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Occurrence and Concentration of AM Fungi in *Chenopodium Quinoa* Willd

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Abstract: ***An assessment of the mycorrhizal association in Chenopodium quinoa willd. Random rhizosphere soil samples were collected from 4 different areas of Telangana state. AM fungi were isolated from rhizosphere soils of Quinoa plants by wet and decanting method (Gerdemann and Nicolson 1963). The percentage of mycorrhizal colonization in each of the plant was calculated by Phillips and Hayman method (1970) and Morpometric technique (Toth and Toth 1982) was used***

Keywords: *Chenopodium quinoa* willd. Telangana state, AM fungi, rhizosphere soils, wet and decanting method, Phillips and Hayman method, Morpometric technique

1. Introduction

Quinoa is the common name for *Chenopodium quinoa* Willd. (Valencia - Chamoro, 2003) that belongs to Chenopodiaceae family and considered as a pseudocereal. It is commonly called as Goosefoot family; it has 102 genera and 1400 species. In India these members are abundantly grown in dry, xerophytic, alkaline soils. The other members of Chenopodiaceae family are City Goosefoot (*Chenopodium urbicum*) is a moderate allergen, Fremont's Goosefoot (*Chenopodium fremontii)* , Lamb's - Quarters and *Chenopodium album*.

Quinoa is the only food crop that contains all the essential amino acids, trace elements and vitamins, and it is also gluten - free. Quinoa is considered as desert beauty of Bolivia and originated from the Andean region of Peru, Bolivia, Ecuador, Colombia and Chile. The life cycle of Quinoa varies from 120 to 240 days and is suited to various environmental conditions. *Chenopodium quinoa* Willd. is a dicotyledonous annual plant usually about 1–2 m (3.3–6.6 ft) height. It has broad, generally pubescent, powdery, smooth (rarely) to lobed leaves normally arranged alternately. The woody central stem is branched or unbranched depending on the variety of plant. The stem may be green, red or purple. The flowering panicles arise from the top of the plant or from leaf axils along the stem. Each panicle has a central axis from which a secondary axis emerges either with flowers (Amaranthiform) or bearing tertiary axis carrying the flowers. The green hypogynous flowers have a simple perianth and are generally self - fertilizing. The fruit is achene, cylindrical to lenticular in shape. The seeds are about 3.54 mm in length and 0.36 mm wide (Hirose Y 2011) diameter and of various colours from white to red or black depending on the cultivar. AM mycorrhizal fungi are soil and root inhabiting fungi which enhance the plant growth by helping in uptake of phosphorus, water and other nutrients H. Marschner & B. Dell (1994) these fungi appear to have a significant role in ecosystem function. According to Eckhard George (2011) AMF helps in translocation of nutrients between hosts through hyphal connection. Mycorrhizae, the symbiotic association of fungi and plants are proven microbes that help in the establishment, nourishment and disease resistance of the crop plants (Mohammad Imad khrieba et. al., 2019). As arbuscular mycorrhizae are promising bio - fertilizers, it is proposed to study the arbuscular mycorrhizal association in Quinoa and to screen establish the indigenous VAM fungi for growing this new crop at Nizamabad, Telangana State.

2. Study Site

The cultivar of Quinoa grown in Telangana state were surveyed for VAM mycorrhizal association. The sites of sample collection are: L1, L2, L3 and L4 they are: T. U Research field, Thipparthy village of Nalgonda, Pedda Adisarlapally village of Mahaboobnagar and Chinthaloor village of Nizamabad district. L1: Chinthaloor is a Village in Jakranpalle Mandal in Nizamabad District of Telangana state, India. Nizamabad is located 23 km., towards east from district head quarters of Nizamabad with moderate crop concentration. L2: Telangana University located in Nizamabad district, with average temperature of 27^0C to 40^0C rainfall is 1108 mm. L3: Mahabubnagar is the second largest district in Telangana State, the area of the district is 18, 432 sq. km., and the recorded rainfall is 209 mm which is very lower than the normal rainfall (Valli Manickam, 2012). Temperature ranges from 28^0C to 32^0C. Agriculture is the main occupation and is primarily crops are paddy, jowar, groundnut, castor, cotton. Nalgonda is one of the highly drought - prone districts of Telangana. Nalgonda district covers an area of about 14, 240 km^2 accounting for 5.18 % of the total area of Telangana. The soils of the district are mainly 'red earths', the temperature ranges from 28^0C to 40^0C, the average rainfall around 772 ppm, the principal crops are paddy, jower, bajra, grams. Commercial crops like chillies, cotton, groundnuts are grown under irrigation (B. Hemamalini *et. al.,* 2010).

3. Material and methods

The rhizosphere soil samples from 4 different fields, taken with the help of widget by taking a block of soil with the plant roots intact. Soil adhering the roots were carefully removed and placed in sterile polythene bags. These soil samples were transported to the laboratory and processed for the quantitative and qualitative studies on Vesicular Arbuscular Mycorrhizal fungi and root colonization.

Chlamydospores, Azygospores and vesicles produced by the VAM fungi were collected by wet and decanting method (Gerdemann and Nicolson 1963), 100g rhizosphere soil samples were taken in 500 ml beaker with sufficient quantity of water and stirred thoroughly to make soil suspension. Soil suspension was passed through sieves of different sizes (450 µm, 250 µm, 106 µm, 75 µm and 53 µm) which were kept one below the other in ascending order.

Soil Analysis:
The rhizosphere soils of Quinoa and the alfisol soil used in the pot and field experiments were analyzed for the following factors.

Soil pH
Ten grams of soil was powdered and mixed with double distilled water in 1: 5 ratios. This was thoroughly shaken and kept aside for an hour. The pH of the supernatant was recorded with the help of Elico pH meter.

Moisture Content
Soil was dried in the oven for measuring the moisture content in the soil 5g of soil samples in triplicate were kept in the hot air oven at 80^0C for 24 hours and dry weights were measured till constant weights were maintained. The moisture of the soil was calculated with the following formula.
% moisture content (MC) =

Weight of moist soil (M) –Weight of dry soil (D)
Weight of dry soil (D)
Available phosphorus (Olsen et. al., 1954), soil nitrogen (Subbarao and Asija, 1956) and potassium (Flame photometer method, Jackson 1958) were estimated.

VAM infection, colonization and establishment:
For the assessment of infection, roots were collected from different fields of Quinoa, which are located in Telangana State.
The plants in the natural fields were carefully uprooted so that the lateral roots and rootlets will come without damage. Well developed roots with root lets were collected from five replicates of each cultivar. These are kept in polythene bags and immediately transferred to the laboratory and 10 to 15 root bits of 2 cm length were selected. These were gently washed in water to remove soil particles. Washing was done with much care so that mycelium and spores which are attached to the root would not be washed away. Washed roots were fixed in FAA (formalin: acetic acid: ethanol, 5: 5: 90) in sterilized bottles. Cleaning and staining of the roots were done by Phillips and Hayman method (Phillips and Hayman, 1970) with slight modifications. The FAA roots were transferred into 10% KOH and left for 48 hrs in tightly closed bottles. After 48 hrs these root bits were boiled in 10% KOH for 45 'min' at 75^0C. Later there were neutralized in 1% HCl and stained with 0.05% trypan blue in lactophenol. Excess stain was removed by keeping the stained roots in lactophenol for 2 'min'. The stained roots were made into slides and observed under the microscope for mycelium, arbuscules and vesicles. The infection, colonization and establishment were studied in two cultivars of Quinoa.

For the quantification of VAM colonization Morpometric technique (Toth and Toth 1982) was used. The VAM fungal infection was counted with the help of superimposing grid system of intersecting lines. The number of points lying over the fungus divided by the number of points lying over the root (Pp) is equal to the volume occupied by the fungus in the root (Vv). The number of points lying over cortical cells containing arbuscules divided by the number of points lying over all cortical cells can also be used to know the extent of arbuscular infection. Similarly vesicles and mycelium were quantified separately.

Therefore:
$\frac{\text{P fungus}}{\text{P root}}$ = **Pp for fungus in root**

And
$\frac{\text{P cells with arbuscules}}{\text{P cortical cells}}$

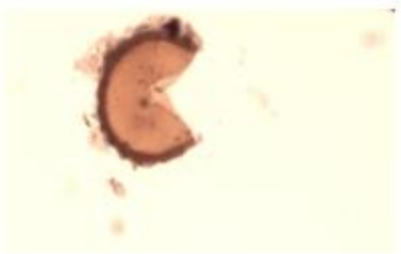
4. Result & Discussion

Table 1. List of VAM mycorrhizal fungi of Quinoa *(Chenopodium quinoa Willd.)*

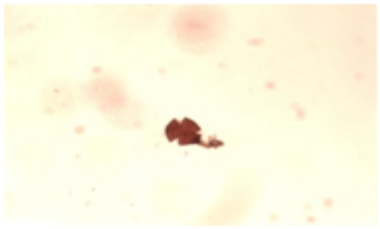
1. Acaulospora delicate
2. Acaulospora nicolsonii
3. Gingospora margarita
4. Glomus aggregatum
5. Glomus ambisporium
6. Glomus claroideum
7. Glomus constrictum
8. Glomus fasciculatum
9. Glomus macrocarpum
10. Glomus microaggregatum
11. Glomus mosseae
12. Glomus viscosum
13. Scutellospora tepuiensis

Photographs of VAM spores isolated from rhizospheric Soil of Quinoa

Aculospora delicate (Fig.6)



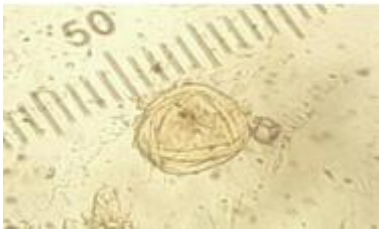
Aculospora nicolsoni (Fig.7)



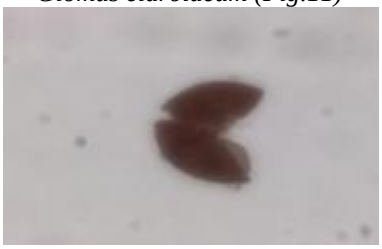
Gingaspora margarita (Fig.8)

Glomus aggregatum (Fig.9)

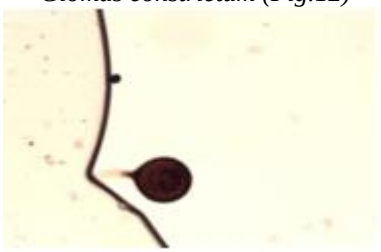
Glomus ambisporam (Fig.10)



Glomus claroideum (Fig.11)

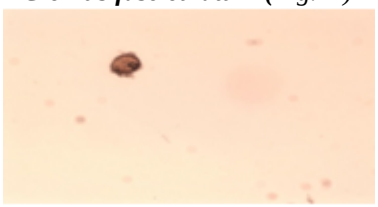


Glomus constrictum (Fig.12)



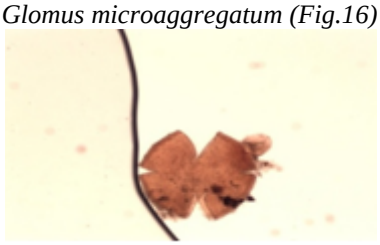
Glomus mosseae (x 200) (*Fig.13)*

Glomus fasciculatum (*Fig.14)*

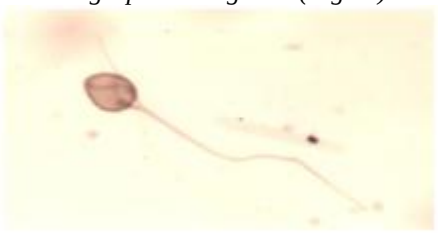


G. macrocarpum (Fig.15)

Glomus microaggregatum (Fig.16)



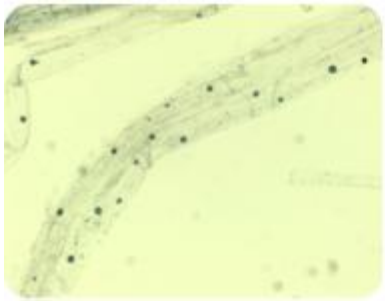
Gingospora margarita (Fig.17)

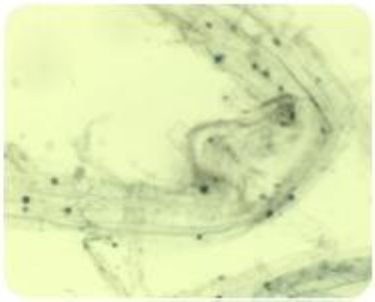


Glomus viscosum (Fig.18)

Scutellospora tepuiensis (Fig.19)

AMF Colonization in Quinoa roots:





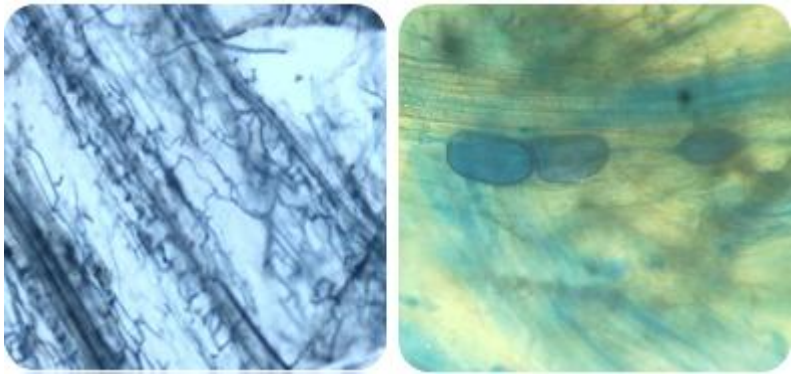


Table 1: VAM Colonization (%) and number of propogules Quinoa Plant

	Factor	L1	L2	L3	L4
1	No. of AMF propogules per 100g Rhizosphere soil	13	14	11	17
2	% of AMF infection	6%	9%	13%	11%

L1: T. U Research field, L2: Nalgonda, L3: Mahaboobnagar, L4: Chinthaloor, (Nizamabad

Quinoa is an important protein crop which has been neglected for its VAM association and its potential to enhance growth and yield. According to (Abiala *et. al.*, 2013), Chenopodiaceae family is a non - mycorrhizal or at least only very sparsely infected. Members of the Chenopodiaceae family are reported as non - mycorrhizal plants. Recently, some species in these families have been reported to have low or in some cases high levels of VAM infection. A sparse vesicular (chlamydospore) infection by *Glomus fasciculatus* was found in some species of Chenopodiaceae, but only when grown in the presence of a mycorrhizal companion plant, citrus or onion. No arbuscules were observed in infected roots. *Chenopodium album* had the highest incidence of infection (5%) (Hirrel *et. al*, 2011).

In This present research work showed better result of Arbuscular mycorrhizalfungi infection in roots of *Chenopodium quinoa* Willd. plants from different study sites of Telangana state. In our study, about 5 to 10% AMF infection was seen in plant Quinoa, (Family: Chenopodiaceae) (Table 4). In this present work, the highest root colonization was observed in quinoa plant roots of Mahaboobnagar. In our study, the lowest root colonization was observed in plants which were collected from T. U. Research field which is showing only 6% of colonization respectively when compare to other sites. According to Hildebrandt et. al (2001) Chenopodium album plants were non – mycorrhizal plants which belongs to chenopodiaceae family, the same result were reported by Abiala et. al., (2013) a sparse AMF infection was identified by the scientist that is only 5% of AMF infection by Hirrel et. al, (2011). In present study also low concentration of AMF infection AMF propgules were identified in Teleganga research field.

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