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Research article

Influence of phosphorus nutrition on growth and metabolism of Duo grass (*Duo festulolium*)

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ABSTRACT

Use of suitable plants that can extract and concentrate excess P from contaminated soil serves as an attractive method of phytoremediation. Plants vary in their potential to assimilate different organic and inorganic P-substrates. In this study, the response of Duo grass (*Duo festulolium*) to variable rates of soil-applied potassium dihydrogen phosphate (KH_2PO_4) on biomass yield and P uptake were studied. Duo grown for 5 weeks in soil with 2.5, 5 and 7.5 g KH_2PO_4 kg^{-1} soil showed a significantly higher biomass and shoot P content of 8.3, 11.4 and 12.3 g P kg^{-1} dry weight respectively compared to plants that received no soil added P. Also, the ability of Duo to metabolize different forms of P-substrates was determined by growing them in sterile Hoagland's agar media with different organic and inorganic P-substrates, viz. KH_2PO_4, glucose-1-phosphate (G1P), inositiol hexaphosphate (IHP), adenosine triphosphate (ATP) and adenosine monophosphate (AMP) for 2 weeks. Plants on agar media with different P-substrates also showed enhanced biomass yield and shoot P relative to no P control and the P uptake was in the order of ATP > KH_2PO_4 > G1P > IHP = AMP > no P control. The activities of both phytase (E.C.3.1.3.26) and acid phosphatases (E.C.3.1.3.2) were higher in all the P received plants than the control. Duo grass is capable of extracting P from the soil and also from the agar media and thus it can serve as possible candidate for phytoextraction of high P-soil.

1. Introduction

Phosphorus is an essential macronutrient that plays a pivotal role in energy transfer, metabolic regulation, and protein activation. Although P is abundant in many soils, it is one among the least available mineral nutrient to the plants, as a significant amount of total soil P (50–80%) exists in organic forms [7]. Inositol penta- and hexaphosphates (phytate) and their derivatives account for a major component of the soil organic P [3]. The availability of organic forms of soil P depends on the mineralization by phosphatase enzymes. To increase soil fertility and P availability to plants, large amounts of P-based fertilizers are applied to soils. Animal feeds are also comprised of phytate, which is the major form of P in plant seeds. Monogastric animals like swine, poultry and fishes are incapable of utilizing phytate. Thus, majority of undigested feed phytate is excreted in manure, which also contributes to redistribution of organic P to the soil. The excess soil P leads to P-enrichment of surface waters through leaching and run-offs [6,22]. As a limiting nutrient in aquatic environments, elevated P levels in aquatic systems may lead to eutrophication and other water quality issues [21].

Recently, use of crop plants to extract and remove excess soil P is emerging as an innovative tool of importance [18]. To decrease the risk of P-run-offs to surface waters, crop plants and forages that are capable of growing and concentrating excess P from soil without external P addition followed by the off-site removal of the above-ground plant parts is the most effective and sound management approach. Utilization of grasses for phytoremediation of P from animal manure impacted soils is already reported [8,9,15]. Newton et al. [14] reported that grasses outperformed broad leafed forages in dry matter yields and nutrient uptake on application of animal manure. Grasses vary in their potential for removal of P from contaminated soils. Many grasses such as tall fescue (*Festuca arundinacea* S.), Italian ryegrass (*Lolium multiflorum* L.) and orchard grass (*Dactylis glomerata* L.) have been used to remove P and N from waste water [1]. Vervoort et al. [27], reported that plant P and N

Abbreviations: AMP, adenosine monophosphate; APase, acid phosphatase; ATP, adenosine-5-triphosphate; $CaCl_2$, calcium chloride; EDTA, ethylene diamine tetra-acetic acid; G1P, glucose-1-phosphate; IHP, myo-inositol hexaphosphate; KH_2PO_4, potassium dihydrogen phosphate; K_2SO_4, potassium sulphate; MES, N-morpholino ethanesulfonic acid; NaOH, sodium hydroxide; P, Phosphorus; PGA, D-3-phosphoglyceric acid; *p*NPP, *p*-nitrophenyl phosphate; RNA, ribonucleic acid; TCA, trichloroacetic acid.

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uptake increased with the rates of animal manure application on blue grass (*Poa pratensis* L.) and tall fescue (*Festuca arundinacea* L.).

Annual ryegrass is the most widely used cool-season annual forage because of its ease of establishment. It is one of the most efficient in extracting P and also highly productive [5]. Enhanced accumulation of P by cultivars of annual ryegrass from P-enriched soil and hydroponic media has already been studied [18,19]. However, annual ryegrasses are not tolerant to freezing temperature and summer drought. Duo grass (*Duo festulolium*) is a hybrid grass resulting from the cross between Meadow Fescue (*Festuca pratensis* H.) and a ryegrass (*Lolium multiflorum* L. or *Lolium perenne* L.) (diploid or tetraploid). It is also very productive forage for grazing and hay production. Recently, *Festuloliums* are gaining popularity as they have enhanced winter hardiness than annual ryegrass and the summer slump associated with rye grass is not severe in *Festuloliums*. Moreover, the P uptake capacity of Duo grass is largely unknown.

In view of the above, the goal of the present study was to gain an insight into the capability of the Duo grass to metabolize P from various P-substrates in the growth media. The responses of the grass seedlings grown in media containing various P-substrates were compared in terms of growth, shoot P content and activities of phytase and acid phosphatase (APase) enzymes. The P uptake capacity of Duo grass grown in the presence of varying concentrations of added P in the soil in the form KH_2PO_4 was also studied.

2. Results

2.1. Growth and P uptake by Duo grass seedlings in KH_2PO_4-enriched soil

Duo grass seedlings were planted in soil amended with KH_2PO_4 at the rate of 2.5, 5 and 7.5 g kg^{-1} soil and grown for 5 weeks along with control that received no KH_2PO_4 treatment. Fig. 1 shows the growth response of the grass seedlings to different application rates. Significant differences in biomass ($P < 0.05$) were observed between control and the plants grown in KH_2PO_4-supplemented soil (Fig. 1). Highest fresh weight in grass seedlings was measured in plants that were grown in P-enriched soil containing 7.5 g KH_2PO_4. There is a gradual increase in biomass of seedlings with respect to an increase in the content of KH_2PO_4 in the soil. Grass seedlings grown in the presence of KH_2PO_4 in the soil also accumulated significantly higher amount of P in their tissues compared to the control plants (Fig. 2). The highest shoot P content of 12.3 g kg^{-1} shoot dry weight was noticed in plants grown in soil enriched with 7.5 g KH_2PO_4 kg^{-1} soil. Plants grown in the presence of 2.5 g

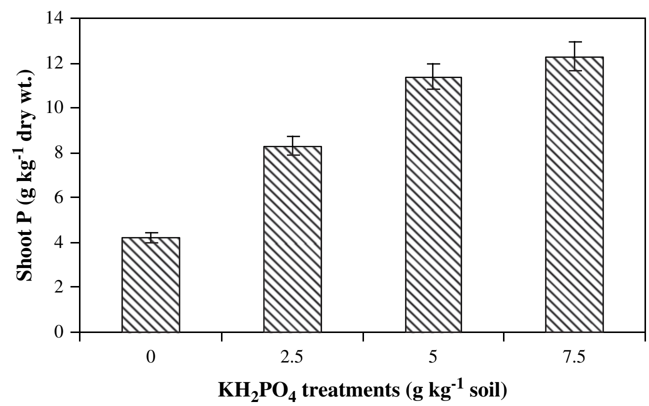

Fig. 2. P uptake in shoots of Duo grass grown for 5 weeks in soil treated with KH_2PO_4 (0–7.5 g kg^{-1} soil).

KH_2PO_4 showed 8.2 g shoot P kg^{-1} dry weight, while seedlings supplied with 5 g KH_2PO_4, showed a shoot P accumulation rate of 11.4 g kg^{-1} shoot dry weight. Control plants assimilated only 4.2 g P kg^{-1} dry weight in the shoot. Duo grass plants grown in soil amended with 2.5, 5 and 7.5 g KH_2PO_4 kg^{-1} soil showed P uptake of 0.82, 1.14 and 1.23% (dry weight) respectively.

2.2. Growth and P uptake by duo grass supplied with various P-substrates

Dry weights of Duo grass seedlings grown for 2 weeks in agar media supplemented with different organic and inorganic P-substrates (5 mM each) are shown in Fig. 3. Significantly highest shoot biomass was measured in plants grown in ATP-supplemented media followed by plants grown in KH_2PO_4. Seedlings grown in IHP and AMP also yielded comparably equivalent biomass, though lower than the above mentioned treatments. The lowest seedling dry weight for P-treatment was recorded for plants grown in G1P-fortified media compared to the other treatments. In general, grass seedlings subjected to different P-treatments significantly ($P < 0.05$) accumulated higher tissue biomass relative to the control plants that received no P (Fig. 3).

Fig. 4 depicts the pattern of P uptake and accumulation in the shoots of grass seedlings subjected to various P-treatments in the growth media. According to the data presented here, the highest shoot P content was noticed in grass seedlings grown in media supplemented with ATP as P-substrate. Duo grass plants grown in

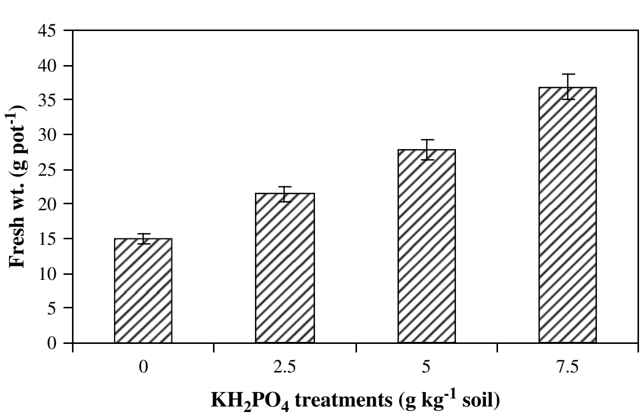

Fig. 1. Biomass yield of Duo grass grown for 5 weeks in soil treated with KH_2PO_4 (0–7.5 g kg^{-1} soil). Values represent mean of three replicates ± standard error of the mean.

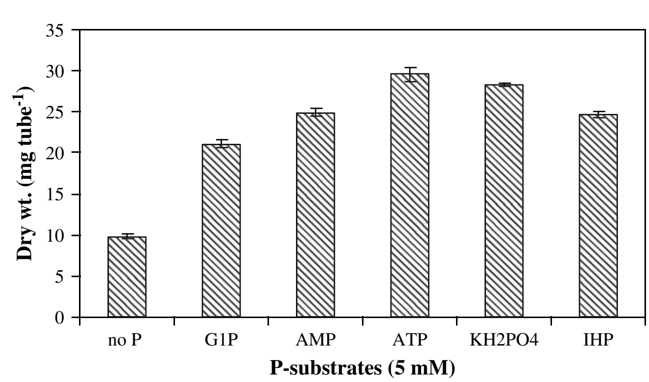

Fig. 3. Biomass yield in Duo grass grown for 2 weeks in Hoagland's agar media. The media contained either no added P or P-supplied as one of the following P-substrates (5 mM): glucose-1-phosphate (G1P), adenosine monophosphate (AMP), adenosine-5-triphosphate (ATP), potassium dihydrogen phosphate (KH_2PO_4) and inositol hexaphosphate (IHP).