

Role of endomycorrhizae, rhizobacteria and compost to improve phosphorus availability in onion

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Abstract

Phosphorus (P) is 2nd most important macronutrient for the growth of plants. The soils of Pakistan are alkaline and calcareous in nature which causes precipitation of P and making it less available to the plants. To overcome this issue, excessive P fertilization is used, some part of fertilizer is used by plants while remaining is either fixed as unavailable form or lost due to runoff. So deficiency of P affects the plant growth while runoff of excess fertilizer may result in eutrophication of our aquatic systems. Use of arbuscular mycorrhizal fungi (AMF) may be an appropriate method to enhance the P uptake efficiency of plants and reduce the use of P fertilizers. Plant growth promoting rhizobacteria (PGPR) are also well known for solubilization of P. Therefore, a pot experiment was conducted in 2019 at Research Farm of Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan to check the efficiency of compost, PGPR and AMF to enhance availability of P in onion. The impact of PGPR and AMF were evaluated separately and in combination. At harvest, data regarding plant fresh and dry weight, onion bulb circumference, bulb fresh and dry weight, chlorophyll a and b, carotenoids, and P concentration in plants were analyzed. Results showed that combined inoculation of endomycorrhizae and rhizobacteria improved plant fresh and dry weight, onion bulb circumference, bulb fresh and dry weight, chlorophyll a and b, carotenoids. Overall, integrated application of endomycorrhizae and rhizobacteria enhanced P uptake by 77% as compared to control treatment.

Keywords: PGPR, Endomycorrhizae, Compost, Phosphorus uptake, Onion

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Introduction

Phosphorus is the second most important nutrient for

plant growth after nitrogen (Raghothama, 1999). Phosphorus plays an important role in plant metabolism and development. It is an important part



of multiple processes like synthesis of nucleic acid, fixation of nitrogen, glycolysis, formation and stability of membrane, redox reactions, carbon metabolism, cell signaling, energy transfer and generation and enzyme activation or inactivation (Rengel and Zhang, 2011).

Balanced uptake of P enhances many developmental processes like fruiting, flowering, root growth and shoot growth (Williamson et al., 2001). It is a necessary part of nucleic acids, phospholipids, nicotinamide adenine dinucleotide phosphate (NADPH), adenosine triphosphate (ATP) and sugar phosphates which are very important compounds of the process of photosynthesis (Hammond and White, 2008). Even, very minute deficiency in P has very large impacts on development and growth of plants (Carstensen et al., 2018).

Pakistan soils have pH greater than 7.0 and have more than 3% CaCO₃ (Niaz et al., 2007). The most suitable pH for all vegetables is between 5.5 to 7.0. Onion is one of the important vegetable used throughout the year in every house. Onion bulbs and green leaves are used in preparation of vegetables or eaten raw. They are used in sauces, soups or for seasoning foods. Onions play a role in preventing heat stroke and several other ailments. Onion bulbs are rich in carbohydrates, phosphorus and calcium. Onion are grown in several cities of Pakistan, like Vehari, Hyderabad, Kasur, Sanghar, Nawabshah, Mirpurkhas, Badin, Dadu, Ghotki, Kalat, Chaghi, Turbat, Swat, Jaffarabad etc. (Khokhar, 2018). Moving from less to extreme P deficiency, 90% of Pakistani soil suffers from P deficiency (Alam et al., 1994). Inorganic P is the only bioavailable form for uptake by plants but it is less available due to chemical fixation and making complexes with different elements at low as well as high pH (Shen et al., 2011). Due to chemical fixation of P, excessive P fertilization is done to fulfill plant requirements but only 20% of applied P is utilized by plants (Cordell and White, 2015). These fixation reactions reduce the solubility of P by converting it into compounds which are less soluble and ultimately reduce the P availability to plants (Leytem and Mikkelsen, 2005). Less availability of P fertilizer can reduce crop yield and overuse of P fertilization can cause eutrophication of aquatic systems (Baker et al., 2015).

There are several different methods to enhance the availability of P like organic amendments, agronomic practices, P efficient cultivars, phosphate solubilizing bacteria, arbuscular mycorrhizal fungi, composting

etc. (Kunwar et al., 2018). One of the most appropriate solutions is the use of mycorrhizal fungi because it helps in the uptake of P by plants and produce high yield under limited supply of P (Marschner, 1995). Mycorrhizae is a symbiotic relationship in which soil derived nutrients are supplied by external mycelium of a fungus to the roots of plants (Balestrini et al., 2015). The AMF enhance the availability and uptake of P by plants through different mechanisms. Like network of hyphae helps to extend the root system of plants and explore more soil volume in order to get access to the available P (Elbon and Whalen, 2015). Arbuscular mycorrhizal fungi (AMF) changes the pH of soil in root zone and release organic acids (Tawaraya et al., 2006) which leads to the solubilization of P minerals and release phosphate ions (Kirk, 1999). The AMF releases phosphatase which is an enzyme used for mineralization of organic phosphate into phosphate ions which diffused faster into the hyphae (Koide and Kabir, 2000).

Plant growth promoting rhizobacteria (PGPR) are located in root area, and thus increase plant growth (Ahemad and Kibret, 2014). The PGPR have some direct and indirect mechanisms. These mechanisms include the synthesis and excretion of enzymes, organic acids, biologically active substances and hormones. Such P solubilizing bacteria enhance plant growth, yield and uptake of nutrients (Glick, 2005). Microbially mediated biologically active substances and hormones increase P uptake by stimulating growth of plant roots (Sharma et al., 2013).

In relationship of fungus and bacteria, bacteria improves the AMF activity, while in return, fungus supports bacteria to mobilize P from immobile sources and to fix nitrogen (Nadeem et al., 2017). Mycorrhizae inoculation with different strains of bacteria shows positive impact on the growth of plants. Bacteria also enhance the penetration of fungus into the roots (Jeffries et al., 2003). A study was executed by Tanwar et al. (2012) to find the combined interaction of *Pseudomonas fluorescens* and endomycorrhizae fungi on bell paper (*Capsicum annuum* L.) under different levels of P. They concluded that their combined effect enhanced the root colonization of arbuscular mycorrhizae, P uptake, and activity of phosphatase with the lowest level of P fertilizer which ultimately enhanced the yield of *Capsicum annuum* L.

Compost increases P availability directly because it is an important source of P for the growth of plants (Prasad, 2013) as well as indirectly by effecting



microbial activities, producing organic acids, blocking fixing sites for P. This increased P mineralization and ultimately P uptake by plants enhance growth of plants (Khan and Joergensen, 2009). Addition of compost enhances soil organic material and activity of microbes. These microbes decompose organic matter and feed on them. Due to decomposition process, organic acids are released and these organic acids help to make symbiotic relationship with AMF (Kumar et al., 2012). Keeping in view the importance of PGPR, AMF and compost, present experiment was performed to find out the impact of AMF and PGPR along with compost on P uptake and growth of onion in order to reduce our dependence on chemical fertilizers and protect our environment.

Material and Methods

An experiment was conducted to evaluate the combined effect of AMF, rhizobacteria and compost to enhance the efficiency of P to improve growth and yield of onion. Furthermore, to suppress the wastage of P by efficient utilization of added P in the wire house under ambient conditions. Strain of *G. intraradices* and rhizobacteria and compost were used alone or in combination. Each treatment was repeated thrice. In each pot, 10 kg of soil was filled. Onion nursery was taken from private farm. For inoculation of *Glomus Intraradices* (AMF), same quantity of inoculum was added in the respective pots and then nursery was placed over it. For inoculation with bacteria, first of all 250 mL of Lauria Bertani (LB) broth was prepared, bacterial strain was mixed and left in orbital shaker for 3 days at 28°C temperature. Then onion bulbs were dipped in the inoculum for 1 hour and transplanted to the respective pots. Compost was used as a source of organic matter at a rate of 2% in respective treatment as per plan. Recommended dose of N:P:K for onion at the rate of 50:35:25 kg ha⁻¹, respectively, was applied to each pot except one treatment containing *G. intraradices*, rhizobacteria and compost in combination. Good quality water was used for irrigation. Crop was harvested after 3 months. The growth parameters like bulb weight, bulb size, fresh weight and oven dry weight were recorded. Chlorophyll a and b, carotenoids (Arnon,

1949) and SPAD value (SPAD-502 m) was determined.

Soil and plant were analyzed for P concentration by Olsen method (Olsen, 1954) and Yellow color method (Jackson, 1958), respectively. Data was statistically analyzed by complete randomized design (CRD) under factorial arrangements using computer-based statistical software Statistix-8.1 (Analytical Software, Tallahassee, USA). Treatment means were compared using Honestly Significant Difference (HSD) (Montgomery, 2013).

Results

Growth parameters

Glomus intraradices (endomycorrhizae) and plant growth promoting rhizobacteria along with compost were effective in increasing the growth parameters. The combined inoculation of *G. intraradices* and PGPR enhanced the plant fresh weight by 61% and 74%, plant dry weight by 65% and 82%, bulb fresh weight by 34.18% and 42.98%, bulb dry weight by 60% and 66% as compared to sole application of *G. intraradices* or rhizobacteria, respectively. Similarly, bulb circumference was increased by 19% and 22% when AMF and PGPR were used together as compared to their alone application. However, an increase of 26% plant fresh weight, 15% plant dry weight, 20% bulb fresh weight, 28% bulb dry weight and 8% bulb circumference were observed when AMF and PGPR were applied together as compared to the plants which were treated with AMF and compost. Similarly, dual inoculation of endomycorrhizae and rhizobacteria increase the plant fresh weight by 32%, plant dry weight by 18%, bulb circumference by 11%, bulb fresh weight by 23% and bulb dry weight by 34% in contrast to application of rhizobacteria along with compost.

Table-1: Properties of pre-analyzed soil (used in pot experiment)

Characteristics	Unit	Value
pH _s		7.7
EC _e	dS/m	1.41
Saturation percentage	%	37.8
Available phosphorus	mg/kg	6.58



Table-2: Impact of endomycorrhizae and PGPR along with compost on growth parameters of onion.

Treatments	Plant fresh weight (g pot ⁻¹)	Plant dry weight (g pot ⁻¹)	Bulb fresh weight (g pot ⁻¹)	Bulb dry weight (g pot ⁻¹)	Bulb circumference (cm)
Control	37.0 e	8.3 e	19.5 e	6.2 e	12.3 e
Mycorrhizae	50.2 d	10.9 d	24.7 cd	7.6 d	13.9 cd
Bacteria	46.3 d	9.9 de	23.1 d	7.3 de	13.4 d
M + B	80.9 a	18.2 a	33.1 a	12.2 a	15.9 a
M + O.M	64.1 b	15.8 b	27.3 b	9.5 b	14.9 ab
B + O.M	61.2 bc	15.3 b	26.9 b	9.0 bc	14.8 bc
M + B + O.M (no fertilizer)	54.9 cd	12.7 c	25.6 bc	7.7 cd	14.3 b-d

Mean sharing the same letter(s) do not differ significantly at $P \leq 0.05$

Table-3: Impact of endomycorrhizae and PGPR along with compost on physiological and chemical parameters of onion.

Treatments	Chlorophyll a (mg/L)	Chlorophyll b (mg/L)	Carotenoid (mg/L)	SPAD value	Plant phosphorus uptake (mg/L)
Control	24.1 f	18.8 e	1.57 d	48.4 e	1.66 d
Mycorrhizae	29.8 de	22.2 c-e	2.01 cd	60.3 d	2.00 b-d
Bacteria	27.5 e	20.79 de	1.88 cd	58.2 cd	1.88 cd
M + B	38.9 a	32.0 a	3.09 a	75.5 a	2.94 a
M + O.M	35.3 b	27.3 b	2.57 b	70.6 ab	2.66 ab
B + O.M	33.8 bc	25.3 bc	2.35 b	69.0 a-c	2.41 a-c
M + B + O.M (no fertilizer)	31.2 cd	23.9 b-d	2.09 bc	64.4 b-d	2.18 b-d

Mean sharing the same letter(s) do not differ significantly at $P \leq 0.05$

M = Mycorrhizae

B = Bacteria

O.M = Organic matter

Physiological parameters

In present experiment, inoculation of PGPR, *G. intraradices* and compost significantly enhanced all the physiological parameters. Improvement in chlorophyll "a" and chlorophyll "b" were recorded when treated with endomycorrhizae and PGPR with recommended dose of fertilizer i.e. upto 30%, 41% and 43%, 53% as compared to the application of AMF or rhizobacteria alone, respectively. Similar results were observed in case of SPAD value when inoculated with PGPR and endomycorrhizae together i.e. 25% and 29%. Percent increase in plant carotenoid contents by 53% and 64% was observed when treated with *G. intraradices* and PGPR together in contrast to application of AMF or PGPR solely. Similarly, combined application of arbuscular mycorrhizae along with rhizobacteria increased chlorophyll "a" by 10% and 15%, chlorophyll "b" by 17% and 26%, SPAD value by 6% and 9% and carotenoids by 20% and 31% as compared to sole application of endomycorrhizae or PGPR, respectively.

Chemical parameters

In present trial, all the treatments showed significant ($P \leq 0.05$) increase in P uptake as compared to the control. But maximum uptake was observed when plants were treated with endomycorrhizae and rhizobacteria together with recommended dose of fertilizer which was 46% and 56% in contrast to alone inoculation of AMF or PGPR, respectively. The treatment in which endomycorrhizae and rhizobacteria were applied together, showed 10% and 21% more P uptake as compared to the treatment in which endomycorrhizae + compost or rhizobacteria + compost were used.

Discussion

Production of every crop is highly depends upon phosphate fertilizers. However, only 20% of applied P is utilized by plants (Cordell and White, 2015). So, the application of biological mean like endomycorrhizae or rhizobacteria is one of the appropriate methods to



enhance P availability (Kunwar et al., 2018). It is due to different mechanisms like phosphorus solubilization, greater soil volume exploration and rapid phosphorus movement into hyphae of mycorrhizae (Bolan, 1991). Similarly, PGPR enhance phosphorus uptake by releasing several organic acids e.g. fumeric acids, butyric acids, citric acids etc. These acids liberate P from its compounds (Kapoor, 1995). Even compost itself is a source of P and also improves microbial activity in order to enhance P availability (Khan and Joergensen, 2009). Similarly, in present research, plants treated with *G. intraradices* and PGPR along with compost showed improvement in plant growth parameters. Combined application of endomycorrhizae and rhizobacteria enhanced the growth of plants which might be due to the enhanced uptake of water and nutrients by AMF due to development of hyphae (Chen et al., 2016). It could be also due to the ability of PGPR to produce phytohormones which ultimately increase nutrient uptake and thus improve photosynthetic rate and crop yield (Gholami et al., 2009). The inoculation of arbuscular mycorrhizae fungi enhanced the growth parameters (shoot fresh and dry weight, root fresh and dry weight) of leek (Kahiluoto and Vestberg, 1998), while PGPR inoculated bitter guord plants showed better yield, increased growth of root length, dry root mass and quality (Kumar et al., 2012).

Moreover, it was observed by Mehrvarz et al. (2008) that alone application of PSB enhanced biological yield but inoculation of same bacteria with AMF gave maximum grain mass of barley. In present pot trial, application of PGPR and *G. intraradices* improved the physiological parameters of plant. This might be due to the more harvesting of light during symbiotic relationship, which ultimately enhanced the efficiency of photosynthesis (Wu et al., 2010), due to the enhanced uptake of P and magnesium by mycorrhizae inoculated plants (Zhu et al. 2014). Also this might be due to the nitrogen fixation ability of rhizobacteria, which enhanced the chlorophyll contents in soybean plants (Wu et al., 2006). Different bacterial strains like Azobacter, Pseudomonas and Azospirillum enhanced the chlorophyll a, chlorophyll b and total chlorophyll in plants (Al-Erwy et al., 2016).

In present study, *Glomus intraradices* and PGPR inoculated plants showed maximum uptake of P. This might be due to the development of fungal hyphae which helped in the uptake of P (Gong et al., 2013). Moreover, this can be also due to the ability of PGPR to mobilize the insoluble P by synthesizing organic

acids, chelating substances, growth hormones and enhanced nitrogen fixation rate (Khan et al., 2007). The inoculation of Pseudomonas and AMF together enhanced the total concentration of P in plants (Akhtar and Siddiqui, 2008).

In present research, application of PGPR with compost or *G. intraradices* with compost also enhanced all parameters as well as P uptake as compared to control. These results are in accordance to Perner et al. (2006), who found that application of AMF with compost enhanced the growth, potassium and P uptake. This can be due the uptake of macro and micro nutrients which reduced 50% of availability on chemical fertilizers (Yildirim et al., 2011).

Conclusion

The use of PGPR, endomycorrhizae and compost can be used to enhance P availability in onion. It was concluded in the light of present study that the use of PGPR, AMF along with compost enhanced the growth and P uptake in onion as compared to control. The use of PGPR along with AMF maximum enhanced the growth as well as P uptake by onion. So, by using this method our dependency on chemical fertilizer can be reduced in future.

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Contribution of Authors

- Shahzad F: Conducted research, collected and analyzed data
Asghar HN: Planned and supervised research
Mushtaq Z: Helped in execution of research
Hadayat A: Collected and analyzed data
Zuhra N: Collected and analyzed data
Ahmad R: Analyzed data
Ali MA: Provided host soil of arbuscular endomycorrhizae fungi and helped in data collection

