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(RESEARCH ARTICLE)

Effect of Vasicular-Arbuscular Mycorrhiza (VAM) and PGPR on plant growth response of two cultivars of *Chenopodium quinoa* Willd (INIA – 427, INIA- 431) in both field and pot experiments

Prashanthi Sandepogu [1,*] and M. Mamatha [2]

[1] *Lecturer in Department of Botany, TSWRDC (W), Nizamabad, Telangana, India.*
[2] *Professor in Forest Botany at FCRI, Mulugu, Hyderabad, Telangana, India.*

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Abstract

Experiments were conducted to approach the effect of interaction of *Glomus mosseae*, *Glomus aggregatum* (VAM) and *Pseudomonas aeruginosa* (PGPR) on the growth, percentage of mycorrhizal infection and plant growth parameters of *Chenopodium quinoa* Willd. Which belongs to Chenopodiacea family considered as a healthy food because of its good source of many nutrients, which when consumed with other foods can be an important part of a balanced diet. Quinoa is most known for its protein content compared to other plant foods. The present research work conducted at Telangana University, Nizamabad, Telangana state from November 2016 to March 2017. The research work carried by two cultivars of *Chenopodium quinoa* Willd. The two cultivars has shown highest yield and significant increase in N, P, K levels, plant dry weight, fresh weight and in total plant biomass and also estimated that the difference between grains yield per plant, protein content of the grains, weight of 100 grains over the control was recorded when the experimental plants were subjected to VAM or PGPR alone or in combination. VAM propogules were established successfully in sterile soils and the effectiveness of PGPR inoculants were appreciably increased in association with VAM. Especially dual infection of VAM and PGPR expressed a higher beneficial effect on root and shoot development, percentage of mycorrhizal infection and plant growth parameters than either inoculum alone.

Keywords: *Glomus mosseae*; *Glomus aggregatum*; *Pseudomonas aeruginosa*; *Chenopodium quinoa* Willd; NPK; Plant growth parameters

1. Introduction

The microbial activity in the rhizosphere, on the surface and in the tissue of roots is extremely important for the plant growth and yield. VAM fungi which include a group of important soil fungi are ubiquitous throughout the world and are known to improve the plant growth through better uptake of nutrient like NPK. [1- 4] and water resistance to drought and increased tolerance or resistance to root pathogens. They also improve the activity of N fixing in the root zone [5]. Probiotic influence of symbiotic nitrogen fixers and those of VAM occurring in association with major agricultural crops have been well reviewed [6].

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 [7]. Several scientists reported that Quinoa consumption recovers from severe bouts of malnutrition [8].

Even after 7 decades of Independence, malnutrition remains an important health hazard and it needs to be tackled by cultivating protein rich crops. Indian food is predominantly vegetarian and carbohydrate rich with cereals as major

* Corresponding author: Prashanti Sandepogu
Lecturer in Department of Botany, TSWRDC (W), Nizamabad, Telangana, India.

component. Pulses serve as the main source of proteins. But the production of pulses is declining and sometimes we are dependent on import of pulses. Quinoa has become an attractive alternative to traditional carbohydrate rich cereal crops as it provides proteins, minerals and vitamins unlike other cereals. In view of the UNO call to popularize the crop and to find better protein source outside the pulses, the study was undertaken to assess its growth under climatically conditions of Telangana state. Present study investigates the effect of interaction of VAM and PGPR on growth and yield of Quinoa in pot experiments as well as in field experiments in sterile soil conditions.

2. Material and methods

Quinoa cultivars INIA – 431 and INIA 427 were used as experimental plants. The experimental soil had pH 6.8 with a loam texture and was deficient in available P having 3.5 ppm Olsen's P method [9] sterile soil was taken in pot experiments which is autoclaved at 121 ^{0}C for 15 mints at 15lbs pressure and VAM propagules and infected root pieces were added around the seeds placed in 1-inch-deep holes at the time of sowing. There were 8 replicants for each inoculation treatment 1. Control 2. *Glomus mosseae* 3. *Glomus aggregatum* 4. *Glomus mosseae* +*Glomus aggregatum* 5. PGPR 6. PGPR + *Glomus mosseae* 7. PGPR + *Glomus aggregatum 8*. PGPR + *Glomus mosseae* + *Glomus aggregatum.* The experimental plants were maintained in greenhouse at 25 ^{0}C and 35 ^{0}C were irrigated with tap water as and when required. Plants were uprooted after 30, 60, 90 and 120days for observations on growth parameters like plant length estimation of the NPK levels.

2.1. Estimation of total nitrogen root & shoot, protein content in (seed)

The oven dried shoot and root samples were estimated for nitrogen content by alkaline permanganate method [10]. The instrument is KELPLUS automatic nitrogen distillation unit. The shoot and root samples were taken, weighed 0.1 g of plant sample and transferred into 100 ml, added 2 ml of Conc., H_2SO_4 by using 2 ml acid dispenser and kept it for overnight for the digestion until a clear solution was obtained. The plant sample was added with 1 g catalyst mixture. Then plant sample transferred to KELPLUS digestion unit and heated initially at 200 ^{0}C by increasing the temperature up to 450 ^{0}C for 30 'min'. It was cooled and transferred to the distillation unit. Taken 20 ml of 4% boric acid which has mixed indicator in 150 ml conical flask and kept it under the receiver tube and placed receiver tube in the boric acid, and switched alkali addition to add sodium hydroxide for 25 ml, distillation unit is stetted for 3 'min' for the process. After the completion of distillation for 3 'min' the conical flask was taken out and titrated with 0.02N H_2SO_4 till the blue color changes into pink color which was the end point.

The nitrogen content in plant sample was calculated as follows:

Weight of sample = 0.1 g
Normality of H_2SO_4 = 0.02N
Titration value (TV) = Sample titration value – Blank titration value.

$$\% \text{ N } = \frac{\text{T. V. x } 0.00028}{0.1} \text{x } 100 \ = \ 0.28 \text{ x T. V}$$

2.2. Estimation of phosphorus and potassium

Other than N, remaining plant samples were digested in diacid or triacid mixture. Taken 1 g of powdered plant material in 100 ml Conical flask,100 ml of diacid mixture (9: 4 ratio of AR grade concentrated HNO_3: $HCLO_4$) was added and kept it for overnight. The flask was placed on hot plate for digestion for 1 hour, the heat is maintained at 100 ^{0}C the temperature was increased to 200 ^{0}C and this temperature was maintained until production of red NO_2 fumes appeared and the liquid became colorless, allowed for cooling then added 20 ml of distilled water and transferred into 100 ml volumetric flask and adjusted the volume up to 100 ml with distilled water, and filtered through Whatman NO.1 filter paper.

This solution was used for the estimation of Phosphorus and Potassium.

2.3. Estimation of phosphorus

Transferred 5 ml of the plant sample into 50 ml volumetric flask, added 5 ml of Barton's reagent the yellow color was developed in 20 'min' the volume adjusted up to 25 ml. The intensity of yellow color was recorded in Spectrophotometer at 420 nm against a blank which contained the entire reagent but not the plant sample, whose volume was substituted by distilled water.

Phosphorus content was calculated by using standard curve by using KH_2PO_4.

$$\% \text{ P in plant sample } = \text{ Sample concentration in ppm X} \frac{\text{Final volume (50 ml) x 100 x1}}{\text{Wt of sample (1g) x aliquot (5ml) x 106}} = \textit{Sample concentration in ppm x } 0.1$$

(Obtained from standard Curve)

2.4. Estimation of potassium

Prepared 1000 ppm K stock solution which is dissolved in 1.907 g of analytical grade KCl in distilled water and adjusted up to 1 liter.

1000 ppm K stock solution was taken in 0, 1,2,4,6, and 10 ml volumetric flasks and adjusted up to 0, 20,40,60,80 and 100 ppm K with distilled water.

By adjusting the filter and the gas, the readings were recorded by flame photometer at 440 nm of K on horizontal axis.

$$\% \text{ P in plant sample } = \text{Sample concentration in ppm (R) x} \frac{\text{100 X 100}}{\text{Wt of sample (1g) x 106}} = \text{ R x 0.1}$$

3. Results

Table 1 Effect of VAM and PGPR treatments on plant length, phosphorus, nitrogen, potassium content in two cultivars of Quinoa (*Chenopodium quinoa* Willd.) (Field Experiments)

TREATMENT	D1				D2				D3				D4				D5			
	PL	N	P	PS	PL	N	P	PS	PL	N	P	PS	PL	N	P	PS	PL	N	P	PS
CONTROL	3.4	2.3	4.2	3.5	15.3	8.23	13.0	23.0	23.0	14.5	15.2	32.0	34.9	40.8	22.0	56.9	23.0	54.0	17.0	40.0
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	3.4	2.3	4.2	3.5	15.3	9.2	12.0	20.0	23.0	14.0	10.0	34.0	8.0	8.0	7.9	34.0	35.0	18.0	23.0	26.0
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; PL: Plant length, N: Nitrogen, P: Phosphorus, PS: Potassium; *All the values are means of five replicates.

Dual infection with VAM and PGPR resulted in maximum yield and plant growth. Effect of PGPR and VAM fungi on plant growth (shoot, root and total plant length) was studied in two cultivars of Quinoa. All the treatments proved effective in improving plant length in both the cultivars (Table 1). Combination of *G. mosseae* and *P. aeruginosa* treatments showed better performance over *G. aggregatum.* Combined treatments were significantly different over control in field conditions as well as in pot experiments. Results suggest that the effect of different amendments in both field and pot experiments were of similar in nature. Increase in the potassium content of whole plant was significantly improved in both cultivars in all the treatments. A high amount of potassium was recorded in combined treatment of three organisms in cultivar INIA - 431 over INIA - 427. All the treatments were significantly different over control in cultivar INIA-431 under field conditions. There was no significant difference in cultivar INIA - 431 over cultivar INIA-427 under field and pot experiments. The total plant nitrogen showed a moderate increase over control in all the treatments in both cultivars. Combined treatments were more effective in improving the nitrogen content than the individual treatments. Results are significantly different over control. Of the nitrogen content in shoots and roots only root nitrogen is influenced significicantly by the combined treatments. The plant nitrogen showed a moderate increase over control in all the treatments in both cultivars. Combined treatments were more effective in improving the nitrogen content than the individual treatments. Results are significantly different over control. Of the nitrogen content in shoots and roots only root nitrogen is influenced significicantly by the combined treatments. [Table1 and 2].

Rather than in few cases, a significant increase in growth parameter over control was witnessed when the experimental plants were subjected to different treatments (Table - 1). Plant length was recorded. A significant increase in N,P,K levels were recorded when the both cultivars were subjected to dual inoculation of PGPR and VAM followed by VAM and PGPR inoculation alone failed to exhibit a significant difference over control in respect all growth parameters of experimental plant. Similar results were obtained when the above cultivars were treated with VAM inoculation alone or in combination with PGPR.

Table 2 Effect of VAM and PGPR treatments on plant length, phosphorus, nitrogen, potassium, two cultivars of Quinoa (*Chenopodium quinoa* Willd.) (Pot experiments)

TREATMENT	D1				D2				D3				D4				D5			
	PL	N	P	PS	PL	N	P	PS	PL	N	P	PS	PL	N	P	PS	PL	N	P	PS
CONTROL	3.7	2.9	3.6	2.00	4.2	3.2	4.0	2.06	10.00	4.78	3.98	2.87	14.98	5.11	15.23	25.97	25.99	16.78	13.00	59.00
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	3.9	2.90	3.5	2.00	4.22	3.20	4.22	2.00	12.99	4.50	3,65	2.22	12.54	5.12	13.87	23.00	23.00	21.4	12.00	79.87
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; PL: Plant length, N: Nitrogen, P: Phosphorus, PS: Potassium; *All the values are means of 8 replicates.

4. Discussion

VAM propogules (*Glomus mosseae*, *Glomus aggregatum*) in the present investigation established successfully in sterile soils. The effectiveness of PGPR as single inoculants was apparent and appreciably increased in association with VAM fungus. Basically, dual infection of symbionts in the present study bring in to play a higher beneficial effect on substantial development in total plant length N,P,K levels and crop yield than either inoculum alone. VAM fungi have reported to increase the growth of plants by enhancing nutrient uptake [11] through a reduction of the distance that nutrients must diffuse to plant root [12] by accelerating the rate of nutrient absorption and nutrient concentration at the absorbing surface especially P [13] and finally by chemically modifying the availability of nutrient for uptake by plants through mycorrhizal hyphae. Experimental plants subjected to a dual inoculation of VAM and PGPR developed extensive root system, higher mycorrhizal infection. These factors ensured the plants with increased availability of water and nutrients especially phosphorus, Nitrogen and Potassium which are important for the growth and productivity. It is, therefore, natural for experimental plants in the present study to induce improved growth responses and yield under dual inoculation treatment. Similar results were reported by several investigators [14-16].

It has been documented that mycorrhizal fungi assist plants in accumulating higher concentration of phosphorus [17] and later known to have a positive effect on both growth parameters and yield of the experimental plant. This may be attributed to a better phosphorus nutrition of experimental plants by introduced VAM propogules rather than by VAM propogules endophyte population already present in the soil.

The possibility of inoculating the field crops with selected strains of VAM more effective than those already present in the field soil has tremendously improved the prospect of successfully exploring VAM and PGPR infection for better yields of crop plants, especially those grown under low moisture and P conditions. However, the obligate nature of VAM fungi still remains as a major constraint in large scale adaptation of this technology under field and pot conditions.

The effect of VAM fungus *Glomus fasciculatum* in *Catharanthus roseus* L. under different phosphate regions increased the length of the shoot, root length, number of leafs, fresh weight and dry weight and chlorophyll content of VAM inoculated plants were significantly high in comparison with non-VAM inoculated plants [18]. In the present study the effect of VAM fungi and PGPR on the growth of Quinoa was assessed by measuring total plant length and NPK levels. All the treatments including VAM fungi and PGPR in different combinations significantly increased the growth of the plant

in both the cultivars of Quinoa. Early root infection by VAM fungi shows significant effect on plant growth in short season crop plants like sunflower, groundnut, sesamum, and safflower. In Quinoa, VAM infection was evident from third week of its growth. Preliminary screening for symbiotic efficiency of VAM fungi against Quinoa revealed *G. mosseae* and *G .aggregatum* to be better organisms and hence were selected for assessing their effect on plant growth. Prerequisite for increased benefits from VAM symbiosis. Vasicular arbuscular mycorrhizal fungi except a positive influence on plant growth and vigor mainly through enhanced nutrient uptake. Mycorhrizal dependency defined by [19] in 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 height the effect of *G. mosseae, G. aggregatum* and *P. aeruginosa* on the growth and yield of two cultivars of Quinoa was investigated in pot and field experiments.

Several workers found beneficial effects in terms of growth and yield due to VA mycorrhizal inoculation in legumes [20] ground nut, [21] soyabeen. Performance of *G. mosseae* was better than *G. aggregatum* while *P. aeruginosa* also significantly enhanced plant growth with *G. mosseae* than *G. aggregatum* in combination treatments in general and triple combination treatments in particular exhibited more growth over control and individual treatments. The present results confirm the earlier findings on other protein seed crops and suggest a beneficial interaction between VAM fungi and PGPR.

Vasicular arbuscular mycorrhizal fungi enhance plant growth mainly by maximum nutrient uptake. Phosphate, sulfur, nitrogen, potassium and other nutrients are required for plants in large amounts but these are available in minute quantities in soil solution due to the fact that the inorganic phosphate ions get bounded with soil colloids A normal plant can absorb phosphorus from the zone of 0.2 mm around the root the zone depends up on the depletion of phosphorus. The uptake of the phosphorus depends upon surface area of root system more surface of root system increases the phosphorus uptake [22] by exploring the larger volume of soil. VAM fungi play an important role in for plant phosphorus addition by increasing soil volume [23] through their fungal hyphae. VAM fungi are the modified root systems because of the extensive network of extrametrical mycelium which provides an additional root surface for the acquisition of phosphorus. Mycorrhizal plants without addition of phosphorus were several times better than the non-mcorrhizal plants in any soil. Mycorrhizal symbiosis makes a ecological interaction with the plants. They get carbon from the plants in return plants gets micro and macronutrients from the fungus. The present result also show higher internal concentrations of phosporus in the plants which are inoculated with *G. mosseae* and *G. aggregatum*. Suggesting the efficiency of *G. mosseae* over *G. aggregatum*. Similar results have been reported earlier from different plants such as *Mentha arvensis* [24]. Interestingly in the present study the internal phosphorus concentrations were further enhanced by the combined treatments. This suggests that synergistic activity between VAM fungi as well as the growth promoting rhizobacterium. The beneficial influence of VAM on nutrient uptake in maize [25], soya been [26]. In the present study nitrogen, phosphorus and potassium contents were estimated by tissue analysis in VAM and *P. aeruginosa* inoculated plants and non-inoculated controls. All the inoculated plants showed higher levels of N, P, and K. Increase in the content of N, P, and K was significantly different in various treatments in comparison with UN inoculated control.

Mycorrhizal plants showed higher levels of internal nitrogen than non-mycorrhizal control. Increase in nitrogen content is significantly different from mycorrhizal plants. Percentage of root colonization was significantly higher at different growth stages with the corresponding increase in the levels of internal nitrogen. Similar findings were reported earlier by various workers on different plants.

Increase in nitrogen content of mycorrhizal plants in the present study may be explained as an indirect consequence of the enhanced phosphorus uptake. An increased level of potassium was observed in various treatments of mycorrhizal plants in the present study in comparison with non-mycorrhizal plants. However, the incensement was not significantly similar between various treatments. In the present study, plant growth promoting rhizobacteria, *P. aeruginosa* also exhibited positive response in enhancing the plant growth. Seed bacterization resulted in the enhanced levels of N, P and K in the tissues (shoot/root) of both the cultivars of Quinoa. Increase in N, P, K levels was also evident in combination treatments with VAM fungi, *G. mosseae* and *G. aggregatum*. In present results the combination of PGPR and VAM fungi supported maximum improvement in the plant growth and yield. This gives the possibility of using composite bio- fertilizers for better yields.

4.1. VAM – PGPR interactions

The symbiotic association of VAM and PGPR seems to be loosely or tightly with the host plants and the mycorrhizal fungi and most likely play a key role in mycorrhizal function.[27] introduced the term helper bacteria and defined as those bacteria, which support mycorrhizal establishment. Under natural conditions, bacteria associated with

mycorrhizal fungi colonize the surface of extra-radical hyphae or at least in some fungal taxa, live in the cytoplasm as endo-bacteria. The plant provides the fungus with photosynthatically derived carbohydrates, while the fungus supplies the plants roots with nutrients [28]. Changes in bacterial communities may also be driven by complex interactions between plant species and fungal species involved [29, 30]. The increased host phosphorus provided by fungus supports rhizobial production of nitrogenenase enzymes, which are important for nitrogen fixation [31]. The enhanced nitrogen status of the plant promotes further development of mycorrhizal symbiosis. The bio-fertilizer properties of plant growth promoting bacteria are frequently ascribed to their ability to increase the bioavailability. [32] explained that both PGPR and VAM fungus increased plant biomass as well as tissue nitrogen and phosphorus content. In tomato plants the phosphorus content was increased when the roots are inoculated with the VA fungus *G. etunicatum*, or with phosphorus solubilizing bacterium *Enterobacter agglomerans* [33]. The highest nitrogen and phosphorus uptake was observed when tomatoes were inoculated with the both organisms, suggesting that bacteria and VA fungi might together increase the rate of nutrient uptake by the plants.

Rhizosphere microflora might stimulate or inhibit the colonization and establishment of introduced bacterial biocontrol agents such as fluorescent pseudomonads. Biocontrol agent might also stimulate mycorrhizal infection and reduces the utilization of chemical fertilizers as demonstrated by [34]. [35] Reported that PGPR increases the uptake of N and P plays a vital role as Biofertilizer alone or in combination with VAM fungi. PGPR influences spore germination and VAM colonization [36] and triggers vesicle and arbuscules formation, besides increasing the biomass and chlorophyll of the host plant. PGPR with VAM fungi can modify or improves the establishment of mycorrhizal symbiosis and these bacteria referred as mycorrization helper bacteria (MHB), MHB showed better growth of Fungus and mycorrhizal formation. Specific strains of *Pseudomonas* have been shown increase in the growth and yield of some agricultural crops [37].

5. Conclusion

Results of the pot and field experiments were confirmed the effect of VAM and PGPR, the results were appeared under field conditions in response to various treatments. All the treatments showed significant increase in the length of shoot, root and total plant in both the cultivars. A result of plant fresh and dry weight reveals an increase in plant growth in both the cultivars of Quinoa INIA - 431 and INIA - 427 with different treatments. All the treatments were significantly different over control. Combination of all the three organisms proved effective significance form the other individual treatments.

Compliance with ethical standards

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