Comparative potency of single and multiple inocula of VAM fungi for Quinoa (*Chenopodium quinoa* Willd.)

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Abstract **-** The outcome result of inoculation of two different VAM fungi of Glomus Spp. were studied on two cultlivars of Quinoa was studied in this present research work. The effect Glomus mosseae more in the formation root colonization in two cultivars of Quinoa than Glomus aggregatum in host plant. The formation of spores and sporocarps also abundant in single and multiple inoculations.

Key words: **VAM fungi, *Glomus* Spp. *Glomus mosseae, Glomus aggregatum,* Quinoa.**

Introduction

The importance of VAM fungi for plant growth has now has been fully appreciated. Inocula consisting of single species of a VAM fungus are being tried to ensure a better performance of the crops. A benefit of mixed inocula have been highlighted in the recent years but due to attention has not been paid to explore their practical application and still necessary to pay more attention in production and overcome the problems in marketing of the VAM inocula (Gianinazzi et al. 1990). In the present study, two VAM fungi Viz., *Glomus mosseae* (Nicolson. & Gerd) Gerdemann and Trappe, *Glomus aggregatum* (Shenck, 1990) evaluated individually as well as in different combinations for their potentiality as VAM inoculation for a protein rich crop, Quinoa which belongs to chenopodiaceae family. 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 (Gordillo, 2016). VAM fungi are eco friendly bio-fertilizers which enrich the soils and increases the efficiency of plants in phosphate utilization by formation of dense root clusters (Cheng et al. 2011; Lambers et.al. 2011). Mycorrhizae show a symbiotic association with all terrestrial plants (Parniske, 2008). 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. 2014). 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). Due to lack of existing literature on the mycorrihzal colonizagion in Quinoa, albeit sparse colonization in *Chenopodium album* has been reported, it is proposed to study the native mycorrhizae association with the rhizosphere soils and its colonization in the roots for assessing it as a prospective biofertilizer. Therefore in the present work the interaction between the plant and VAM fungi, *G.mosseae* and *G.aggregatum* and their effects on the growth of Quinoa have been investigated.

Materials and Methods:

Quinoa cultivars INIA – 431 and INIA – 427 were raised in field pits (13 X 3m) with soil (sandy clay loam soil with pH 7.3, organic matter 0.72%, and indigenous spore population of *Glomus* spp.) from surface sterilized *Rhizobium* treated seeds. Before sowing the seeds, inocula of different VAM fungi was added to the soil at the rate of 150 g soil inoculums/pits having 350 – 400 spores/ 100 g soil. Following treatments with five replications were included in the study. The VAM fungi were picked up from the indigenous soil in the form of spores and sporocarps. They were identified, among them *G. mosseae* and *G. aggregatum* were selected for the mass multiplication.

Preparation of Starter Inoculum:

Single spore pure inoculums of each VAM fungi were elevated by using funnel technique (Menge and Timmer 1982). The funnel was filled with 1:1 ratio of sterilized soil and sand mixture. Single spore VAM fungal spores were added to this mixture. Then seeds of Quinoa were sown in the funnel and watered at regular intervals. After 30 days seedling, roots were analyzed for VAM colonization and abundance of VAM fungal spores. Then these pure inoculum was transferred to field for the mass multiplication. Field beds were prepared at Agricultural Research field at Telangana University for the mass multiplication of VAM fungi.

The field level VAM multiplication bed measurements:

- 3 - 6.5 kg of inoculum.
- 13 X 3m of plastisheet.
- Requires 12 weeks of period to increase number of spores and colonization.
- In 1st Bed 68% VAM propagules were calculated.
- In 2nd Bed 97% VAM propogules were calculated.
- In 3rd Bed 54% VAM propogules were calculated.

TREATMENTS

1. Soil without inoculum (Control).
2. Soil with inoculum of *Glomus mosseae* (150 g).
3. Soil with inoculum of *Glomus aggregatum* (150 g).
4. Soil with inoculums of *Glomus mosseae* (75 g) and inoculum of *Glomus aggregatum* (75 g).
5. Soil with inoculum of *Glomus mosseae* (50 g) and inoculum of *Glomus aggregatum* (50 g).

All the treatments were maintained under greenhouse conditions. Plants were uprooted periodically and percentage of colonization of mycorrhizal root was recorded by methods of Phillips and Hyman (1970). The spores were extracted from the rhizosphere soil of Quinoa by the methods of Gerdemann and Nicolson (1963) and spore count of rhizosphere soil was recorded. Shoot dry biomass was recorded.

VAM infection, colonization and establishment:

For the assessment of infection, roots were collected from the agriculture research field of Quinoa, which are located in Telangana State.

The plants in the research field 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 they 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 (INIA - 431 and INIA - 427).

QUANTIFICATION OF VAM COLONIZATION

For quantification of VAM colonization morpometric technique (Toth and Toth 1982) [12] 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:

$$\frac{\text{P fungus}}{\text{P root}} = \textbf{Pp for fungus in root}$$

And

$$\frac{\textbf{P cells with arbuscules}}{\textbf{P cortical cells}}$$

The data were statistically analyzed by the methods of analysis of variance (Hicks, C.R. & Turner K.V, 1999) and critical difference at 5% level of significance was calculated.

Result and Discussion

The VAM inoculants evaluated for Quinoa individually as well as in different combinations caused an improvement not only in mycorrhizal colonization in root, spore count in rhizosphere soil. However, as expected the magnitude of improvement varied with the VAM inoculants and their combinations. While maximum improvement in spore count was caused by an inoculum *of G. aggregatum*, maximum improvement was caused by a combination of *G. mosseae* and *G. aggregatum*. Combined inoculum of all the two VAM fungi was most effective in improving mycorrhizal colonization.

Considering the yield to be an important parameter of crop performance, comparatively higher yield in Quinoa due to double inoculants is noteworthy and deserves due to attention and further exploration. Repeated observations of this kind with other crops will confirm the superiority of multiple inocula and open a new avenue to achieve better productivity in crops will confirm the superiority of multiple inocula and open a new avenue to achieve better productivity in crops. Colonization of roots by VAM fungi in different treatments was recorded in INIA-427, INIA-431 at 30 days, 60 days, 90 days, 120 days and 150days. The percentage of colonization was given in Tables.Root colonization in the form of vesicles and arbuscules was evident in 60 day plants of both the cultivars of Quinoa. *G. mosseae* was showed better colonization than *G. aggregatum.* Colonization was not affected by different combination treatments. Arbuscule number was increased

with the age of the plant. Similarly number of vesicles also increased with the increase in plant age. Colonization reached maximum in 120days plants. INIA - 421 plant recorded over 90% colonization in double combination with *G. mosseae* and *G. aggregatum.*

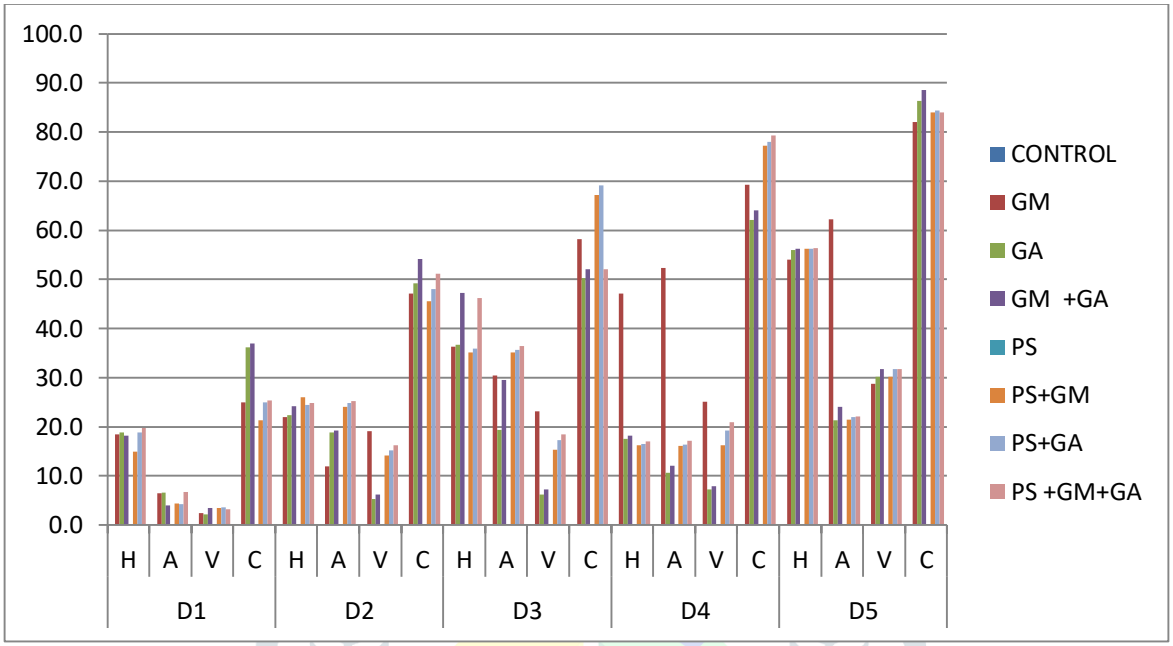
Similar changes in colonization were observed in INIA - 421 with *G. mosseae* were recorded. There was a better colonization with *G. mosseae* than *G. aggregatum.* Arbuscule number was more in 90 and 120 days plants. Maximum colonization was recorded in 120 days plants. Colonization was relatively more in INIA - 421 than INIA - 437 in different treatments. Root colonization is more in combination treatments with *G. mosseae* and *G. aggregatum*.

Table 1. VAM Colonization (%) in INIA - 431 of Quinoa (*Chenopodium quinoa* Willd.)

TREATMENT	D1				D2				D3				D4				D5			
	H	A	V	C	H	A	V	C	H	A	V	C	H	A	V	C	H	A	V	C
CONTROL	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
GM	18.5	6.5	2.5	25.0	22.0	12.0	19.1	47.1	36.3	30.4	23.2	58.2	47.1	52.4	25.1	69.3	54.0	62.3	28.7	82.0
GA	18.8	6.6	2.2	36.2	22.4	18.9	5.3	49.2	36.7	19.4	6.2	50.2	17.5	10.6	7.2	62.1	56.0	21.3	30.2	86.4
GM +GA	18.2	4.0	3.5	37.0	24.2	19.3	6.2	54.1	47.2	29.6	7.2	52.1	18.2	12.1	7.9	64.1	56.3	24.0	31.8	88.6
PS	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PS+GM	15.0	4.4	3.5	21.3	26.0	24.1	14.2	45.5	35.2	35.2	15.4	67.2	16.3	16.1	16.3	77.2	56.2	21.4	30.2	84.0
PS+GA	18.8	4.2	3.6	25.0	24.4	24.8	15.2	48.1	35.9	35.7	17.3	69.2	16.5	16.4	19.2	78.0	56.3	22.0	31.8	84.4
PS +GM+GA	19.8	6.8	3.2	25.4	24.8	25.3	16.2	51.2	46.2	36.4	18.5	52.1	17.0	17.1	21.0	79.3	56.4	22.1	31.8	84.0

PS - *Pseudomonas aeruginosa*; **GM –** *Glomus mosseae*; **GA** – *Glomus aggregatum.*
D1 – 30 days; D2 – 60 days; D3 – 90 days; D4 – 120days; D5 – 150 days.
H-Hyphae; A- Arbuscules; V-Vasicules; C-Colonization.
*All the values are means of five replicates.

Table 2. Bar graph for root colonization (%) of INIA-431 of Quinoa (*Chenopodium quinoa* Willd).



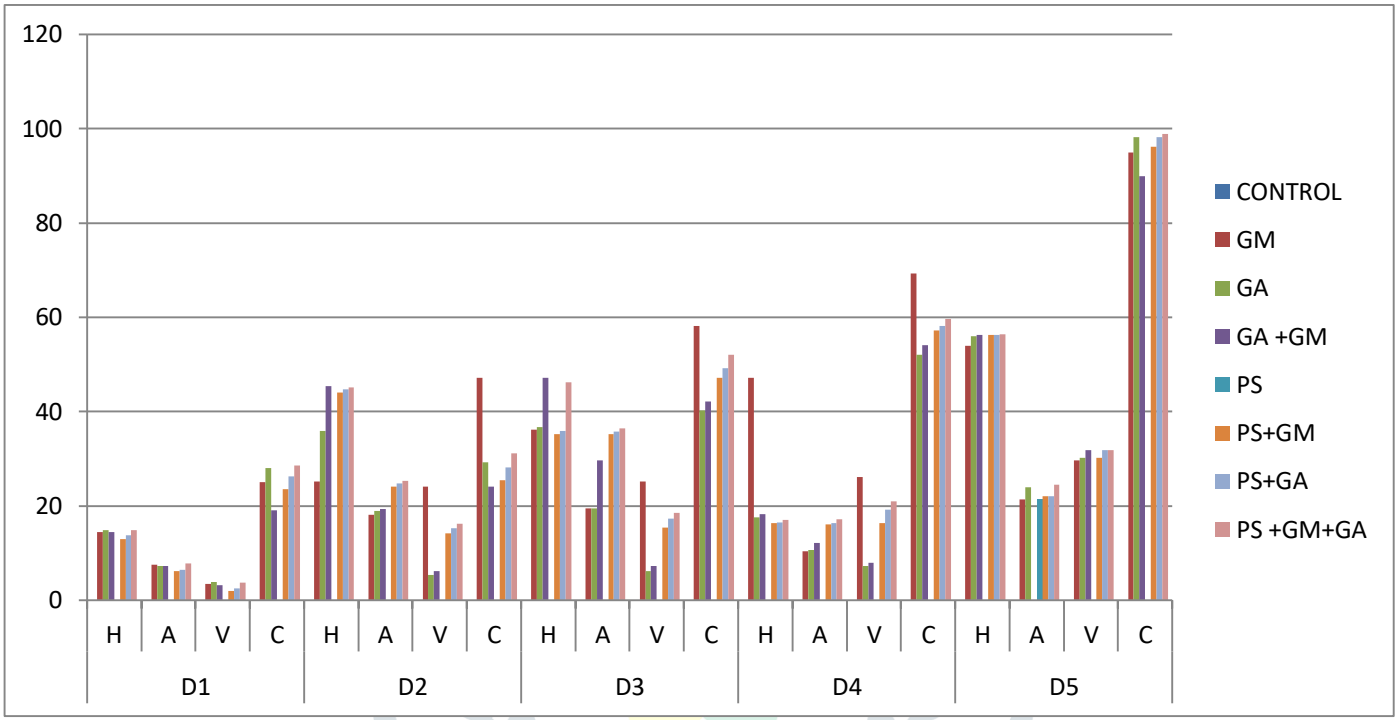
X Axis - Results.
Y Axis - Intervals.
D1 – 30 days; D2 – 60 days; D3 – 90 days; D4 – 120days; D5 – 150 days.
H-Hyphae; A- Arbuscules; V-Vasicules; C-Colonization.
PS - *Pseudomonas aeruginosa*; **GM** - *Glomus mosseae*; **GA** – *Glomus aggregatum*

Table 3. VAM Colonization (%) in INIA – 427 of Quinoa (*Chenopodium quinoa* Willd.)

TREATMENT	D1				D2				D3				D4				D5			
	H	A	V	C	H	A	V	C	H	A	V	C	H	A	V	C	H	A	V	C
CONTROL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GM	14.5	7.5	3.5	25	25.2	18.1	24.1	47.1	36.2	19.4	25.2	58.2	47.1	10.4	26.1	69.3	54	21.3	29.7	95
GA	14.8	7.2	3.8	28	35.9	18.9	5.3	29.2	36.7	19.4	6.2	40.2	17.5	10.6	7.2	52.1	56	24	30.2	98.2
GA +GM	14.4	7.2	3.2	19	45.4	19.3	6.2	24.1	47.2	29.6	7.2	42.1	18.2	12.1	7.9	54.1	56.3	0	31.8	90
PS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21.4	0	0
PS+GM	13	6.2	2	23.5	44.1	24.1	14.2	25.5	35.2	35.2	15.4	47.2	16.3	16.1	16.3	57.2	56.2	22	30.2	96.2
PS+GA	13.8	6.4	2.5	26.2	44.7	24.8	15.2	28.1	35.9	35.7	17.3	49.2	16.5	16.4	19.2	58.2	56.3	22.1	31.8	98.2
PS +GM+GA	14.8	7.8	3.7	28.6	45.1	25.3	16.2	31.2	46.2	36.4	18.5	52.1	17	17.1	21	59.6	56.4	24.5	31.8	98.9

PS - *Pseudomonas aeruginosa*; *GM-Glomus mosseae*; **GA** -*Glomus aggregatum*.
D1 – 30 days; D2 – 60 days; D3 – 90 days; D4 – 120days; D5 – 150 days.
H-Hyphae; A- Arbuscules; V-Vasicules; C-Colonization.
*All the values are means of five replicates.

Table 4. Bar graph of root colonization (%) in INIA-427 of Quinoa (*Chenopodium quinoa* Willd.)



X Axis - Results.
Y Axis - Intervals.
D1 – 30 days; D2 – 60 days; D3 – 90 days; D4 – 120days; D5 – 150 days.
H-Hyphae; A- Arbuscules; V-Vasicules; C-Colonization.
PS - *Pseudomonas aeruginosa*; **GM** - *Glomus mosseae*; **GA** – *Glomus aggregatum*.

ACKNOWLEDGMENT

I take opportunity to thanq my supervisor Dr.M.Mamatha, Professor, Forest College and Research Institure, Mulugu, Hyderabad, Telangana, for Numours discussions and valuble suggestions and help to prepare this research paper for publication. I am very much thank full to UGC for providing me RGNF Fellowship to carry out my research.

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