Evaluation of inoculum arbuscular mycorrhizal fungi in Brachiaria decumbens

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Abstract. Arbuscular Mycorrhizal Fungi (AMF) can help plants to uptake nutrients, especially phosphorus, so that plants will produce sustainably. The study aimed to evaluate the inoculum of AMF obtained by hydroponic techniques, such as spray, drip, and NFT (Nutrient Film Techniques), with different nutrients Hyponex red and AB mix on Brachiaria decumbens grass. The experimental design used randomized complete design with 7 treatments namely control, SP1 (Spray/Hyponex red), SP2 (Spray/AB mix), NP1 (NFT/Hyponex red), NP2 (NFT/AB mix), DP1 (Drip/Hyponex Red), DP2 (Drip/ AB mix). Variables measured were production of shoot dry matter; content, and uptake of phosphorus, nitrogen, and crude protein. The results showed that AMF inoculum significantly (p<0.05) increased shoot dry matter production, phosphor, nitrogen, and protein content when compared with control. Phosphorus uptake in SP2 gave the best results but not significantly different from NP1, NP2, and DP1, but significantly different (p<0.05) with SP1, DP2, and control. The AMF inoculum significantly (p<0.5) increased nitrogen and protein uptake when compared to control. The conclusion that AMF inoculum increased shoot dry matter, phosphor, nitrogen, and protein content, and uptake of phosphorus, nitrogen, and protein. The inoculum that has been produced can be used by the community to increase productivity.

1. Introduction

The development of forage on sub-optimal land has an extensive opportunity when the availability of land decreases. Indonesia has quite a lot of suboptimal land, including acid land, dry land, saline land, peatland, and post-mining land. Indonesia owns a marginal land area of 149.5 million ha [1], which has various constraints in terms of utilization, namely high P fixation, reduce the solubility of P and Mo, reduce the concentration of elements N, Mg, Ca and K, increasing the engagement of Al, Mn, and Fe, inhibits root growth and absorption of water, increases the leaching of nutrients [2] [3]. Suboptimal land has the root problem in achieving sustainable crop productivity. The existence of acidic land in Indonesia is relatively high, covering 30% or 0.51 million km² of the land area of Indonesia, which is spread over the regions of West Java, Sumatra, Kalimantan, Sulawesi, and Irian Jaya. Arbuscular Mycorrhizal Fungi (AMF) are soil borne fungi that can improve plant to increase the nutrient uptake, and resistance for abiotic stress. The use of biological fertilizers such as AMF is an alternative for solving problems in sub-optimal land. AMF can help plants to supply, and absorb P elements, that are low in availability on acid soils because of the ability of AMF to adapt in acidic soils [4]. The power of AMF to improve plant nutrient status at this time can be used as an alternative strategy to replace some of the fertilizer required by plants grown on sub-optimal land. The use of AMF has been proven to help plants grow in suboptimal soil conditions such as acid land, dry land, saline land, and degraded land so that it can increase crop production and quality through the various roles of AMF. AMF have been
promoted as a natural tool to maintain and promote sustainable agriculture due to their role as natural biofertilizers shown to increase the content of N, P, K, and Ca and Mg plants [5][6][7], increasing fresh matter production, dry matter production, root biomass of Arachis glabrata, Centrosema pubescens, and Pueraria javanica [8], increasing the drought resistance of Desmodium sp in the early growth phase and Macropthilium scabra [9][10], increasing the growth and production of Setaria splendida grass [11], increased canopy and root dry weight of B. brizantha, B. decumbens, B. humidicola and Panicum maximum, as well as showing high dependence on AMF on dry matter production and uptake P on acid soils [12] and as bio-protectants against fungal, bacterial, and nematode pathogens [13][14][15].

Increasing the capacity of marginal land for forage sources can be realized with the availability of AMF biological fertilizer and can support forage productivity. The AMF availability as natural fertilizer requires large scale production support. AMF production technology has developed since the known role of AMF for plants. In general, there are three categories for AMF production techniques, namely (1) classical production systems using soil or sand media, (2) culture systems without substrates such as hydroponics and aeroponics with the AMF inoculum production of a relatively clean and (3) the in vitro culture system based on cut roots or called root organ culture (Root Organ Culture / ROC). Plant growth and AMF are supported by fertigation techniques is a combination of watering and fertilizing. The application of this technique contributes to achieving better results and quality with increased efficiency of fertilizer use [16]. Several advantages of fertigation compared to conventional fertilization, namely the flexibility and easier management, cost-effectiveness, the potential for increased uniformity distribution and efficiency of fertilizer use, less fertilizer loss due to a decrease in osmotic pressure, and the possibility of sharing of use fertilizers during the growing season [17]. The AMF inoculum production requires an extensive quantity production system with good quality. Using the proper technique, AMF inoculum production is required utilizing technology, environmental factors, and more effective management. This research aimed to evaluate the AMF inoculum using a hydroponic system and fertigation with appropriate nutrient solutions on Brachiaria decumbens grass. B decumbens is one type of pasture grass that is resistant to acid soils and an important introduced forage grasses [12].

2. Materials and methods

2.1. Materials
This research was conducted in Laboratory Agrostology. Division of Forage and Pasture Science and Technology, Department of Nutrition Science and Feed Technology, Faculty of Animal Science, Bogor Agricultural University. The materials used in this study were B. decumbens var. Mulato pols, the AMF inoculum, was obtained from the Laboratory Agrostology, zeolite, and soil latosol as a medium, Hyponex Red and AB Mix nutritional solutions, distilled water, trypan blue, KOH, HCl, and glycerol. Equipment used in this research were autoclave, digital scale, microscope, stratified filter (1000 μm, 250 μm, 125 μm, 45 μm), plastic pot, spray fertigation, drip fertigation and NFT (Nutrient Film Techniques) fertigation system units.

2.2. Methods

2.2.1. Inoculum preparation
The inoculum used comes from the production of inoculum starter with hydroponic treatment such as spray (S), NFT (N), and drip (D) with two different types of fertilizers, namely Hyponex red (P1), and AB mix (P2). The inoculum used consisted of 5 types of AMF such as Glomus manihotis, Glomus etunicatum, Glomus sp, Gigaspora margarita, and Acaulospora tuberculata.

2.2.2. Land preparation, planting, maintenance and harvesting
The AMF inoculum of each treatment was tested the effectiveness is using the Brachiaria decumbens var Mulato grass. The pols cuttings were used as planting materials and consisted of 2 pols/pot. B. decumbens var Mulato was planted in latosol soil media 5 kg/pot and added with AMF inoculum as much as 20 g/pot. Maintenance is carried out by watering using well water 500 ml/ pot. Plant growth was observed for 12 weeks after planting (MST). B. decumbens var Mulato is harvested by chopping
the shoot and oven-dried at 60 °C for 2 days for obtaining shoot dry weight and then analyzing the content of phosphor, Nitrogen and protein from the shoot.

2.3. Experimental design and data analysis
The experimental design used in this research was a completely randomized design with seven treatments and ten replications for shoot dry matter production, and three replications for Nitrogen, Phosphor, and protein content. The treatments used in this research, namely:
- Control = no inoculant
- SP1 = inoculant from spray fertigation + Hyponex Red nutrient solution
- SP2 = inoculant from spray fertigation + AB Mix nutrient solution
- NP1 = inoculant from NFT fertigation + Hyponex Red nutrient solution
- NP2 = inoculant from NFT fertigation + AB Mix nutrient solution
- DP1 = inoculant from drip fertigation + Hyponex Red nutrient solution
- DP2 = inoculant from drip fertigation + AB Mix nutrient solution

Analysis of variance (ANOVA) was used to analyze the data and the Duncan Multiple Range Test was used to analyze the significant data.

2.4. Parameters observations
Parameters measurement and observations consist of shoot dry biomass production, nitrogen, phosphorus, and crude protein content, as well as nitrogen, phosphorus, and crude protein uptake. Production of dry shoot biomass was taken by cutting the shoot between the roots and stems about 1-2 cm from the soil surface, then the shoot obtained was dried in the sun for two days and then in an oven with a temperature 60 °C for two days. Nitrogen levels were analyzed using the Kjeldahl method (AOAC, 2005). Phosphorus levels were analyzed using micro-colorimetric methods. Uptake of Nitrogen and phosphorus was calculated by multiplying the nitrogen and phosphorus content with the dry shoot biomass production. Production of Crude protein was calculated by multiplying the crude protein content with the dry shoot biomass production

3. Results and discussion
The results of the Evaluation of AMF inoculum on shoot dry matter, P, N, and Protein content can be seen in Table 1. The results showed a significant increase (p <0.05) in dry shoot matter, levels of phosphorus, nitrogen, and protein. The highest increase of dry shoot matter was 138% in DP1, but not significantly different from SP1 (116%), SP2 (106%), NP1 (126%), NP2 (126%), and DP2 (70%). Inoculation with AMF affected on the concentration of P, the highest concentration of P was observed in SP2 with an increased value of 16.8%, but not significantly different from control. The highest nitrogen content was in SP1 treatment with an increase of 40.54% when compared with control, but not significantly different when compared with SP2 (30.63%), NP1 (15.32%), NP2 (18.82%), DP1 (24.32%), and DP2 (34.32%). The highest protein content was SP1 with increasing about 41.10 % when
compared with control, but not significantly different from SP2 (31.55%), NP1 (16.06%), NP2 (19.10%), DP1 (25.18%), and DP2 (34.59%). The evaluation of AMF inoculum on spore number, P, N and protein uptake can be shown in Table 2. The highest spore number in SP2. The best P absorption was SP2 with increasing about 188%, and showed a significant difference with DP2 (92%), SP1 (83%), and control, but did not show a significant difference with NP1 (115%), NP2 (146%) and DP1 (158%). The highest nitrogen uptake was SP1 with increasing about 314% when compared with the control, but not significantly different when compared to SP2 (247%), NP1 (238%), NP2 (295%), DP1 (312%), and DP2 (198%). The highest protein uptake was SP1, with increasing about 314% when compared with control, but not significantly different from SP2 (248%), NP1 (239%), NP2 (296%), DP1 (313%), and DP2 (199%).

Table 2. Evaluation of arbuscular mycorrhizal fungi inoculum on spore number, P, N, and protein uptake

| Treatment | Spora Number of Inoculum (50 g) | Uptake P (g/pot) | Uptake N (g/pot) | Uptake Protein (g/pot) |
|-----------|---------------------------------|------------------|------------------|------------------------|
| Control   | 0.0156 ± 0.006 e                 | 0.333±0.035 b    | 2.077 ± 0.22 b   |
| SP1       | 2047.90 ± 1106.23                | 0.0286±0.005 b   | 1.380±0.041 a    | 8.607 ± 0.26 a         |
| SP2       | 2687.00 ± 1237.66                | 0.0450±0.005 a   | 1.157±0.065 a    | 7.220 ± 0.41 a         |
| NP1       | 2023.30 ± 833.40                 | 0.0336±0.003 ab  | 1.126±0.235 a    | 7.047 ± 1.47 a         |
| NP2       | 1783.00 ± 518.04                 | 0.0383±0.003 ab  | 1.316±0.184 a    | 8.223 ± 1.15 a         |
| DP1       | 1487.40 ± 553.19                 | 0.0403±0.015 ab  | 1.373±0.456 a    | 8.587 ± 2.85 a         |
| DP2       | 2067.40 ± 717.37                 | 0.0300±0.003 b   | 0.993±0.157 a    | 6.207 ± 0.98 a         |

Notes:  S = Spray  N = NFT  D = Drip  P1 = Hyponex red  P2 = AB mix

Accumulation of dry matter can be caused by inoculation of the AMF. The AMF was inoculated in plants that can increase atmospheric carbon fixation by increasing the sink effect and movement of photo assimilation from the part to the roots. The increase in dry shoot weight in this study was higher than Karti’s research about 34 % [5], still not similarly inoculation of commercial mycorrhizal products did not significantly increase the maize shoot dry matter compared with control, because of a high level of P deficiency[18]. The increase in N and P in panicle can be influenced by AMF with increasing transfer shoot biomass under the lower of fertilizer [19]. The increase in shoot dry matter production can be due to an increase in phosphorus, nitrogen, and protein content and uptake [5]. AMF inoculation can improve growth, and development with increased photosynthetic activities and others leave functions with uptake of N, P, and C. AMF-inoculated soil forms significantly higher extra-radical hyphae than do the non-AMF-treated soils [20]. Nutrient uptake, plant growth and development can be improved due to the external hypha of AMF [21]. Plants inoculated with AMF will increase their plant nutrition because the availability and transport of nutrients increases [22]. Mineral essentials such as N, P, K, Ca, Zn, S can be transported to the host plant by AMF symbiosis. The symbiotic of AMF with plants can improve phosphorus and nitrogen acquisition under limiting conditions [23]. The inadequate nutrition in deficient nutrient soil can be absorbed by AMF and transport to the host plant [24]. The increasing of immobile nutrient uptake can be done by AMF especially phosphorus. AMF can uptake and transport mineral nutrients in almost all plants [25]. The maize plant was inoculated with AMF can increase the rate of Pi uptake [26]. The increasing of Pi uptake mechanism in plants by AMF inoculated is P movement faster into mycorrhizal hyphae; the solubilization of soil phosphorus is achieved by increasing the P ions affinity; by decreasing of concentration required for P absorption; by the release of organic acids and phosphatase enzymes and by increasing the surface area for absorption nutrients [27]. Nitrogen is primary source of soil nutrition and is required for plant growth and development. Nitrogen is required for premier growth and as a limiting factor in higher plants. Another research has explained that AMF can absorb and transfer N to host plants, and translocation of N into seeds is enhanced from heading to maturity [28]. The symbiotic of AMF increased the percentage of N derived from the atmosphere in the total N biomass of faba bean grown in the mixture but not in pure stand, and AMF can increase N transfer about 20% [29] was lower than in this research about 40 %. The number
of spores did not show a significantly differ from all fertigation treatments and differences in fertilizers. The highest spore count was the SP2. The high number of spores will cause high colonization of AMF to increase the P, N and protein content [5]. Plants that are symbiotic with AMF will increase levels of phosphorus, nitrogen, and protein levels and will also increase the uptake of phosphorus, nitrogen, and crude protein so that it will increase the yield of photosynthetic in the photosynthetic process, and the result increase biomass production from plants [5]. Plants that are symbiotic with AMF will increase macro and micro nutrients plant so that it will increase plant biomass production.

4. Conclusion
The conclusions of this research that AMF inoculum increased dry shoot matter production, phosphor, nitrogen, and protein content, and uptake of phosphorus, nitrogen and protein in Brachiaria decumbens

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