	58.4±3.3a	53.2±5.1a	42.4±3.6b	
	ProtocatechuicAcid	39.0±2.0a	11.8±0.4	23.1±1.2b	
Benzoic Acid Derivatives	Gallic acid	26.9±1.3a	19.8±0.8b	5.5±0.06c	
	Para- Hydroxybenzoic acid	20.1±0.8b	13.1±0.5c	28.8±1.4a	
	Syringic acid	18.4±1.0a	Not detected	4.5±0.02b	
	Subtotal	162.8	97.9	104.3	
Phenolic Acids	Chlorogenic acid	160.8±9.8a	26.8±2.1b	22.5±1.1b	
	Caffeic acid	88.9±5.0a	61.5±4.9b	10.6±0.6c	
	Cinnamic acidderivatives	Ferullic acid	70.1±5.1a	31.4±2.4c	37.5±2.5b
		para - Coumaric acid	40.9±3.2a	21.4±1.3c	24.5±1.8b
		Sinapic acid	9.7±0.09a	Not Detected	3.7±0.02b
Subtotal	370.4	141.1	98.8		
Total	533.2	239.0	203.1		

Experimental Evidence of medicinal qualities

The seed extracts have been demonstrated to dissolve calcium oxalate crystals *in vitro* (3, 7) with aqueous fraction performing the best (3) as well as the seed extract helps dissolve renal calculi in Lithiasic rat model (5). The seeds have been seen to contain quite a high amount of Phytic acid (6) and it has been suggested that Phytic acid may be one of the constituents responsible for antiurolithic activity of *M. uniflorum* (9). According to one report, dietary Phytate may be a safe and important agent to reduce the risk of kidney stone formation in young women (23). Various other activities of the plant such as antimicrobial activity, anti – helminthic activity, anti-inflammatory and analgesic effect, anti – diabetic activity, anti – cholitic activity, anti – histaminic activity, anti – peptic – ulcer activity, antioxidant activity and free – radical scavenging activity, diuretic activity, haemolytic activity, hepatoprotective activity etc. have been reported (10) and the plant has also been considered as non – toxic. *M. uniflorum* leaves have been reported to have antiurolithic activity (7).

Microflora associated with *Macrotyloma uniflorum*

Little amount of data available about microflora associated with the *M. uniflorum* plant. Since this is a leguminous plant, it generally harbours root nodule forming rhizobia just like other legumes, as well as shows good mycorrhization. Some data regarding microflora includes mycorrhizae, rhizobia, seed borne microflora, plant growth promoting microbes (PGPMs) including plant growth promoting fungi (PGPF), plant growth promoting rhizobacteria (PGPR) and endophytes.

Mycorrhizae

Mycorrhizae are symbiotic association between fungi and plant roots. They cause little or no damage to plant roots but help the plant to gain nutrients from soil (29). The term ‘Mycorrhizae’ was first coined by Dr. A. B. Frank (30). Infection of roots with mycorrhizal fungi extends the zone in the soil, from which the plant can access the nutrients (31). Mycorrhizae can roughly be classified into three types; i.e., Endomycorrhiza, Ectomycorrhiza and Ectendomycorrhiza. Mycorrhizae or similar structure (Paramycorrhiza *sensu* Strullu-Derrien and Strullu) (32, 33) are known to occur in fossil records as early as 407 million years old Devonian Rhynie Chert bed, where Glomeromycotina and Mucoromycotina used to form root-like associations with rootless earliest land plants like *Aglaophyton* and *Horneophyton* (34). These were reported in both sporophytes and gametophytes of early land plants (33). They are somewhat allied to modern day Arbuscular Mycorrhizal symbiosis. An earliest of the known fungus, *Glomites rhynensis* is known to produce arbuscules, intra- and inter- cellular aseptate hyphae and glomoid spores with bilayered walls (35). However, the known oldest fossil ectomycorrhiza dates back to 50 million years ago only (36), and probably evolved through a separate evolutionary route (32), although fossil Ascomycota were known from as early as 400 million years old Rhynie chert, with perithecia scattered beneath the epidermis in the rhizomes and the upright

stems of the early land plant *Asteroxylon* (37). Fossil records are known from 443 – 359 million years old (38) *Tortotubus protuberans* which is one of the earliest land – dwelling organism (39), a fungus of uncertain nature (40), but showing parallelism with modern Basidiomycetes (39). The oldest generally accepted Basidiomycetes are known in fern stems from Mississippian (Lower Carboniferous) strata of France (41).

Arbuscular Mycorrhizae or AM fungi or AMF (one kind of endomycorrhiza) belong to Glomeromycota that forms mycorrhizal associations with plant roots. They are obligate mutualistic symbionts on plants and depend on plant for fatty acid. Sometimes refer to as VAM (Vesicular – Arbuscular – Mycorrhiza), however, since members of Gigasporaceae does not produce vesicles (42). VAM or AMF are very much ubiquitous in occurrence, i.e., nearly all group of terrestrial plants show this type of symbiosis. AMF inoculants are being widely used as biofertilizers to improve plant health, vigour, yield and resistance from stress and disease through stimulating the Systemic Acquired Resistance (SAR) pathway.

AM – fungal colonisation is known to extend the nutrition depletion zone in the soil, indicating a broadening of the region from which plant can access the nutrients (43), imparting Mycorrhiza Induced Resistance (MIR) (44) and improving stress tolerance such as various abiotic stresses such as drought stress (45), salt stress (46), cold stress (47-49), heat stress (50), light stress (51), water – logging stress (52), heavy metal stress (53) and Oxidative stress (54, 55).

Some AM fungi including *Glomus aggregatum*, *G. constrictum*, *G. deserticola*, *G. margarita*, *Acaulospora morrawae*, *Sclerocystis rubiformis* and *Scutellospora calospora* have been reported to present in mycorrhizosphere of *M. uniflorum*. *M. uniflorum*, inoculated with *G. deserticola* has shown significant increase in nutrient uptake and biomass production compared to uninoculated plant set (56). Inoculation of *G. fasciculatum* on *M. uniflorum*, grown in soil in iron mine area has been reported to increase nodule formation, nodule fresh weight (75%), nodule dry weight (100%), shoot dry weight (94.4%) (57). Under water stress condition, application of *G. fasciculatum* and *G. mosseae* in soil has been observed to improve nodulation in *M. uniflorum* and increase nodule number, nodule weight and leghaemoglobin content in nodules (58). It has been demonstrated that crop rotation (maize – horse gram/rice rotation) is the most effective in promoting indigenous AMF population which significantly increases crop yield and total productivity (59).

Microscopic views of mycorrhizal association with *M. uniflorum* roots have been captured in our lab (60-63) which are presented in Fig. 4.

Rhizobia

Rhizobia are symbiotic nitrogen-fixing (diazotrophic) Gram – negative bacteria capable of colonizing plant roots of Leguminosae and forming root nodules in legumes. Rhizobia are paraphyletic group, consisting of alpha and beta proteobacteria (64). They are allied to *Agrobacterium*

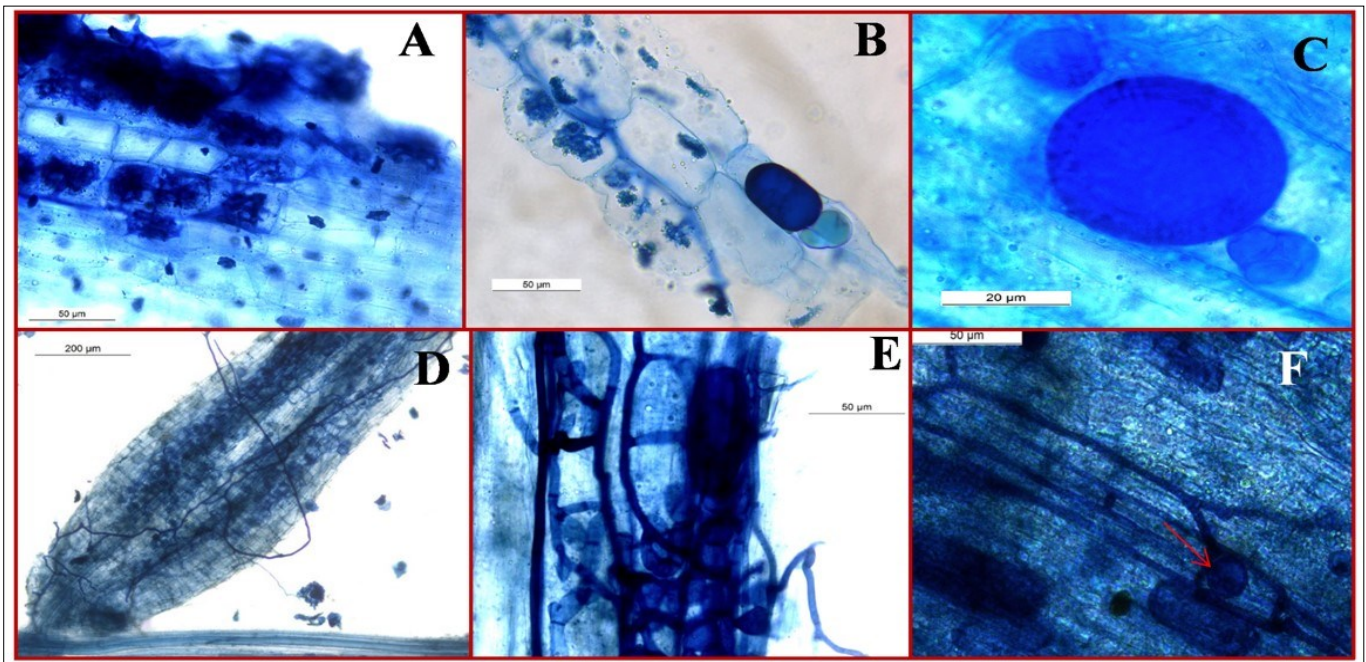


Fig. 4. Microscopic view of mycorrhizal association associated with *Macrotyloma uniflorum* (where, **A**- arbuscular mycorrhizal association, **B**- intracellular arbuscular mycorrhizal association, **C**, **F**- mycorrhizal spore, **D**- mycorrhiza with extraradical and intraradical hyphae, **E**- arbuscular mycorrhizal association and dark septate endophyte). Photographs A-D by Rajarshi Rit, distributed under a CC BY 4.0 license.

tumifaciens that is widely used in transgenic approach (65). Rhizobia were first discovered in 1988 by Beijerinck who isolated root nodule bacteria in pure culture from nodules of leguminous plants and showed the nitrogen fixation capacity of those (64). They are predominantly aerobic chemo-organotrophs. Some of them are easy to culture, where as some are non culturable (66). Although conventionally root nodules have been reported from legumes but there are more diversities than this. The interaction of rhizobia (*Rhizobium*, *Mesorhizobium*, *Bradyrhizobium*) with Non – legumes has been reported (67). A broad range of nitrogen fixing rhizobia are non – nodule forming endophytes (68). Whereas non – legume plant such as *Parasponia* may form nodules with *Bradyrhizobium* sp. (69). Sometimes rhizobia form stem nodule in tropical legumes (70). *Rhizobium rhizogenes*, a pathogenic strain of *Rhizobium* has been shown to attack a broad range of hosts causing hairy root disease and transferring genetic material (T-DNA) to plants (71). *Rhizobium* sp. NGR234, *Sinorhizobium meliloti* and *Mesorhizobium loti* has also found to transfer T-DNA (72). Rhizobia improve crop productivity by plant growth promoting (PGP) characteristics such as direct food supply nitrogenous matter by fixing nitrogen, as well as solubilising phosphate, producing siderophores, stimulating growth by producing IAA etc. (64). Phylogeny of naturally occurring rhizobia from *M. uniflorum* roots have been studied (73). All the isolates were of slow grower, *Bradyrhizobium* type. Some of them were previously reported exclusively from African continent and may have biogeographical implications. Some of the isolates have showed their ability to cross infect and nodulate several other legumes like, Soybean and several species of *Vigna* (73). Application of fast grower type rhizobia isolated from wild leguminous plants (several species of *Canavalia* sp., *Crotalaria* sp. and *Derris* sp.) on *M. uniflorum* and some other legumes has been reported to improve plant biomass and nodulation (74). However, *Rhizobium*

pusence AS05 (MT645243) isolated from contaminated sites has been reported to mitigate Cr (VI) stress effect on *M. uniflorum* var. Madhu (75). It has been documented that rhizobia associated with *M. uniflorum* are involved in phytoremediation of Nickel (76). The potential of *Rhizobium* strains in improving plant height, number of branches, number of nodules, fresh weight, dry weight, nitrogen content, nitrogen uptake, number of pods per plant and seeds per pod of *M. uniflorum* has been demonstrated (77). Rhizobia, obtained from root nodules of *M. uniflorum* Lam verdc. have been found to exert antagonistic and antibacterial activity through the production of Bacteriosin (78). Moreover, rhizobia associated with *M. uniflorum* (Lam.) verdc. have been considered as important agents for building plant tolerance to toxicity of Iron, Nickel, Lithium and Aluminium (76, 79- 81).

PGPMs (PGPF and PGPB)

The term PGPMs and all its subsets are presented in Fig.5.

PGPMs are microbes that promote plants growth,

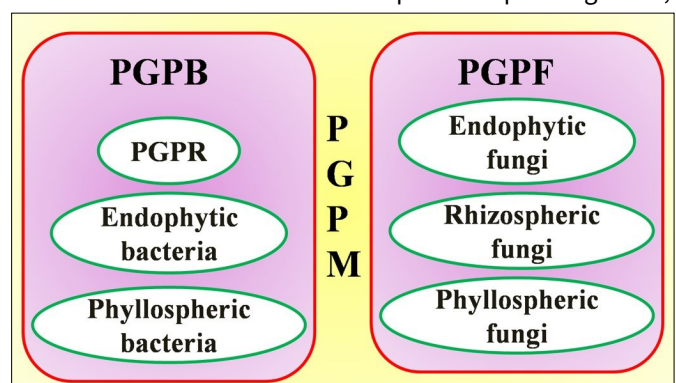


Fig. 5. PGPM and all its subsets. PGPM, PGPB, PGPR and PGPF represent Plant Growth Promoting Microbes, Plant Growth Promoting Bacteria, Plant Growth Promoting Rhizobacteria and Plant Growth Promoting Fungi respectively. PGPM includes PGPB and PGPF. PGPR, endophytic bacteria and phyllospheric bacteria remain under PGPB. PGPF include endophytic fungi, rhizospheric fungi and phyllospheric fungi.

preferably in friendly manner. However, the term PGPM (82) has been quite interchangeably used as PGPR. PGPMs have been considered as separate group than the Biocontrol Agents (BCA), where PGPM activity indicates direct plant growth promoting activity (82). Although it has been acknowledged that same organism can have PGPM activity and BCA activity on the same time. However, any means of improved plant protection (including microbial biocontrol agents or microbial biopesticides) within PGPM has been included (83).

PGPMs are expected to be beneficial. There are many disease-causing microbes that promotes plant growth, or hypertrophy and hyperplasia; such as the fungus *Gibberella fujikuroi* that causes bakanae disease of rice, or the bacteria *Agrobacterium tumefaciens* that causes crown gall disease; they are not generally considered as PGPMs, however, many PGPMs are hypovirulent strains of well-known plant pathogens (84). PGPMs exert plant growth promoting (PGP) traits. PGP activities are sometimes broadly classified into direct and indirect activities (85).

Direct plant growth promotion occurs when a bacterium facilitates the acquisition of essential nutrients or modulates the level of hormones within the plant (86). Direct PGP activities include:

- A. Stimulating Plant Growth – IAA synthesis, Gibberellin synthesis, Polyamine synthesis etc.
- B. Increasing Nutrient Bioavailability – Nitrogen fixation, Ammonia Production, Phosphate Solubilisation, Siderophore secretion etc.
- C. Stress Alleviation – ACC Deaminase activity, ROS Scavenging activity, Antioxidant production etc.

Indirect promotion of plant growth occurs when a PGPB decrease the damage to plants caused by various phytopathogens (86). Indirect PGP activity includes mycoparasitism, antibiosis, induced systematic resistance (ISR), exopolysaccharide production, antipathogenicity and protective enzyme production (87).

The term PGPF, in its broadest sense, are group of fungi that promotes growth of plant, regardless of the location (Rhizospheric or Phyllospheric or Endophytic) or habitat of the fungus. However, according to a more restrictive definition such as by Elizabeth Bent, PGPF excludes mycorrhizal fungi (84). However, a broader view considers mycorrhizal fungi within PGPF (88).

The term PGPB (Plant Growth Promoting Bacteria) in its broadest sense, is any kind of (rhizospheric or phyllospheric or endophytic) bacteria that promotes plant growth. PGPB can be classified into two subgroups – PGPR (those typically found in rhizosphere) and Plant-growth-promoting bacterial endophytes (those are found within any of the tissues of host plant) (89).

The term PGPR was first used by Klopper and co-workers around 1970 (90). Rhizobacteria can create three effects: beneficial, neutral, or deleterious (91). The term PGPR is defined as rhizobacteria beneficial to the plant (92). Induced Systemic Resistance has been reported to

mediate by PGPR and PGPF (84). PGPR are non-pathogenic bacteria that colonize in the rhizosphere, around root tissues and improve plant growth and yield (87). Phosphate is one of the important factors for plant growth. Phosphate solubilizing bacteria convert insoluble form of phosphate to soluble-plant usable form (93). Phosphate solubilizing bacteria from rhizosphere of *M. uniflorum* have been reported (94).

Endophyte

Endophytes are microbes that inhabit inside plant tissue, and usually with no visible manifestation to plant, and often have beneficial (mutualistic) relations with plants (95). Commonly they are either fungi or bacteria (95), but can sometimes be algae or anything else (96). Some evidence is telling that many endophytic fungi (97) and bacteria (98, 99) are not culturable (100) - perhaps they grow exclusively inside plant host. Endophyte occurrence may vary greatly according to geographic location (101, 102), season (103, 104), a range of host species (95, 105) and tissue or organ (105) and their localization may be intracellular (106) or intercellular (107). Endophytes have been reported to occur in xylem vessels, inner tissues of flower, fruits, or seeds and even pollen grains of pine (95). Endophytes may transmit through a horizontal mode (infected plant to uninfected plant) or a vertical mode (parent plant to offspring plant) mode and may involve interaction with insects (105). A large portion of endophytes are also can show plant growth promotion (PGP) functions and can protect the plant against biotic and abiotic stress (95). Many endophytes and other PGPMs are nowadays being used as biofertilizers with less harmful and more sustainable activities than chemical fertilizers. Their beneficial activities are already proven. Now, our civilization needs to find various endophytic-PGPM and other PGPMs to enhance plant growth and metabolite production in a sustainable way. They usually locate in the intercellular space of the plant tissue though they can form more intrusive structures toward plant cells. They are thought to have very complex mechanisms to establish an association with plant tissues. The associations are often mutualistic-symbiotic, i.e., besides taking some advantage from the plants, they help the plant in some way, such as vegetative growth promotion (95, 108), nutrient absorption, improvement of stress-tolerance and disease resistance, secondary metabolite productions (of the endosymbiont's own) (109, 110) and increase the production of plant secondary metabolites that protect plants against herbivores, including insects and grazing animals (111). Neurotoxic alkaloids produced by clavicipitaceous endophytes protect grasses from grazing herbivores (95). Plants seemingly bear complex mechanisms to recognise and harbour the endophytes (112) but little is known about the mechanisms.

Endophytic associations are very ancient and probably occurring from the time of origin of land plants, and perhaps played role in land colonisation by plants (113). These associations have been changed lot with time (114). Among bacterial endophytes, some common groups are alpha-proteobacteria, beta-proteobacteria, gamma-proteobacteria, firmicutes, actinobacteria etc. Among

fungal endophytes, members of Glomeromycota and Ascomycota are common. *Bacillus aryabhattai* AS03 and *Rhizobium pusense* AS05, native nodule endophytic bacteria have been considered as important agents to minimize Chromium (Cr) toxicity on *M. uniflorum* (75). Application of endophytic bacterial strains *Bacillus* and *Rhizobium* has been proved to improve photosynthetic performance of *M. uniflorum*, grown in Cr contaminated soil (115). Several members of the genus *Bradyrhizobium* have been reported as symbionts of *M. uniflorum* that are also capable of nodulating other plants (73).

Conclusion

Although this plant *Macrotyloma uniflorum* (Lam.) Verdc., is able to form vesicular arbuscular mycorrhizal association, the diversity and distribution of naturally occurring mycorrhizae in this plant needs to be worked out. The plant naturally harbours the rhizobia mostly the members of *Bradyrhizobium* and apparently, they have been found capable of infecting and nodulating several other leguminous crops. However, *Rhizobium pusense* has been known to occur. Little is known about rhizospheric and phyllospheric microbes especially those with PGP activities. This opens a wide door of opportunity including exploration of symbiosis and nodulation genes, exploration of symbiosis mechanism, exploration of biodiversity and distribution of different microbes (Endophyte, PGPR, PGPF etc), improving yield and medicinal constituents by applying endophytes, PGPR, PGPF etc., metabolite documentation of endophytes, PGPF etc, studying potential of using *M. uniflorum* for soil restoration and cross – infecting other pulse crops for nodulation, improvement of nitrogen fixation from the native rhizobia from this plant and studying the potential of *M. uniflorum* to use as a host for propagation of VAM fungi.

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Authors contributions

Special contribution of SD was on concept and title of the review. The literature review was performed by RR and DC. All authors (RR, DC, CM, and SD) discussed the manuscript among themselves. Data collection and manuscript preparation were by RR and DC. Photography was done by RR. Formatting, sketching and arrangement of total manuscript were done by DC. Reference formatting was done by RR, DC and CM. Finally, all authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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