PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 10(3): 300–311
https://doi.org/10.14719/pst.2307

HORIZON
e-Publishing Group
HePG

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

**Rajarshi Rit, Debapriya Choudhury, Chandrama Mukherjee & Sikha Dutta***

Applied and Molecular Mycology and Plant Pathology Laboratory, CAS Department of Botany, The University of Burdwan, Purba Bardhaman 713 104, West Bengal, India

*Email: sikha.bu.academia@gmail.com

OPEN ACCESS

**ARTICLE HISTORY**

Received: 18 December 2022
Accepted: 10 May 2023

Available online
Version 1.0 : 24 June 2023
Version 2.0 : 01 July 2023

Check for updates

**Additional information**

**Peer review**: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

**Reprints & permissions information** is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

**Publisher's Note**: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Indexing**: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting



**CITE THIS ARTICLE**

Rit R, Choudhury D, Mukherjee C, Dutta S. 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. Plant Science Today. 2023;10(3): 300–311. https://doi.org/10.14719/pst.2307

## 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.

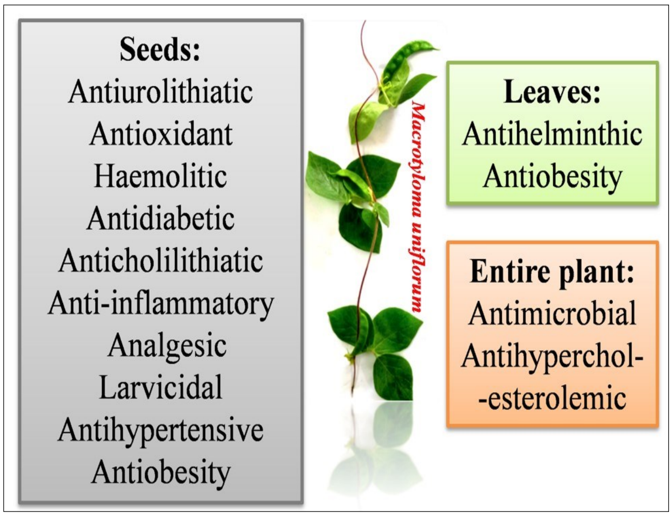


**Fig. 1.** Medicinal properties of *Macrotyloma uniflorum*.

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 *M. uniflorum* | Description |
|---|---|
| *M. uniflorum* var. *uniflorum* | It is probably a native to Indian subcontinent, found wild in southern Asia and Namibia. Cultivated widely in tropics. Pods 6-8 mm wide. |
| *M. uniflorum* var. *stenocarpum* (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. |
| *M. uniflorum* var. *verrucosum* 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. |
| *M. uniflorum* var. *benadirianum* (Chiov.) Verdc. | Occur in East Africa (Somalia, Kenya) at sea level altitude, on sand dunes and thin soils on coral rag. |

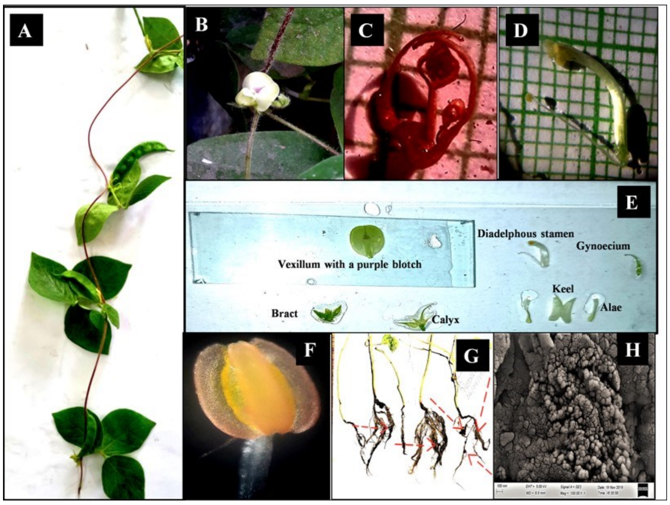


**Fig. 2.** Morphological characters of *Macrotyloma uniflorum* (where, **A**, **B**, **C**, **D**, **E**, **G**, **H** represent plant body, flower, flower transverse section, diadelphous stamen, floral parts, anther, root nodule and SEM of root nodule respectively). Photographs C, D, F by Rajarshi 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 trifoliate, 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. Trifoliate, 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, zonitriporate.

### 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, anticancerous 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 |
| | Valine | 6.47 |
| Total ash value | | 4.68 % (w/w) |
| Essential minerals (mg/g) | Calcium | 1.01 |
| | Magnesium | 0.4-1.90 |
| | Phosphorus | 0.13-4.20 |
| Vitamins (µg/g) | Vit B1 (Thiamin) | 4.2 |
| | Vit B2 (Riboflavin) | 0.9 |
| | Vit B3 (Niacin) | 15 |
| Fatty acids (%) | Oleic acid | 8.9 – 16.8 |
| | Linoleic acid | 40.3 – 45.6 |
| | Linolenic acid | 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 Antiuriolithic (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.

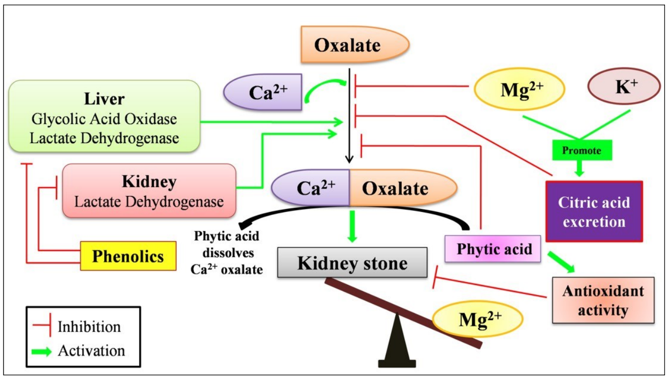


**Fig. 3.** Magnesium depletion provokes kidney stone formation. $Mg^{2+}$ competes with $Ca^{2+}$ to bind with oxalate, thus does not let to form. Magnesium, along with potassium, promotes excretion of citric acid that interfere calcium oxalate crystallisation. Phenolics interfere the certain enzymes 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 |
| Phenolic Acids | Benzoic Acid Derivatives | 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 |
| | | 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 |
| | Cinnamic acidderivatives | 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 |
| | | 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 rhizhobia 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 mycorhizosphere 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*

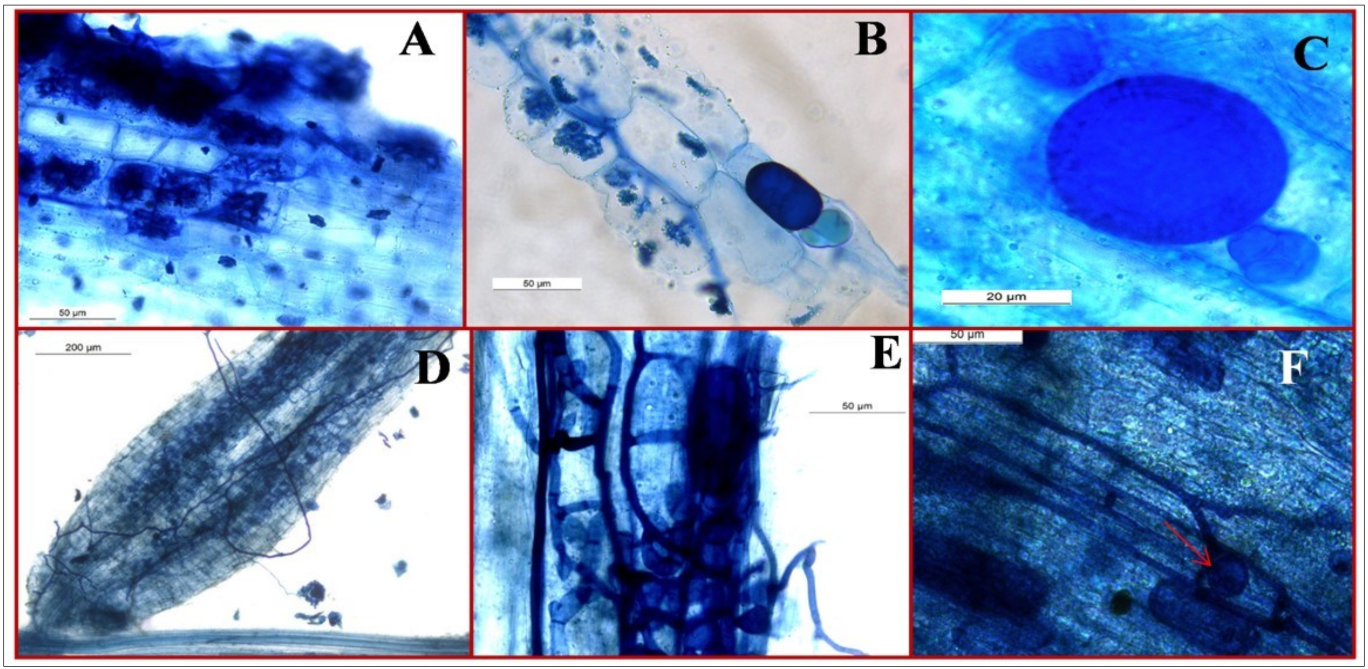


**Fig. 4.** Microscopic view of mycorrhizal association associated with *Macrotyloma uniflorum* (where, **A**- arbuscular mycorrhizal association, **B**- intracellular arbuscular mycorrhizal association, **C**, **F**- mycorrhizal spore, **D**- mycorrhiza with extraradical and intraradical hyphae, **E**- 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,

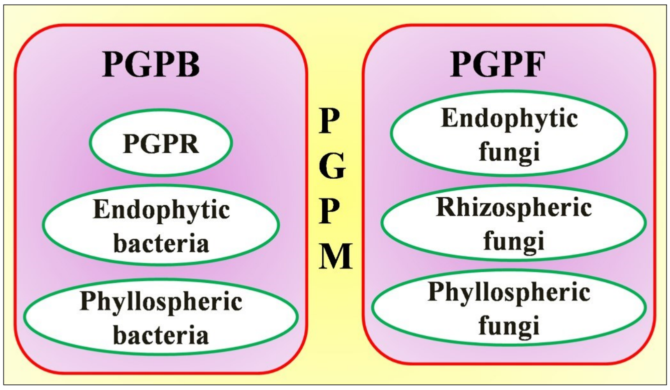


**Fig. 5.** PGPM and all its subsets. PGPM, PGPB, PGPR and PGPF represent Plant Growth Promoting Microbes, Plant Growth Promoting Bacteria, Plant Growth Promoting Rhizobacteria and Plant Growth Promoting Fungi respectively. PGPM includes PGPB and PGPF. PGPR, endophytic bacteria and phyllospheric bacteria 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 coworkers 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 pusence* has been known to occur. Little is known about rhizospheric and phyllosperic 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.

## Acknowledgements

All the authors are really grateful to all the lab members of Applied and Molecular Mycology and Plant Pathology Laboratory, CAS Department of Botany, University of Burdwan for active cooperation. We are thankful to Mr. Abhijit Roy, fluorescence lab, USIC department BU for his very kind permission to handle the Leica DM - 1000 microscope. The authors are thankful to UGC, Govt. of India for financial support.

## 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.

**Ethical issues**: None.

## References

1. Sangeetha M, Gayathiri K, Sharanya VK, Shyam Prakash G, Gopi Sudheer kumar J, Vimalavathini R, Kavimani S. Phytoconstituents with antiurolithic activity -A review. International Journal of Biological and Pharmaceutical Research. 2015;6(12):1001-07.
2. Soundararajan P, Mahesh R, Ramesh T, Begum VH. Effect of *Aerva lanata* on calcium oxalate urolithiasis in rats. 2006 Dec;44 (12):981-86.
3. Atodariya U, Barad R, Upadhyay S, Upadhyay U. Anti-urolithiatic activity of *Dolichos biflorus* seeds. Journal of Pharmacognosy and Phytochemistry. 2013 Jul 1;2(2):209-13.
4. Brink M, Belay G, De Wet JM. Plant Resources of Tropical Africa 1: Cereals and Pulses. Wageningen, The Netherlands: PROTA Foundation; 2006.
5. Saha S, Verma RJ. Antinephrolithiatic and antioxidative efficacy of *Dolichos biflorus* seeds in a lithiasic rat model. Pharmaceutical Biology. 2015 Jan 2;53(1):16-30. https://doi.org/10.3109/13880209.2014.909501
6. Prasad SK, Singh MK. Horse gram-an underutilized nutraceutical pulse crop: a review. Journal of Food Science and Technology. 2015 May;52(5):2489-99. https://doi.org/10.1007%2Fs13197-014-1312-z
7. Kachru A, Bisht M, Baunthiyal M. *In vitro* evaluation of antineprolithiatic activity of leaves and seeds of *Macrotyloma uniflorum* on dissolution or removal of kidney stones. Res J Pharmacognosy and Phytochem. 2016 Mar 28;8(1):05-12. https://doi.org/10.5958/0975-4385.2016.00002.9
8. Ahmed S, Hasan MM, Mahmood ZA. *Macrotyloma uniflorum* (Lam.) Verdc. (Papilionaceae): A review of medicinal uses, phytochemistry and pharmacology. World Journal of Pharmacy and Pharmaceutical Sciences. 2016;5(2):51-62.
9. Ahmed S, Hasan MM, Mahmood ZA. *Macrotyloma uniflorum* (Lam.) Verdc, *Phaseolus lunatus* Linn, *Phaseolus vulgaris* Linn. seeds: Nature's potential candidates against urolithiasis by virtue of multidimensional pharmacology. World Journal of Pharmacy and Pharmaceutical Sciences. 2016 Jun 12;5(8):289-300. https://doi.org/10.7897/2277-4572.0516
10. Rlds R, Erhss E. Medicinal and nutritional values of *Macrotyloma uniflorum* (Lam.) verdc (kulattha): A conceptual study. Global Journal of Pharmacy and Pharmaceutical Sciences. 2017;1(2):44 -53. http://dx.doi.org/10.19080/GJPPS.2016.01.555559
11. Yadav RL, Yadav DV, Duttamajumder SK. Rhizospheric environment and crop productivity: A review. Indian Journal of Agronomy. 2008 Mar 1;53(1):1-17.
12. Murphy C, Fuller DQ. Seed coat thinning during horsegram (*Macrotyloma uniflorum*) domestication documented through synchrotron tomography of archaeological seeds. Scientific Reports. 2017 Jul 14;7(1):1-9. https://doi.org/10.1038/s41598-017-05244-w
13. . Rit R, Macrotyloma uniflorum flower TS [Image File]. 2019 Nov 13 [appeared online 2021 June 22]. Available from: https://commons.wikimedia.org/wiki/File:Macrotyloma_Uniflorum_flower_TS.jpg , distributed under a CC BY 4.0 license
14. Rit R, Macrotyloma uniflorum dissected flower – Androecium [Image File]. 2019 Nov 13 [appeared online 2021 June 22]. Available from: https://commons.wikimedia.org/wiki/

File:Macrotyloma_uniflorum_dissected_flower-_Androecium_01.jpg , distributed under a CC BY 4.0 license .

15. Rit R, Macrotyloma Uniflorum androecium [Image File]. 2019 Nov 13 [Appeared online 2021 June 22]. Available from: https://commons.wikimedia.org/wiki/File:Macrotyloma_Uniflorum_androecium_02.jpg , distributed under a CC BY 4.0 license .
16. Verdcourt B. A new genus of Leguminosae-Phaseoleae. Kew Bulletin. 1970 Jan 1;24(2):322. https://doi.org/10.2307/4103055
17. Verdcourt B. Studies in the Leguminosae-Papilionoïdeae for the 'Flora of tropical East Africa': III. Kew Bulletin. 1970 Jan 1;379-447. https://doi.org/10.2307/4102845
18. Shobhana MC, Kulkarni M. Kulatha: therapeutic approach-acomprehensive review. International Journal of Ayurveda and Pharma Research. April 2017;5(4):89-93.
19. Sreerama YN, Neelam DA, Sashikala VB, Pratape VM. Distribution of nutrients and antinutrients in milled fractions of chickpea and horse gram: seed coat phenolics and their distinct modes of enzyme inhibition. Journal of Agricultural and Food Chemistry. 2010 Apr 14;58(7):4322-30. https://doi.org/10.1021/jf903101k
20. Kumar A, Singh B, Raigond P, Sahu C, Mishra UN, Sharma S, Lal MK. Phytic acid: Blessing in disguise, a prime compound required for both plant and human nutrition. Food Research International. 2021 Apr 1;142:110193. https://doi.org/10.1016/j.foodres.2021.110193
21. Wu SE, Hashimoto-Hill S, Woo V, Eshleman EM, Whitt J, Engleman L, Karns R, Denson LA, Haslam DB, Alenghat T. Microbiota-derived metabolite promotes HDAC3 activity in the gut. Nature. 2020 Oct;586(7827):108-12. https://doi.org/10.1038/s41586-020-2604-2
22. Okazaki Y, Sekita A, Katayama T. Intake of phytic acid and myo-inositol lowers hepatic lipogenic gene expression and modulates gut microbiota in rats fed a high-sucrose diet. Biomedical Reports. 2018 May 1;8(5):466-74. https://doi.org/10.3892/br.2018.1079
23. Curhan GC, Willett WC, Knight EL, Stampfer MJ. Dietary factors and the risk of incident kidney stones in younger women: Nurses' Health Study II. Archives of internal medicine. 2004 Apr 26;164(8):885-91. https://doi.org/10.1001/archinte.164.8.885
24. Grases F, Prieto RM, Gomila I, Sanchis P, Costa-Bauzá A. Phytotherapy and renal stones: the role of antioxidants. A pilot study in Wistar rats. Urological research. 2009 Feb;37(1):35-40. https://doi.org/10.1007/s00240-008-0165-1
25. Grases F, Simonet BM, Vucenik I, Prieto RM, Costa - Bauzá A, March JG, Shamsuddin AM. Absorption and excretion of orally administered inositol hexaphosphate (IP6 or phytate) in humans. Biofactors. 2001;15(1):53-61. https://doi.org/10.1002/biof.5520150105
26. Grases F, Prieto RM, Simonet BM, March JG. Phytate prevents tissue calcifications in female rats. Biofactors. 2000;11(3):171-77. https://doi.org/10.1002/biof.5520110303
27. Patel VB, Acharya N. Effect of *Macrotyloma uniflorum* in ethylene glycol induced urolithiasis in rats. Heliyon. 2020 Jun 1;6(6):e04253. https://doi.org/10.1016/j.heliyon.2020.e04253
28. Grases F, Rodriguez A, Costa-Bauza A. Efficacy of mixtures of magnesium, citrate and phytate as calcium oxalate crystallization inhibitors in urine. The Journal of Urology. 2015 Sep 1;194(3):812-19.https://doi.org/10.1016/j.juro.2015.03.099
29. Moore D. David Moore's World of Fungi: where mycology starts; 2016. http://www.davidmoore.org.uk/assets/mostly_mycology/diane_howarth/am.htm
30. Trappe JM. AB Frank Mycorrhizae: the challenge to evolutionary and ecologic theory. Mycorrhiza. 2005 Jun;15(4):277-81. https://doi.org/10.1007/s00572-004-0330-5
31. Hopkins WG, Hüner Norman PA, editors. Introduction to Plant Physiology. 4th ed. Wiley; 2009.
32. Strullu-Derrien C, Strullu DG. Mycorrhization of fossil and living plants. Comptes Rendus Palevol. 2007 Nov 1;6(6-7):483-94. https://doi.org/10.1016/j.crpv.2007.09.006
33. Krings M, Harper CJ, Taylor EL. Fungi and fungal interactions in the Rhynie chert: a review of the evidence, with the description of *Perexiflasca tayloriana* gen. et sp. nov. Philosophical Transactions of the Royal Society B: Biological Sciences. 2018 Feb 5;373(1739):20160500. http://doi.org/10.1098/rstb.2016.0500
34. Strullu-Derrien C, Selosse MA, Kenrick P, Martin FM. The origin and evolution of mycorrhizal symbioses: from palaeomycology to phylogenomics. New Phytologist. 2018 Dec;220(4):1012-30. https://doi.org/10.1111/nph.15076
35. Lalica MA, Tomescu AM. The early fossil record of glomeromycete fungi: New data on spores associated with early tracheophytes in the Lower Devonian (Emsian; c. 400 Ma) of Gaspé (Quebec, Canada). Review of Palaeobotany and Palynology. 2022 Mar 1;298:104590. https://doi.org/10.1016/j.revpalbo.2021.104590
36. Johnson NC, Gehring CA. Mycorrhizas: symbiotic mediators of rhizosphere and ecosystem processes. In: Cardon ZG, Whitbeck JL, editors. The rhizosphere. Academic Press. 2007 Jan 1; pp. 73-100. Academic Press. https://doi.org/10.1016/B978-012088775-0/50006-9
37. Taylor TN, Hass H, Kerp H. The oldest fossil ascomycetes. Nature. 1999 Jun; 399(6737):648. https://doi.org/10.1038/21349
38. Nagy LG, Varga T, Csernetics Á, Virágh M. Fungi took a unique evolutionary route to multicellularity: Seven key challenges for fungal multicellular life. Fungal Biology Reviews. 2020 Dec 1;34(4):151-69. https://doi.org/10.1016/j.fbr.2020.07.002
39. Smith MR. Cord-forming Palaeozoic fungi in terrestrial assemblages. Botanical Journal of the Linnean Society. 2016 Apr 1;180(4):452-60. https://doi.org/10.1111/boj.12389
40. Auxier B, Bazzicalupo A, Betz E, Dee JM, Le Renard L, Roushdy MM, Schwartz C, Berbee M. No place among the living: phylogenetic considerations place the Palaeozoic fossil *T. protuberans* in Fungi but not in Dikarya. A comment on M. Smith (2016). Botanical Journal of the Linnean Society. 2016 Dec 1;182(4):723-28. https://doi.org/10.1111/boj.12479
41. Halbwachs H, Harper CJ, Krings M. Fossil Ascomycota and Basidiomycota, With Notes on Fossil Lichens and Nematophytes. Journal: Encyclopedia of Mycology. 2021 Jan 1;378-95.https://doi.org/10.1016/B978-0-12-819990-9.00048-2
42. Moore D, Robson GD, Trinci AP. 21st century guidebook to fungi. Cambridge University Press; 2020 May 8. https://doi.org/10.1017/9781108776387
43. Li XL, George E, Marschner H. Extension of the phosphorus depletion zone in VA-mycorrhizal white clover in a calcareous soil. Plant and Soil. 1991 Sep;136(1):41-48. https://doi.org/10.1007/BF02465218
44. Cameron DD, Neal AL, van Wees SC, Ton J. Mycorrhiza-induced resistance: more than the sum of its parts?. Trends in Plant Science. 2013 Oct 1;18(10):539-45. https://doi.org/10.1016/j.tplants.2013.06.004
45. Bahadur A, Batool A, Nasir F, Jiang S, Mingsen Q, Zhang Q, Pan J, Liu Y, Feng H. Mechanistic insights into arbuscular mycorrhizal fungi-mediated drought stress tolerance in plants. International Journal of Molecular Sciences. 2019 Aug 27;20(17):4199. https://doi.org/10.3390/ijms20174199

46. Dastogeer KM, Zahan MI, Tahjib-Ul-Arif M, Akter MA, Okazaki S. Plant salinity tolerance conferred by arbuscular mycorrhizal fungi and associated mechanisms: a meta-analysis. Frontiers in Plant Science. 2020;1927. https://doi.org/10.3389/fpls.2020.588550

47. Chu XT, Fu JJ, Sun YF, Xu YM, Miao YJ, Xu YF, Hu TM. Effect of arbuscular mycorrhizal fungi inoculation on cold stress-induced oxidative damage in leaves of *Elymus nutans* Griseb. South African Journal of Botany. 2016 May 1;104:21-29. https://doi.org/10.1016/j.sajb.2015.10.001

48. Ma J, Janoušková M, Li Y, Yu X, Yan Y, Zou Z, He C. Impact of arbuscular mycorrhizal fungi (AMF) on cucumber growth and phosphorus uptake under cold stress. Functional Plant Biology. 2015 Nov 4;42(12):1158-67. https://doi.org/10.1071/FP15106

49. Ma J, Sun C, Bai L, Dong R, Yan Y, Yu X et al. Transcriptome analysis of cucumber roots reveals key cold-resistance genes induced by AM fungi. Plant Molecular Biology Reporter. 2018 Feb;36(1):135-48. https://doi.org/10.1007/s11105-018-1066-2

50. Zhu X, Song F, Liu F. Arbuscular mycorrhizal fungi and tolerance of temperature stress in plants. In: Wu QS, editors. Arbuscular mycorrhizas and stress tolerance of plants. Singapore: Springer. 2017 Apr 8; p. 163-94. https://doi.org/10.1007/978-981-10-4115-0_8

51. Mathur S, Jajoo A. Arbuscular mycorrhizal fungi protects maize plants from high temperature stress by regulating photosystem II heterogeneity. Industrial Crops and Products. 2020 Jan 1;143:111934. https://doi.org/10.1016/j.indcrop.2019.111934

52. Tuheteru FD, Wu QS. Arbuscular mycorrhizal fungi and tolerance of waterlogging stress in plants. In: Wu QS, editors. Arbuscular Mycorrhizas and stress tolerance of plants. Singapore: Springer. 2017; p. 43-66. https://doi.org/10.1007/978-981-10-4115-0_3

53. Adeyemi NO, Atayese MO, Sakariyawo OS, Azeez JO, Sobowale SP, Olubode A, Mudathir R, Adebayo R, Adeoye S. Alleviation of heavy metal stress by arbuscular mycorrhizal symbiosis in *Glycine max* (L.) grown in copper, lead and zinc contaminated soils. Rhizosphere. 2021 Jun 1;18:100325. https://doi.org/10.1016/j.rhisph.2021.100325

54. Nath M, Bhatt D, Prasad R, Gill SS, Anjum NA, Tuteja N. Reactive oxygen species generation-scavenging and signaling during plant-arbuscular mycorrhizal and *Piriformospora indica* interaction under stress condition. Frontiers in Plant Science. 2016 Oct 21;7:1574. https://doi.org/10.3389/fpls.2016.01574

55. Wu QS, Zou YN, Abd-Allah EF. Mycorrhizal association and ROS in plants. In: Ahmad P, editors. Oxidative damage to plants. Academic press. 2014 Jan 1; p. 453-75. https://doi.org/10.1016/B978-0-12-799963-0.00015-0

56. Bohra S, Singh J, Mathur N, Vyas A. Increased nutrient uptake and productivity of *Dolichos biflorus* by mycorrhizal fungi. J Arid Legumes. 2005;2:187-89.

57. Patanaik R, Sahu S, Padhi GS, Mishra AK. Effect of inoculation of vesicular arbuscular mycorrhiza on horsegram (*Macrotyloma uniflorum*) grown in soil in iron mine area. Indian Journal of Agricultural Sciences (India). 1995;65(3):186-90.

58. Savitha MM, Anitha P, Sumalatha BS, Tejavathi DH. Effect of mycorrhizal association on nodule number, mass, leghaemoglobin and free proline content i*n Macrotyloma uniflorum* (Lam.) Verdc. under PEG induced water stress. International Journal of Current Microbiology and Applied Sciences. 2016;5(4):165-74. http://dx.doi.org/10.20546/ijcmas.2016.504.021

59. Maiti D, Toppo NN, Variar M. Integration of crop rotation and arbuscular mycorrhiza (AM) inoculum application for enhancing AM activity to improve phosphorus nutrition and yield of upland rice (*Oryza sativa* L.). Mycorrhiza. 2011 Nov; 21(8):659-67. https://doi.org/10.1007/s00572-011-0376-0

60. . Rit R. Vesicular Arbuscular Mycorrhizae. [Image file]. 2019 Aug 08 [Available online 2021 June 22]. Available from: https://commons.wikimedia.org/wiki/File:Vesicular_Arbuscular_Mycorrhizae_40X0031_01.jpg , distributed under a CC BY 4.0 License .

61. Rit R. Vesicular Arbuscular Mycorrhizae. [Image file]. 2019 Aug 08 [Available online 2021 June 22]. Available from: https://commons.wikimedia.org/wiki/File:Vesicular_Arbuscular_Mycorrhizae_40X0031_03.jpg , distributed with a CC BY 4.0 License

62. . Rit R. Vesicular Arbuscular Mycorrhizae. [Image file]. 2019 Aug 08 [Available online 2021 June 22]. Available from: https://commons.wikimedia.org/wiki/File:Vesicular_Arbuscular_Mycorrhizae_40X0031_07.jpg , distributed with a CC BY 4.0 License

63. Rit R. Vesicular Arbuscular Mycorrhizae. [Image file]. 2019 Aug 08 [Available online 2021 June 22]. Available from: https://commons.wikimedia.org/wiki/File:Vesicular_Arbuscular_Mycorrhizae_40X0031_04.jpg , distributed with a CC BY 4.0 license

64. Maheshwari DK, Agarwal M, Dheeman S, Saraf M. Potential of rhizobia in productivity enhancement of *Macrotyloma uniflorum* L. and *Phaseolus vulgaris* L. cultivated in the Western Himalaya. In: Maheshwari D, Saraf M, Aeron A, editors. Bacteria in Agrobiology: Crop Productivity. Berlin, Heidelberg: Springer. 2013 May; p. 127-65. https://doi.org/10.1007/978-3-642-37241-4_6

65. Young JP, Crossman LC, Johnston AW, Thomson NR, Ghazoui ZF, Hull KH, Wexler M, Curson AR, Todd JD, Poole PS, Mauchline TH. The genome of *Rhizobium leguminosarum* has recognizable core and accessory components. Genome Biology. 2006 Feb;7(4):1-20. https://doi.org/10.1186/gb-2006-7-4-r34

66. Muresu R, Polone E, Sulas L, Baldan B, Tondello A, Delogu G, Cappuccinelli P, Alberghini S, Benhizia Y, Benhizia H, Benguedouar A. Coexistence of predominantly nonculturable rhizobia with diverse, endophytic bacterial taxa within nodules of wild legumes. FEMS Microbiology Ecology. 2008 Mar 1;63(3):383-400. https://doi.org/10.1111/j.1574-6941.2007.00424.x

67. Mehboob I, Naveed M, Zahir ZA. Rhizobial association with non-legumes: mechanisms and applications. Critical Reviews in Plant Science. 2009 Nov 4;28(6):432-56. https://doi.org/10.1080/07352680903187753

68. Ochieno DM, Karoney EM, Muge EK, Nyaboga EN, Baraza DL, Shibairo SI, Naluyange V. *Rhizobium*-linked nutritional and phytochemical changes under multitrophic functional contexts in Sustainable Food Systems. Frontiers in Sustainable Food Systems. 2021 Jan 11;4:604396. https://doi.org/10.3389/fsufs.2020.604396

69. Dupin SE, Geurts R, Kiers ET. The non-legume *Parasponia andersonii* mediates the fitness of nitrogen-fixing rhizobial symbionts under high nitrogen conditions. Frontiers in Plant Science. 2020 Feb 7;10:1779. https://doi.org/10.3389/fpls.2019.01779

70. Cocking EC, Kothari SL, Batchelor CA, Jain S, Webster G, Jones J et al. Interaction of rhizobia with non-legume crops for symbiotic nitrogen fixation nodulation. In: Fendrik I, del Gallo M, Vanderleyden J, de Zamaroczy, MA. *Azospirillum* VI and related microorganisms Berlin, Heidelberg: Springer. 1995; p. 197-205. https://doi.org/10.1007/978-3-642-79906-8_20

71. Muranaka T, Saito K. Production of pharmaceuticals by plant tissue cultures. Comprehensive natural products II: Chemistry and Biology. 2010;3:615-28. http://dx.doi.org/10.1016/B978-008045382-8.00065-4

72. Christie PJ. Agrobacterium and plant cell transformation. The Desk Encyclopedia of Microbiology. 2003 Dec 11;10. https://doi.org/10.1016/B978-012373944-5.00115-2

73. Appunu C, Ganesan G, Kalita M, Kaushik R, Saranya B, Praba-

vathy VR, Sudha N. Phylogenetic diversity of rhizobia associated with Horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) grown in South India based on glnII, recA and 16S-23S intergenic sequence analyses. Current microbiology. 2011 Apr;62(4):1230-38. https://doi.org/10.1007/s00284-010-9823-y

74. Arun AB, Sridhar KR. Symbiotic performance of fast-growing rhizobia isolated from the coastal sand dune legumes of west coast of India. Biology and Fertility of Soils. 2004 Dec;40:435-39. https://doi.org/10.1007/s00374-004-0800-0

75. Dhali S, Pradhan M, Sahoo RK, Mohanty S, Pradhan C. Alleviating Cr (VI) stress in horse gram (*Macrotyloma uniflorum* var. Madhu) by native Cr-tolerant nodule endophytes isolated from contaminated site of Sukinda. Environmental Science and Pollution Research. 2021 Jun;28(24):31717-30. https://doi.org/10.1007/s11356-021-13009-2

76. Edulamudi P, Antony Masilamani AJ, Vanga UR, Divi VR, Konada VM. Nickel tolerance and biosorption potential of rhizobia associated with horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.). International Journal of Phytoremediation. 2021 Sep 19;23(11):1184-90. https://doi.org/10.1080/15226514.2021.1884182

77. Paikara B, Paliwal AK, Singh RN, Gupta DK. Response of rhizobium strains on horse gram (*Dolichos biflorus* L.) in northern hill region of Chhattisgarh.

78. Edulamudi P, Masilamani AJ, Divi VR, Konada VM. Production bacteriosin by rhizobia obtained from root nodules of *Macrotyloma uniflorum* (Lam.) Verdc. (Horse gram). Bangladesh Journal of Microbiology. 2011;28(2):76-79. https://doi.org/10.3329/bjm.v28i2.11820

79. Edulamudi P, Vanga UR, Konada VM. Iron effect on symbiotic efficiency of horse gram. Acta fytotechnica et zootechnica:: ISSN 1336-9245. 2022 Mar 31;25(1): https://doi.org/10.15414/afz.2022.25.01.54-59

80. Edulamudi P, Vanga UM, Konada VM. Lithium stress tolerance of horse gram [*Macrotyloma uniflorum* (Lam.) Verdc.] plants in association with rhizobia. Thai Journal of Agricultural Science. 2022 Oct 31;55(1):65-72.

81. Prabhavati E, Masilamani AJ, Divi VR, Vishnuvardhan Z, Vanga UR, Konad VM. Symbiotic efficiency, biosorption and the growth of rhizobia on Horse gram plants under aluminium stress. Acta Biologica Slovenica. 2019;62(1):77-86.

82. Avis TJ, Gravel V, Antoun H, Tweddell RJ. Multifaceted beneficial effects of rhizosphere microorganisms on plant health and productivity. Soil Biology and Biochemistry. 2008 Jul 1;40(7):1733-40. https://doi.org/10.1016/j.soilbio.2008.02.013

83. Schlaeppi K, Bulgarelli D. The plant microbiome at work. Molecular Plant-Microbe Interactions. 2015 Mar;28(3):212-27. https://doi.org/10.1094/MPMI-10-14-0334-FI

84. Bent E. Induced systemic resistance mediated by plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF). In: Tuzun S, Bent E, editors. Multigenic and induced systemic resistance in plants. Springer: Boston, MA. 2006; p. 225-58. https://doi.org/10.1007/0-387-23266-4_10

85. del Carmen Orozco-Mosqueda M, Glick BR, Santoyo G. ACC deaminase in plant growth-promoting bacteria (PGPB): An efficient mechanism to counter salt stress in crops. Microbiological Research. 2020 May 1;235:126439. https://doi.org/10.1016/j.micres.2020.126439

86. Glick BR. Plant growth-promoting bacteria: mechanisms and applications. Scientifica. 2012 Oct 11;2012. https://doi.org/10.6064/2012/963401

87. Choudhury D, Tarafdar S, Dutta S. Plant growth promoting rhizobacteria (PGPR) and their eco-friendly strategies for plant growth regulation: A review. Plant Science Today. 2022;9(3):524-37. https://doi.org/10.14719/ pst.1604

88. Varma A, Bakshi M, Lou B, Hartmann A, Oelmüller R. Functions of a novel plant growth-promoting mycorrhizal fungus: *Piriformospora indica*. Agric Res. 2012;1(2):117-31. https://doi.org/10.1007/s40003-012-0019-5

89. Santoyo G, Moreno-Hagelsieb G, del Carmen Orozco-Mosqueda M, Glick BR. Plant growth-promoting bacterial endophytes. Microbiological Research. 2016 Feb 1;183:92-99. https://doi.org/10.1016/j.micres.2015.11.008

90. Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil. 2003 Aug; 255(2):571-86. https://doi.org/10.1023/A:1026037216893

91. Kloepper JW. Plant growth-promoting rhizobacteria (other systems). In: Okon Y, editors. *Azospirillum*/Plant Associations. Boca Raton, FL, USA: CRC Press. 1994;p. 111-18.

92. Kloeppe JW, Rodriguez-Kabana R, Zehnder AW, Murphy JF, Sikora E, Fernandez C. Plant root-bacterial interactions in biological control of soilborne diseases and potential extension to systemic and foliar diseases. Australasian Plant Pathology. 1999 Mar;28(1):21-26. https://doi.org/10.1071/AP99003

93. Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Applied Soil Ecology. 2006 Nov 1;34(1):33-41. https://doi.org/10.1016/j.apsoil.2005.12.002

94. Khatri D, Durgapal A, Gupta SM. Biochemical and molecular characterization of phosphate solubilizing bacteria from the rhizosphere of horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.). Bochem Cell Archives. 2012;12:303-06.

95. Hardoim PR, Van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A et al. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiology and Molecular Biology Reviews. 2015 Sep 1;79(3):293-320. https://doi.org/10.1128/MMBR.00050-14

96. Schulz B, Boyle C. What are endophytes?. In: Schulz BJE, Boyle CJC, Sieber TN, editors. Microbial root endophytes. Berlin, Heidelberg: Springer. 2006; p. 1-13. https://doi.org/10.1007/3-540-33526-9_1

97. Alam B, Lǐ J, Gě Q, Khan MA, Gōng J, Mehmood S et al. Endophytic fungi: from symbiosis to secondary metabolite communications or vice versa?. Frontiers in Plant Science. 2021:3060. https://doi.org/10.3389/fpls.2021.791033

98. Thomas P, Swarna GK, Patil P, Rawal RD. Ubiquitous presence of normally non-culturable endophytic bacteria in field shoot-tips of banana and their gradual activation to quiescent cultivable form in tissue cultures. Plant Cell, Tissue and Organ Culture. 2008 Apr;93(1):39-54. https://doi.org/10.1007/s11240-008-9340-x

99. Thomas P. Intense association of non-culturable endophytic bacteria with antibiotic-cleansed *in vitro* watermelon and their activation in degenerating cultures. Plant Cell Reports. 2011 Dec;30(12):2313-25. https://doi.org/10.1007/s00299-011-1158-z

100. Müller T, Ruppel S. Progress in cultivation-independent phyllosphere microbiology. FEMS Microbiology Ecology. 2014 Jan 1;87(1):2-17. https://doi.org/10.1111/1574-6941.12198

101. Harrison JG, Griffin EA. The diversity and distribution of endophytes across biomes, plant phylogeny and host tissues: how far have we come and where do we go from here?. Environmental Microbiology. 2020 Jun;22(6):2107-23. https://doi.org/10.1111/1462-2920.14968

102. Fang K, Miao YF, Chen L, Zhou J, Yang ZP, Dong XF, Zhang HB. Tissue-specific and geographical variation in endophytic fungi of *Ageratina adenophora* and fungal associations with the environment. Frontiers in microbiology. 2019 Dec 18;10:2919. https://doi.org/10.3389/fmicb.2019.02919

103. Ramírez-Camejo LA. Diversity of culturable endophytic fungi vary through time in *Manihot esculenta* Crantz. Brazilian Journal of Biology. 2022 Jan 5;84. https://doi.org/10.1590/1519-6984.253156

104. Göre ME, Bucak C. Geographical and seasonal influences on the distribution of fungal endophytes in *Laurus nobilis*. Forest Pathology. 2007 Aug;37(4):281-88. http://dx.doi.org/10.1111/j.1439-0329.2007.00509.x

105. Rodriguez RJ, White Jr JF, Arnold AE, Redman AR. Fungal endophytes: diversity and functional roles. New Phytologist. 2009 Apr;182(2):314-30. https://doi.org/10.1111/j.1469-8137.2009.02773.x

106. Thomas P, Sekhar AC. Live cell imaging reveals extensive intracellular cytoplasmic colonization of banana by normally non-cultivable endophytic bacteria. AoB Plants. 2014 Jan 1;6 (plu002):1-12. https://doi.org/10.1093/aobpla/plu002

107. Wang Y, Dai CC. Endophytes: a potential resource for biosynthesis, biotransformation and biodegradation. Annals of Microbiology. 2011 Jun;61(2):207-15. https://doi.org/10.1007/s13213-010-0120-6

108. Salazar-Cerezo S, Martinez-Montiel N, Cruz-Lopez MD, Martinez-Contreras RD. Fungal diversity and community composition of culturable fungi in *Stanhopea trigrina* cast gibberellin producers. Frontiers in Microbiology. 2018 Apr 4;9:612. https://doi.org/10.3389/fmicb.2018.00612

109. Newman DJ, Cragg GM. Endophytic and epiphytic microbes as "sources" of bioactive agents. Frontiers in Chemistry. 2015 May 22;3:34. https://doi.org/10.3389/fchem.2015.00034

110. Zhang HW, Song YC, Tan RX. Biology and chemistry of endophytes. Natural Product Reports. 2006;23(5):753-71. https://doi.org/10.1039/B609472B

111. Pappas ML, Liapoura M, Papantoniou D, Avramidou M, Kavroulakis N, Weinhold A, Broufas GD, Papadopoulou KK. The beneficial endophytic fungus *Fusarium solani* strain K alters tomato responses against spider mites to the benefit of the plant. Frontiers in Plant Science. 2018 Nov 6;9:1603. https://doi.org/10.3389/fpls.2018.01603

112. Carella P, Schornack S. Manipulation of bryophyte hosts by pathogenic and symbiotic microbes. Plant and Cell Physiology. 2018 Apr 1;59(4):656-65. https://doi.org/10.1093/pcp/pcx182

113. Krings M, Taylor TN, Dotzler N. Fungal endophytes as a driving force in land plant evolution: evidence from the fossil record. Biocomplexity of Plant-Fungal Interactions. 2012 Feb 17;5-28. https://doi.org/10.1002/9781118314364.fmatter

114. Saikkonen K, Wäli P, Helander M, Faeth SH. Evolution of endophyte–plant symbioses. Trends in Plant Science. 2004 Jun 1;9 (6):275-80. https://doi.org/10.1016/j.tplants.2004.04.005

115. Dhali S, Acharya S, Pradhan M, Patra DK, Pradhan C. Synergistic effect of *Bacillus* and *Rhizobium* on cytological and photosynthetic performance of *Macrotyloma uniflorum* (Lam.) Verdc. Grown in Cr (VI) contaminated soil. Plant Physiology and Biochemistry. 2022 Nov 1;190:62-69. https://doi.org/10.1016/j.plaphy.2022.08.027