Application of *Glomus sp.* and *Pseudomonas diminuta* Reduce the Use of Chemical Fertilizers in Production of Potato Grown on Different Soil Types

A Nurbaity¹,², E T Sofyan¹ and J S Hamdani¹

¹Faculty of Agriculture, Universitas Padjadjaran, Jl. Raya Bandung Sumedang km.21 West Java, 45363, Indonesia

E-mail: a.nurbaity@unpad.ac.id

Abstract. The use of high chemical fertilizer rates in potato production has been applied on the farm in Indonesia. Application of biofertilizer consists of arbuscular mycorrhizal fungi has been tested to reduce the use of NPK rates in production of potato and to determine whether different soil types will have different response to this biofertilizer. A greenhouse experiment was conducted using mixtures of spores of *Glomus* sp. and inoculant of mycorrhizal helper bacteria *Pseudomonas diminuta*, applied at different rates of NPK fertilizer (0, 25, 50, 75 and 100% of recommended rates) and different soil types (Andisols and Inceptisols). Results of experiment showed that application of *Glomus sp.* and *P. diminuta* reduced the use of NPK up to 50%, where the growth (plant height and tuber number), N,P,K uptake and tuber yields of potato had similar effect to the highest recommendation rate of NPK fertilizer. Inceptisol s in general had better response to the biofertiliser compared to Andisols. Findings from this experiment confirmed the evidences that biofertilizer could reduce the use of chemical fertilizer, and the widely distributed soil in Indonesia such as Inceptisols, is potential to be used as a medium for potato production.

1. Introduction

Potato is one of the most important food plants after rice, hence, some strategies need to be applied to improve the production of potato yield. One of the growing problems in potato production is high demands for fertilizer inputs, as its N, P and K requirements are higher than other vegetable crops [2]. The chemical practice used in intensive agriculture, has brought attention to alternative methods such as applying beneficial microbes for the establishment of a sustainable potatoes cultivation system [12].

Arbuscular mycorrhizal fungus is one of the beneficial microbes that play a vital role in maintaining soil and plant health [7, 11]. Some bacterias, known as Mycorrhizal Helper Bacteria (MHB), can directly stimulate mycorrhizal fungal activities. Enhancement of productivity of potatoes due to application of AMF [16] and MHB has also been reported [6]. Increased plant growth in the presence of mycorrhizal infection has been attributed mainly to the enhanced uptake of P. Previous study has shown that AMF occurred in potato production area in Andisols West Java Indonesia, and several AM fungi isolated from this area were effective as microbial inoculants for potato production system [12].

Andisols in highland areas is the most common soil type used for potato plantation in Indonesia. However, it has important characteristics due to its high capacity to immobilize phosphorus [13] and unstable particle sizes [15]. High rates of agro-chemical application are common to Andisols [13].

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Furthermore, due to the limited high land areas for the potato plantation, it is now essential to find other areas such as medium land-Inceptisols. Inceptisols is widely distributed across the nation, covers about 37.5% of land in Indonesia that need to be maximized for agriculture production. Nevertheless, Inceptisols is known to have poor fertility especially the availability of phosphorus [15], hence improvement of the soil condition is vital prior to the cultivation of potato. Improvement in the productivity of Inceptisols will have significant contribution to the improvement of agricultural productivity in Indonesia.

Production of mycorrhizal propagules, as conducted in this experiment, permits the inoculation of these organisms in plants growing in soils where AMF inoculum levels are reduced [14]. Different type of soils will have different responses to the AMF inoculum and soil type influenced spore density as well as the percentage of mycorrhizal colonization of roots during cultivation period [9].

The purpose of this experiment was to determine the beneficial effects of AMF mixed with MHB on soil nutrients availability, nutrients uptake, growth and yield of potato grown in different soil types at different NPK levels. It was expected that application of this biofertilizer could reduce the use of NPK rates in the production of potato crops cultivated on different soil types.

2. Materials and methods
The experiment was conducted at a greenhouse of Assessment Institute for Agricultural Technology (AIAT) West Java, Indonesia (1.312 m above sea level). Two types of soil, Andisols from Lembang and Inceptisols from Jatinangor, were collected from 0-20 cm depth, air-dried and used as growing medium. The basic properties of these soils are presented in Table 1.

| Table 1. Properties of the soils used in experiment. |
|---------------------------------|-----------|-----------|
| Andisols                        | Inceptisols|
| pH H₂O                          | 6.3       | 6.0       |
| Organic Carbon (%)              | 2.7       | 2.6       |
| Total N (%)                     | 0.5       | 0.4       |
| Available P (ppm)               | 4.3       | 2.5       |
| Available K (mg 100 g⁻¹)        | 18.7      | 18.1      |
| CEC (cmol.kg⁻¹)                 | 51.5      | 25.4      |
| Base Saturation                 | 24.4      | 65.1      |
| Sand (%)                        | 22        | 6         |
| Silt (%)                        | 64        | 37        |
| Clay (%)                        | 14        | 57        |
| Soil Texture                    | Clay loam | Clay      |

Microbial inoculum consisted of AM Fungi and Mycorrhizal Helper Bacteria was used in this experiment. AM fungus species *Glomus sp.* in this study was isolated from potato plantation area in a farm in West Java Indonesia [12]. The spores were mass produced using sorghum as trap plant grown on sterilised zeolite as substrate for 3 months in 20 cm (diameter) x 10 cm (height) pot in a greenhouse. The mycorrhizal inoculum consisted of soil, spores (an average of 50 spores per g), mycelium and infected root fragments [8]. Ten gram of inoculum was incorporated in each pot. The MHB used was *Pseudomonas diminuta* [6]. Twenty mL of microbial suspension with the density of 10⁷ colony forming units mL⁻¹ was mixed with AMF inoculum in each pot at the day of planting.

Certified potato seed-tubers of Atlantic cultivars from Potato Center in Pangalengan West Java were placed after microbial inoculants covered with a layer of soil in the pots containing 10 kg of soil according to the treatment (Andisols or Inceptisols at different rates of NPK). Prior to sowing, the soils were steam sterilized at 121°C to kill indigenous microbes. Seedlings were grown under greenhouse condition with natural light interception, and watered every day to maintain 80 % of water holding capacity.

A factorial design was used with two factors: 1. Combination of fertilizer (FMA + MHB inoculum) combined with NPK fertilizers at 0, 25%, 50%, 75% and 100% recommended rate (0, 250, 500, 750,
100 kg ha⁻¹), and 2. Two type of soils (Andisols and Inceptisols). The experiment, laid out in randomized block design with three replications.

Plants were evaluated for plant height every week, number of total tuber and tuber weight at 12 weeks after planting (WAP). To quantify AMF infection at the vegetative phase (7 WAP), roots were cut into 1-cm lengths and were stored in lactic acid glycerol until further processing by clearing and staining. Percent infection was assessed using the gridline intersect method [3]. Nutrients concentrations were analyzed in soil and plant shoot at maximum vegetative phase (7 WAP) using Kjeldahl apparatus for N, while phosphorus and potassium were determined by acetic acid extraction and measured with spectrophotometer for P and flame photometer K. Chlorophyll content measured by CCM 200 plus (Opti-science.inc®). All data collected were then analyzed by two-way ANOVA using SPSS software (version 12). Differences at the 5% level of significance were tested using Fisher’s Least Significance Difference (LSD) Test.

3. Results
There was a significant difference on root length colonized by AMF when grown on different soil types. However, no significant differences between fertilizer and soil type treatments on the percentage of mycorrhizal colonization on root of potato (Table 2). Mycorrhizal root length colonized of potato grown on Inceptisols was higher than those grown on Andisols.

| Treatments | Mycorrhizal Colonization (%) | Root Length Colonised (m) |
|------------|-----------------------------|---------------------------|
| NPK levels (kg ha⁻¹) |                           |                           |
| 0          | 42                          | 28                        |
| 250        | 51                          | 33                        |
| 500        | 60                          | 40                        |
| 750        | 46                          | 58                        |
| 1000       | 47                          | 41                        |
| LSD 0.5    | 17                          | 26                        |
| Soil Type  |                             |                           |
| Andisols   | 47                          | 28 a                      |
| Inceptisols| 52                          | 52 b                      |
| LSD 0.5    | 11                          | 16                        |

Measurements on soil parameters showed that there were significant differences between NPK levels and type of soils on available P and exchangeable K, but not to pH and total nitrogen (Table 3). There was a notable increased in soil P availability on Inceptisols compared to Andisols. Inceptisols contain clay more than Andisols.

| Treatments | pH | Total N (%) | Available-P (ppm) | Exchangeable-K (cmol/kg) |
|------------|----|-------------|-------------------|--------------------------|
| NPK levels (kg ha⁻¹) |    |             |                   |                          |
| 0          | 6.4| 0.41        | 60.96             | 2.50 a                   |
Increase shoot nutrients content and chlorophyll content index were observed with application of NPK fertiliser. No differences found in shoot nutrient as the effect of two soil types, but the CCI was higher in plant grown on Inceptisols (Table 4). There was positive correlation between content of N in shoot and CCI in this experiment ($r^2 = 0.741$) (data not shown).

**Table 4.** Nutrient uptake in plants (N, P, K) and chlorophyll content index (CCI) as the effect of different rates of NPK and soil types.

| Treatments | N (%) | P (%) | K (%) | CCI   |
|------------|-------|-------|-------|-------|
| **NPK levels (kg ha$^{-1}$)** |       |       |       |       |
| 0          | 3.79  | 0.35  | 2.30  | 18.20 |
| 250        | 4.00  | 0.39  | 2.62  | 19.90 |
| 500        | 3.99  | 0.44  | 2.54  | 20.90 |
| 750        | 4.24  | 0.47  | 3.17  | 22.10 |
| 1000       | 4.18  | 0.41  | 3.02  | 22.60 |
| **LSD 0.5** | 0.39  | 0.09  | 0.96  | 4.29  |
| **Soil Type** |      |       |       |       |
| Andisols   | 4.05  | 0.43  | 2.95  | 20.50 |
| Inceptisols| 4.03  | 0.40  | 2.50  | 23.80 |
| **LSD 0.5** | 0.25  | 0.05  | 0.61  | 2.71  |

Plant height, number of tuber and tuber yield were significantly higher in Inceptisols than Andisols (Table 5). No significant difference was observed on tuber weight per plant between NPK applications. This indicates that plants inoculated with mycorrhizal received benefit of its occurrence which results in similar yield of potato compared to the 100% of recommended rate of NPK.

**Table 5.** Growth and yield of potato as the effect different rates of NPK and soil types.

| Treatments | Plant Height (cm) | Number of tuber per plant | Tuber weight per plant (g) |
|------------|-------------------|---------------------------|---------------------------|
| **NPK levels (kg ha$^{-1}$)** |       |                   |                        |
| 0          | 62.5              | 7.5                       | 74.0                      |
| 250        | 56.6              | 8.8                       | 72.2                      |
| 500        | 59.0              | 6.7                       | 69.2                      |
| 750        | 58.7              | 8.0                       | 76.2                      |
| 1000       | 65.3              | 8.3                       | 93.2                      |
| **LSD 0.5** | 13.2              | 1.6                       | 42.0                      |
| **Soil Type** |      |                   |                        |
| Andisols   | 53.8 a            | 6.5 a                     | 55.0 a                    |
4. Discussion

In general, finding from this experiment showed that potato crops grown on soil inoculated with biofertiliser consisted of AM fungi (Glomus sp.) and mycorrhizal helper bacteria (Pseudomonas diminuta) combined with reduced NPK levels (500-750 kg ha\(^{-1}\)) gave similar response to the crops grown with NPK recommendation rate (1000 kg ha\(^{-1}\)). Interestingly results also found that different soil types (Andisols and Inceptisols) gave significant different responses on potato growth. Plants grown on Inceptisols which known have lower fertility levels gave better response compared to Andisols when biofertiliser applied.

In term of mycorrhizal parameters, difference responses between mycorrhizal colonisation and root length colonised in different soil types (Andisols and Inceptisols) were found. This could be due to the different fertility levels and textures of both soils. Soil texture was one of the most important factors influencing colonization percentage in several plants such as maize, sorghum and peanuts by AMF. Soils with low fertility limit plant development and increase the dependence of plants on mycorrhizal association [4].

Inceptisols as used in this experiment, had more clay than Andisols (Table 1). Although clayey soils are rarely represent the best growth medium for plants, high level of clay can increase cationic exchange capacity, and soil pH. High acidity enhances the potential for cationic percolation, which will immobilize phosphate. The lower the soil pH, the higher the amount of phosphate fixed in the soil, and therefore the lower its availability to the plant [5].

Nutrient uptakes, growth and yield of potato in this experiment were also showed similar response when grown at different levels of NPK (lowest to the highest). Several experiments carried out in P-deficient soils (such as Inceptisols) have shown improved nitrogen-fixation on inoculation of the plants with mycorrhiza [1]. Other works also revealed the increasing of shoot nutrient contents of mycorrhizal plants, ie. mineral nutrient such as potassium is also assimilated more quickly and in greater amounts by mycorrhizal plants [10]. Andisols was generally has higher soil fertility level compared to Inceptisols. The decrease of plant growth and yield in Andisols is probably related to high soil fertility levels, which reduced the dependence of the plants on mycorrhizae and restricted the development of these fungi to their root cortex [14]. Furthermore, this experiment showed that low fertility soils such as Inceptisols, could be more productive when biofertiliser (Glomus sp and Pseudomonas diminuta) applied and gave similar potential yield with Andisols and high level of chemical fertiliser (NPK) which is now widely used for potato production in Indonesia.

5. Conclusion

Application of Glomus sp. and Pseudomonas sp. reduced the use of NPK up to 50%, where the growth, nutrients uptake (N,P,K), and tubers of potato grown on different soil types had similar effect to the highest recommendation rate of NPK fertilizer that being applied by local farmers. Findings from this experiment confirmed the evidences that application of AMF and MHB could reduce the use of chemical fertilizer which support sustainable farming system.

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