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Influence of PGPR and Two Vesicular-Arbuscular Mycorrhizal Fungi on the Biomass of Two Cultivars of *Chenopodium quinoa* Willd. under Pot Conditions

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Abstract: ***The present research work gives the information about the effect of VAM and PGPR on improvement of plant biomass of the Chenopodium quinoa Willd. The research was carried out at research agricultural field at Telangana University, Nizamabad, Telangana state. The pot experiments were conducted during Khari season of 2017 and in 2018 on loamy sand soil to study the effect of phosphorus levels and bio-fertilizers like PGPR and VAM on biomass productivity and uptake of Phosphorus. We adopted completely randomized design (CRD) for these experiments, seven treatments were designed along with control to estimate biomass productivity (0, 15, 30, 40 and 45g/ha) in treatments of bio-fertilizers (control, GM, GA, GM+GA, PGPR, PGPR+ GA, PGPR+GM, PGPR+GM+GA) thereby making sixteen treatment combinations tested in randomized block design with two cultivars of Quinoa () INIA – 431 and INIA-427). Results indicates that application of VAM and PGPR increases the biomass of the plant up to 40 to 45kg/ha significantly increased net weight of root, stem and total plant weight. This biomass content includes weight of stem, root and total plant along with leaves, branches, inflorences and a seed timely till yield of the crop was measured. Interaction effect of VAM like Glomus mosseae and Glomus fasciculatum and (PGPR) Pseudomonas aeruginosa was significantly improved the biomass of the all the parts of the plant both in vegetative and reproductive parts. This present research work indicates that the application of VAM and PGPR better option for the improvement of biomass of the plant to achieve better yield in Chenopodium quinoa Willd. The results were shown in the form of tables and in graphs.***

Keywords: *Chenopodium quinoa* Willd. (INIA-431, INIA-427) *Glomus mosseae*, *Glomus aggregatum Pseudomonas aeruginosa*, VAM. PGPR, VAM, Biomass. Control, GM, GA, GM+GA, PGPR, PGPR+ GA, PGPR+GM, PGPR+GM+GA

1.Introduction

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 (M. J. Koziol, 1992) diameter and of various colours from white to red or black depending on the cultivar.

As millets and pulses are fast disappearing from Indian agriculture, Quinoa may serve as good source of nutrition, if it can be grown in dry land areas of our country. Quinoa is highly nutritious food with sufficient amounts of proteins (Butt, K. U.2016). According to (Martienz et al., 2009), Quinoa is a model crop and is physiologically adapted to stress, particularly due to efficient use of water. Quinoa plays an important role in the diet of women, during prenatal nutrition conditions and for the survival, growth and health of children (Ruales et al., 2002). Several scientists reported that Quinoa consumption recovers from severe bouts of malnutrition (Penagini et al., 2013).

A VAM fungus are known to be beneficial in nutrition and enhances the growth of the host plants. Mycorrhizae, the symbiotic association of fungi and roots of higher plants are proven microbes that help in the establishment, nourishment and disease resistance of the crop plants. 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.

Plant growth-promoting rhizobacteria (PGPR) can decrease use of chemical fertilizers and increases the plant growth like plants shoot length, root length, total plant biomass and yield when associated with plant roots (Lugtenberg B, Kamilova F, 2009). VAM has shown promising results in increasing plant biomass of forest species in China through increased nutrient uptake (Brundrett et al.1995). Therefore, this present study was carried out to investigate the effectiveness of a combination of AM inoculum and PGPR on the growth of *Chenopodium quinoa* Willd. The

Acaulspora spp. *Glomus* spp. and *Scutellospora* association increased the biomass of the *Solanum quitoens* especially when grown in low phosphorus soil (Gonazalez and Osorio, 2015; Casierra-Posada et al., 2013) . In maize, VAM association increased spike dry weight, spike length and dimensions of seed (Berta G et al., 2014).

2.Methods and Methodology

We adopted completely randomized design (CRD) for these experiments. Treatments were selected and randomly allocated to the experimental units belonging to the same group. Set of observations were drawn from the group randomly. This process was repeated with different groups of experimental units and with different treatments. All experiments were maintained in seven treatments along with control.

VAM + *Pseudomonas aeruginosa*

The mycorrhizal inocula was spread 3 cm below the sterilized soil with *Pseudomonas* treated seeds placed above the inocula so that the growing roots pass through the inocula. The control plants were supplied with soil solution i. e., the solution left out after removing VAM spores. Five plants were grown in each plot. Glucose nitrate broth inoculated with *Psedomonas* was added at regular intervals. Plants were carefully uprooted at 30, 60, 90, 120 and 150 days of growth for the estimation of growth parameters. The biomass of the shoot, root and total plant were recorded at regular intervals. The data was collected in replicates of five for each treatment.

Dynamics of growth

The growth characteristics in respect of biomass increment and mean rate of dry matter production were calculated.

a) Biomass increment

As an index of growth character increase in biomass (W) was expressed in terms of dry weight (Sestak *et al*, 1971).

$$\mathbf{W = W_2 - W_1}$$

Where the subscripts 1 and 2 indicate values of W on two occasions. The increase in shoot biomass was calculated using the above formula.

b) Rate of dry matter production (G)

The mean of dry matter production or mean growth rate G over an interval of time from

D_1 to D_2 is given by

$$\mathbf{G = W_2 - W_1 / D_2 - D_1 \ (gram\ day^{-1})}$$

Estimation of plant biomass

Effect of seed bacterization of *P. aeruginosa* and soil treatment with VAM fungi (*G. mosseae, G. aggregatum)* on plant biomass (shoot, root and total plant) in two cultivars of Quinoa in pot experiments was recorded and the results were statistically analyzed by analysis of variance. (Tables: 1a, 1b, 1c, 1a, 2b, 2c, 3a, 3b, 3c).

3.Results

The biomass production in arbuscular mycorrhizal plants was significantly increased in both the cultivars of Quinoa INIA – 431 and INIA – 427 when tested with the uninoculated treatment (Table 1, 2 and 3). According to Lambert et al., 1979; Sailo and Bagyaraj, 2005, they reported that increased plant growth was recorded when the plants were inoculated with VAM mainly through increased uptake of diffusion limited nutrients like P and these reports were good evidences with the results of this present research work in which all the VAM plants showed significant biomass production with higher nutrient contents in comparison with uninoculated replicates.

4.Estimation of Plant Biomass

Effect of seed bacterization of *P. aeruginosa* and soil treatment with VAM fungi (*G. mosseae, G. aggregatum)* on the plant biomass (shoot, root and total plant) in two cultivars of Quinoa in pot experiments were given and the results were statistically analyzed by analysis of variance and presented in Tables 1a, 2b, 3a, 3b, 4a, 4b.

Shoot Biomass

Improvement of shoot biomass was significant in all the treatments in both the cultivars over control Table (1a). Increase in shoot biomass more in PGPR and VAM fungi treated plants. INIA-431 cultivar was more responsive to various treatments than INIA-427. In combined treatments overall improvement in shoot biomass from 120 days to 150days was significantly different from other treatments in INIA-427 cultivar. Similar pattern of biomass improvement was observed in between days in INIA-431 cultivar (Table 1b). Initially only triple and double combination treatments were more effective than other treatments in INIA-431. Subsequently double combination treatments were also proved effective results after 90days growth of the plant.

Root biomass

Root biomass was significantly enhanced by different VAM and PGPR treatments to the cultivar INIA-431 (Table 1a). Double and triple combinations produced better improvement than individual treatments. Initially *G. mosseae* and *P. aeruginosa* boosted the plant growth. However, triple combination treatments established their supremacy over other treatments at subsequent growth stages. In INIA-427 also similar patterns was observed. Some of the treatments did not show significant increase in root biomass at early stages. The triple combination treatments were most effective in overall improvement of root biomass in pot experiments (Table 1b and 1c).

Total biomass

All the treatments were significantly different over control in improving the total biomass of two cultivars of Quinoa in pot experiments (2a). PGPR was most effective in all stages of growth in all cultivars. The combination treatments produced good improvement in total biomass. Triple combination treatments were the most effective in improving the plant biomass of the cultivar INIA-431 and in the cultivar INIA-427 in pot experiments. Effects of different treatments on the cultivars were positive. Both the cultivars were showed different responses where INIA-431 cultivar showed better response over in the cultivar INIA-427.

Table 1a: Effect of VAM and PGPR treatments on root biomass (g/day-^{1}) of dry matter production in INIA-431 of Quinoa (*Chenopodium quinoa* Willd.)

Pot Experiments

TREATMENT	INIA-431									
	Biomass for	Dry matter production W_2-W_1	Biomass for	Dry matter production	Biomass for	Dry matter production	Biomass for	Dry matter production	Biomass for	Dry matter production
	D2 – D1	D2-D1	D3 – D2	W_3-W_2	D4 – D3	W_4-W_3	D5– D4	W_5-W_4	D5 – D1	W_5-W_2
	Period (g/plant)		Period (g/plant)	D3-D2	Period (g/plant)	D4-D3	Period (g/plant)	D4-D3	Period (g/plant	D4-D3
CONTROL	0.37	0.269	0.76	0.684	0.6	0.233	2.27	0.237	4	0.35
GM	0.56	0.5	0.77	0.831	0.87	1.022	0.7	0.757	3.2	0.1125
GA	0.4	6	1.03	0.97	2.2	1.086	0.73	2.83	4.73	0.738
GM+GA	0.36	6.555	0.4	2.175	3.2	1.021	2.17	0.032	6.13	1.19
PS	0.7	4.38	2.37	1.025	1.93	1.347	4.34	0.154	1.68	3.095
PS+GM	2.9	0.0241	2.4	0.134	3.68	0.633	2.33	0.575	11.33	0.323
PS+GA	1.73	2.4681	1.2	1.95	9.9	0.464	1.36	0.005	14.73	0.069
PS+GM+GA	4.13	1.072	10	0.077	6.74	0.746	1.89	6.227	22.76	0.229

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

Table 1b: Analysis of variance for root biomass dry matter in two cultivars of Quinoa (*Chenopodium quinoa* Willd.)

Pot Experiments

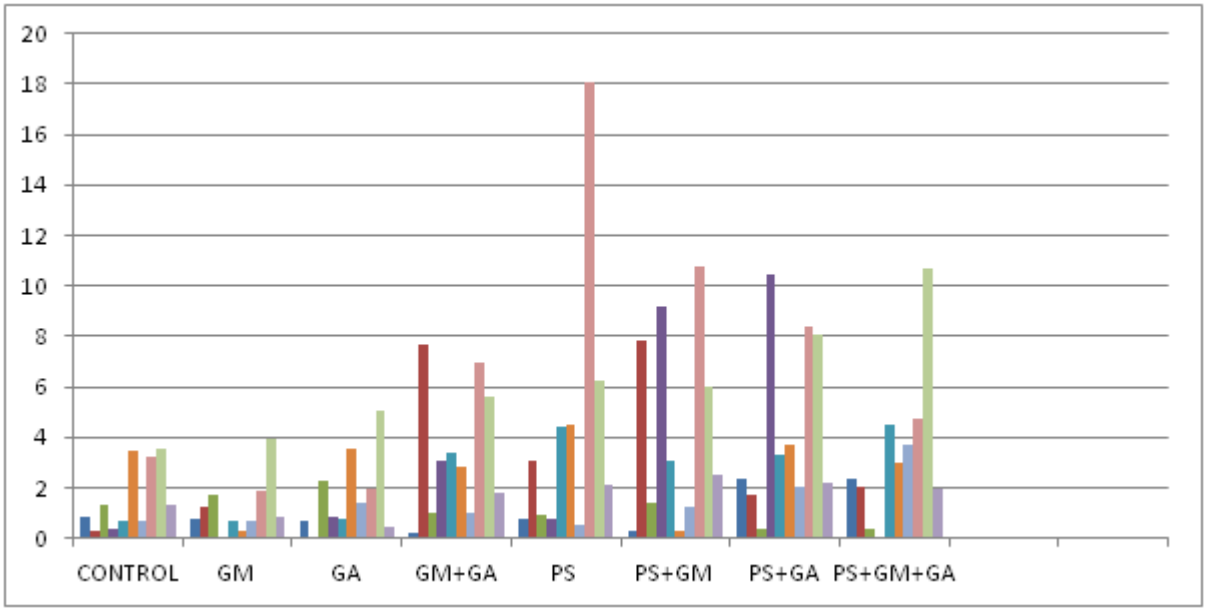
Source of variation	INIA-431				INIA-427			
	Sum of squares	Std. Error	Mean of sum of squares	Std. Dev.,	Sum of squares	Std. Error	Mean of sum of squares	Std. Dev.,
Between Days	1530.56	7.528	30.076	17.758	1498.079	8.173	24.518	19.327
Between treatments	521.838	1.376	7.032	6.33	2506.795	3.738	14.792	16.722
Total	2052.39	8.904	37.108	24.088	4004.874	11.911	39.31	36.049

(P = <0.001) Highly Significant.

Critical Difference (C. D.,) for difference of any two means INIA 431 = 0.02; INIA 427 = 0.78.

Table 1c: Bar graph for root biomass (g/day-^{1}) of dry matter production in INIA-427 of Quinoa (*Chenopodium quinoa* Willd.)

Pot Experiments



X axis-Results.
Y axis –Treatments.
PS-*Pseudomonas aeruginosa*; **GM**-*Glomus mosseae*; **GA** – *Glomus aggregatum*.
D1 – 30 days; D2 – 60 days; D3 – 90 days; D4 – 120days; D5 – 150 days.

Table 2a: Effect of VAM and PGPR treatments on shoot biomass (g/day-^{1}) of dry matter production in INIA-431 of Quinoa (*Chenopodium quinoa* Willd.)

Pot Experiments

TREATMENT	INIA-431									
	Biomass for	Dry matter production W_2-W_1	Biomass for	Dry matter production	Biomass for	Dry matter production	Biomass for	Dry matter production	Biomass for	Dry matter production
	D2 – D1	D2-D1	D3 – D2	W_3-W_2	D4 – D3	W_4-W_3	D5– D4	W_5-W_4	D5 – D1	W_5-W_2
	Period (g/plant)		Period (g/plant)	D3-D2	Period (g/plant)	D4-D3	Period (g/plant)	D4-D3	Period (g/plant	D4-D3
CONTROL	0.37	0.269	0.76	0.684	0.6	0.233	2.27	0.237	4	0.35
GM	0.56	0.5	0.77	0.831	0.87	1.022	0.7	0.757	3.2	0.1125
GA	0.4	6	1.03	0.97	2.2	1.086	0.73	2.83	4.73	0.738
GM+GA	0.36	6.555	0.4	2.175	3.2	1.021	2.17	0.032	6.13	1.19
PS	0.7	4.38	2.37	1.025	1.93	1.347	4.34	0.154	1.68	3.095
PS+GM	2.9	0.0241	2.4	0.134	3.68	0.633	2.33	0.575	11.33	0.323
PS+GA	1.73	2.4681	1.2	1.95	9.9	0.464	1.36	0.005	14.73	0.069
PS+GM+GA	4.13	1.072	10	0.077	6.74	0.746	1.89	6.227	22.76	0.229

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

Table 2b: Analysis of variance for plant shoot biomass of dry matter production (mg/g., dry wt.) in two cultivars of Quinoa (*Chenopodium quinoa* Willd.)

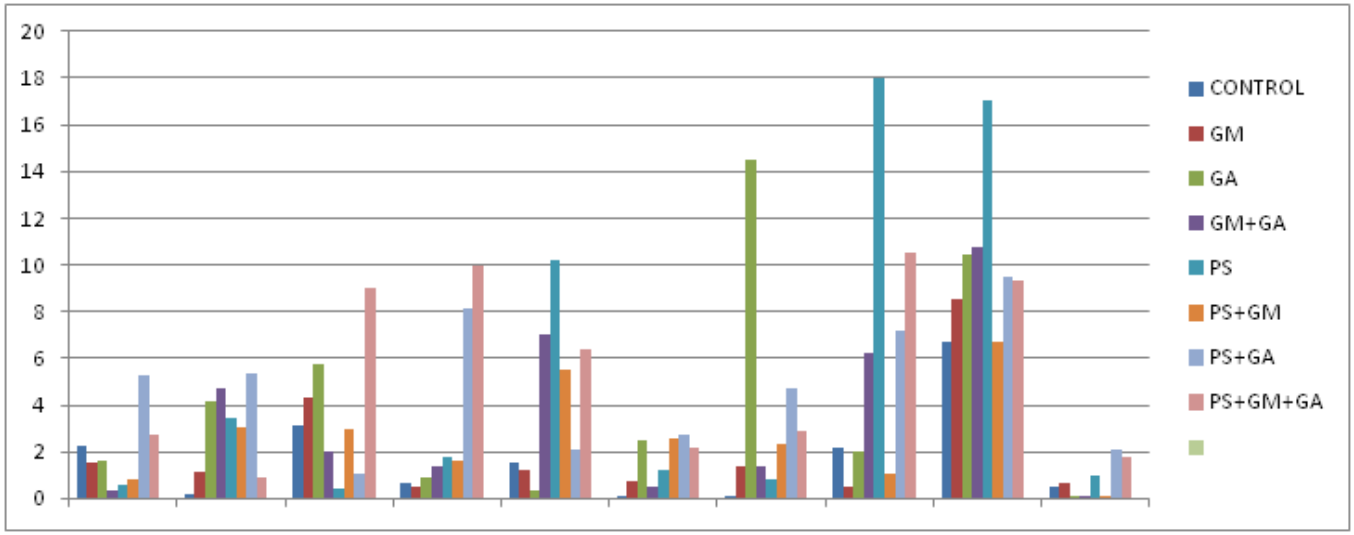
Pot Experiments

Source of variation	INIA-431				INIA-427			
	Sum of squares	Std. Error	Mean of sum of squares	Std. Dev.,	Sum of squares	Std. Error	Mean of sum of squares	Std. Dev.,
Between Days	3157.051*	7.291*	31.855*	20.621*	941.565	2.716	19.798*	7.682
Between treatments	505.012	2.373	11.762	7.503	3593.603*	7.439*	29.56	23.522*
Total	3662.063	9.664	43.617	28.124	4535.168	10.155	49.358	31.204

(P = <0.001) Highly Significant.
Critical Difference (C. D.,) for difference of any two means INIA-431 = 0.18; INIA-427 = 2.97.

Table 2c: Bar graph for shoot biomass (g/day-^{1}) of dry matter production in INIA-431 of Quinoa (*Chenopodium quinoa* Willd.)

Pot Experiments



X axis-Results
Y axis-Intervals
PS-*Pseudomonas aeruginosa*; **GM**-*Glomus mosseae*; **GA** – *Glomus aggregatum*.
D1 – 30 days; D2 – 60 days; D3 – 90 days; D4 – 120days; D5 – 150 days.

Table 3a: Effect of VAM and PGPR treatments on total plant biomass (g/day^{-1}) of dry matter production in INIA – 427 of Quinoa (Chenopodium Quinoa Willd.)

Pot Experiments

TREATMENT	INIA-431									
	Biomass for	Dry matter production W_2-W_1	Biomass for	Dry matter production	Biomass for	Dry matter production	Biomass for	Dry matter production	Biomass for	Dry matter production
	D2 – D1	D2-D1	D3 – D2	W_3-W_2	D4 – D3	W_4-W_3	D5– D4	W_5-W_4	D5 – D1	W_5-W_2
	Period (g/plant)		**Period (g/plant)**	D3-D2	**Period (g/plant)**	D4-D3	**Period (g/plant)**	D4-D3	**Period (g/plant)**	D4-D3
CONTROL	2.23	0.187	3.14	0.652	1.5	0.005	0.08	2.183	6.7	0.49
GM	1.54	1.162	4.33	0.506	1.23	0.737	1.4	0.487	8.5	0.693
GA	1.6	4.15	5.77	0.94	0.37	2.459	14.47	1.991	10.47	0.052
GM+GA	0.34	4.705	2	1.362	7	0.472	1.34	6.234	10.77	0.04
PS	0.6	3.477	0.43	1.776	10.17	1.213	0.83	18.02	17.02	0.985
PS+GM	0.83	3.067	3	1.637	5.52	2.55	2.34	1.035	6.671	0.061
PS+GA	5.27	5.317	1.1	8.15	2.1	2.702	4.74	7.146	9.47	2.126
PS+GM+GA	2.7	0.929	9	10	6.36	2.151	2.85	10.487	9.34	1.74

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

Table 3b: Analysis of variance for total plant biomass in two cultivars of Quinoa (*Chenopodium quinoa* Willd.)

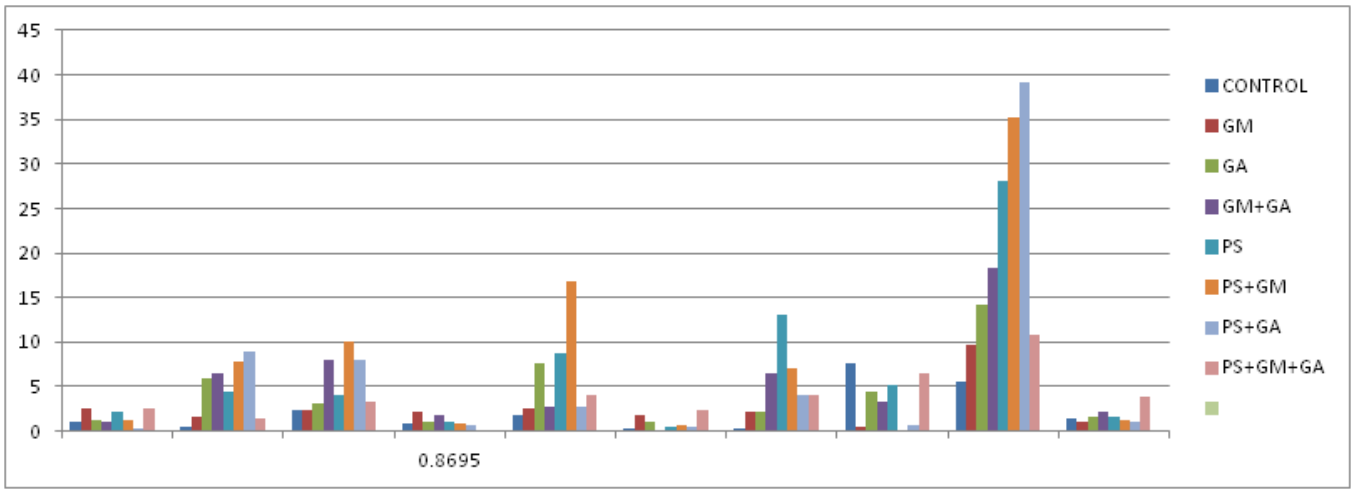
Field Experiments

Source of variation	INIA-431				INIA-427			
	Sum of squares	Std. Error	Mean of sum of squares	Std. Dev.,	Sum of squares	Std. Error	Mean of sum of squares	Std. Dev.,
Between Days	4910.302*	33.621*	49.008*	11.791	5861.753	7.289	49.636*	11.936
Between treatments	2330.36	3.471	13.965	15.522*	8320.692*	7.289	25.208	32.601*
Total	7240.67	37.092	62.973	27.313	14182.45	14.578	74.844	44.537

(P = <0.995) Highly Significant.
Critical Difference (C. D.,) for difference of any two means INIA-431 = 1.06; INIA-427 = 1.08;

Table 3c: Bar graph for total plant biomass (g/day-[1]) of dry matter production in INIA-431 of Quinoa (*Chenopodium quinoa* Willd.)

Field Experiments



X axis-Results.
Y axis-Intervals.
PS-*Pseudomonas aeruginosa*; **GM**-*Glomus mosseae*; **GA** – *Glomus aggregatum*.
D1 – 30 days; D2 – 60 days; D3 – 90 days; D4 – 120days; D5 – 150 days

5.Discussion

Plant rhizosphere facilitates a great number of beneficial micro organisms such as plant growth promoting rhizobacteria (PGPR). Utilization of PGPR as a prospective bio fertilizer is one of the practical significance in enhancing crop yields. Fluorescent *Pseudomona*s bacteria known to produce siderophore compounds, Vitamins and growth hormones like Auxins. PGPR can solubizes ionic phosphorus into soluble phosphorus and VAM fungal hyphae mobilizes and provides Phosphorus to plants. Quinoa (*Chenopodium quinoa* Willd.) is a high protein and fiber crop which can compensate the Rice crop in India. The present study was aimed at investigating VAM association and its impact on growth and yield of Quinoa. Vasicular arbuscular mycorrhizal fungi exert a positive influence on plant growth and vigor mainly through enhanced nutrient uptake. Mycorhrizal dependency as defined by Gerdman, J. W. and Nicolson, T. H. (1963) in the degree to which a plant is dependent on the mycorrhizal association to produce maximum growth or yield. Plant growth is measured using physical parameters such as plant height, shoot and root fresh and dry weights, leaf area index and yield. In the present study, effect of *G. mosseae*, *G. aggregatum* and *P. aeruginosa* on the growth and yield of two cultivars of Quinoa was investigated in pot experiments. Several workers found beneficial effects in terms of growth and yield due to VAM mycorrhizal inoculation in legumes (McKey 1994). (Sprent, 1994; 2001), ground nut (Copetta et al. (2006) Soya been Kapoor et al. (2007). Increase in biomass, dry matter production, plant height and number of leaves resulted due to VAM infection in Soya been variety of JS – 335 with *G. aggregatum* and *G. mosseae* were reported with significance increase in dry weight of pods and yield per plant in pot experiments. (Shashank Ashokrao Tidke 2018).

Performance of *G. mosseae* was better than *G. aggregatum* while *P. aeruginosa* also significantly enhanced plant growth with *G. mosseae* than *G. aggregatum*. Combination treatments in general and triple combination treatments in particular exhibited more growth over control and individual treatments. The present result confirms the earlier findings on other protein seed crops and suggests a beneficial interaction between VAM fungi and Plant Growth Promoting Rhizobacteria.

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