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Elemental stoichiometry indicates predominant influence of potassium and phosphorus limitation on arbuscular mycorrhizal symbiosis in acidic soil at high altitude

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ABSTRACT

The functioning of high-altitude agro-ecosystems is constrained by the harsh environmental conditions, such as low temperatures, acidic soil, and low nutrient supply. It is therefore imperative to investigate the site-specific ecological stoichiometry with respect to AM symbiosis in order to maximize the arbuscular mycorrhizal (AM) benefits for the plants in such ecosystems. Here, we assess the elemental stoichiometry of four *Capsicum* genotypes grown on acidic soil at high altitude in Arunachal Pradesh, India. Further, we try to identify the predominant resource limitations influencing the symbioses of different *Capsicum* genotypes with the AM fungi. Foliar and soil elemental stoichiometric relations of *Capsicum* genotypes were evaluated with arbuscular mycorrhizal (AM) colonization and occurrence under field conditions. AM fungal diversity in rhizosphere, was estimated through PCR-DGGE profiling. Results demonstrated that the symbiotic interaction of various *Capsicum* genotypes with the AM fungi in acidic soil was not prominent in the study site as evident from the low range of root colonization (21–43.67%). In addition, despite the rich availability of carbon in plant leaves as well as in soil, the carbon-for-phosphorus trade between AMF and plants appeared to be limited. Our results provide strong evidences of predominant influence of the potassium-limitation, in addition to phosphorus-limitation, on AM symbiosis with *Capsicum* in acidic soil at high altitude. We also conclude that the potassium should be considered in addition to carbon, nitrogen, and phosphorus in further studies investigating the stoichiometric relationships with the AMF symbioses in high altitude agro-ecosystems.

1. Introduction

The increasing global population has put a great deal of pressure on cultivators to manage the agriculture systems more intensively in order to increase the food production. Consequently, intensified cropping systems have been replacing the subsistence-based conventional agriculture systems (Tiwari et al., 2008). In this context, the high-altitude agro-ecosystems require more attention because their functioning may be constrained by the harsh environmental conditions, such as low temperatures, acidic soil, and low nutrient supply (Robinson, 2002). Arbuscular mycorrhizal fungi (AMF), which are associated with more than 80% of the land plants and which occur globally in most terrestrial ecosystems (Treseder and Cross, 2006) were at one point believed to dominate only in ecosystems characterized by mineral soils with lower altitude (Read, 1991). The subsequent studies have reported that the AMF were dominant mycorrhizal forms at high altitudes as well (Jefwa et al., 2009; Liu et al., 2011; Li et al., 2014). The AMF may be an important driving force in improving the soil conditions in challenging high-altitude agriculture systems. However, it is imperative to collect nutrient dynamics data in such environments before AMF strategies could be applied successfully in high-altitude agro-ecosystems, as these dynamics are strongly intertwined with the AMF efficiency. The nutrient dynamics in a given ecosystem may be explained using the theory of ecological stoichiometry, an approach that seeks to understand the ecological dynamics in terms of the material balance of interacting organisms in the environment (Sterner and Elser, 2002). Although the plant growth requires more than two dozen elements and the majority of them can be supplied through AMF symbiosis, the most extensively studied relationships are those involving the carbon (C):nitrogen (N):phosphorus (P) ratio, because these three elements are tightly interlinked in their biochemical functioning.

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However, potassium (K), which is also an important element required in great quantities for plant growth, has received little attention with regard to its role in ecological stoichiometry. K is one of the most important inorganic solutes and has a crucial role in the processes such as osmoregulation, stomatal regulation, cell extension, solute transport in the xylem, and photosynthesis. A large number of studies unequivocally showed that the presence of AMF increases the uptake of K along with the other elements in plants (Cimen et al., 2010). Porras-Soriano et al. (2009) reported that the inoculation of olive plants with AMF increased the plant growth and the plants' abilities to acquire N, P, and K from non-saline as well as saline media. A higher K^+/Na^+ ratio is one of the determinants of plant salt tolerance (Thomas et al., 2003). Therefore, K should be included in studies of elemental stoichiometry.

Although the significance of relative proportions of elements required for the growth performance of plants has been highlighted by Liebig's law of the minimum (Liebig, 1843), the relative needs of plants for different elements are poorly quantified, and the dependencies between the elements are not well-understood (Ågren, 2008). Some studies have attempted to define the stoichiometric relationships and, have explored the general biogeographic patterns of optimal ratios in different ecosystems (Güsewell, 2004; Knecht and Göransson, 2004). However according to Ågren (2008), in spite of the well-defined stoichiometric relationships, observations of the stoichiometric relationships in actual plants and ecosystems indicate considerable variability. This proposition also finds support from a study conducted by Zhao et al. (2014), in which the average values of leaf N and P and the C:N:P ratios of 175 plant species on Changbai Mountain differed from those reported at a regional scale, whereas leaf C did not differ in this respect. Their study also showed that the N:P ratio was 19% lower on Changbai Mountain than that recorded earlier for Chinese flora (Zhang et al., 2012). Similarly, in European wetlands, the published data on plant species at multiple sites indicated that high N:P ratios are rare in naturally occurring species (Güsewell and Koerselman, 2002; Güsewell et al., 2005).

It has also been observed that during the second half of the 20th century, many aquatic ecosystems shifted from a phosphorus-limited state to a nitrogen-limited state because of anthropogenic P inputs (Fisher et al., 1995; Reddy et al., 1999). Moreover, leaf stoichiometry is understood to vary along altitudinal gradients, and, non-linear relationships between the leaf stoichiometric traits and altitude have been detected. For example, Fisher et al. (2013) reported that leaf N and P first increased and then decreased with increasing elevation in the Peruvian Andes. It is also postulated that site-specific element availability or limitation may be an important source of stoichiometric variability in natural ecosystems that does not affect overall plant growth performance. When elements are available, plants take up all elements in excess of their minimum requirements for growth, thereby increasing the variability in element ratios and masking the variability in the amount of elements required for plant metabolism (Ågren, 2008).

In view of the preceding discussion, it is apparent that investigating the site-specific ecological stoichiometric relationships with respect to AMF symbiosis is necessary in order to develop strategies for maximizing mycorrhizal benefits to plants. These relationships are more important in the high-altitude agriculture systems because the availability of soil nutrients is relatively poor. The data may help not only to elucidate the altitudinal patterns of elemental stoichiometry but also to model nutrient cycling in ecosystem. Therefore, the current study was conducted in order to assess the elemental stoichiometric relationships of four *Capsicum* genotypes grown on acidic soil at high altitude in Arunachal Pradesh, India. It was tried to identify the predominant resource limitations influencing the arbuscular mycorrhizal association with different *Capsicum* genotypes, using various statistical and graphical tools.

2. Materials and Methods

2.1. Field trials, growing conditions, and sampling

Field trials were conducted from May to August 2011 at Defence Research Laboratory (DRL) field station, Salari (27°34′ N and 92°40′ E) in the West Kameng district of Arunachal Pradesh. The topography of the area is mostly mountainous with three principal mountain chains belonging to the Sela range, Bomdila range, and Chaku range. The Bomdila range, in which Salari is located, has an average height of 2743.2 m above mean sea level (http://westkameng.nic.in/geography.asp; accessed 05.07.14.). The most part of West Kameng district has a moderately sloping terrain with the average annual rainfall ranging between 1900 and 2200 mm. It comes under the agro-climatic zone 'Thermic perhumidmidhills and valleys' (http://arunagri.com/climate.asp; accessed 07.07.14.).

The seeds of four *Capsicum* genotypes were procured from the local markets in Tezpur (bell pepper and chilli), Imphal (Naga chilli), and Dimapur (Naga chilli) and raised to seedlings in nursery at the study site. Naga chilli, also known as 'Bhut Jolokia' in Assam, is a highly pungent chilli species native to the northeastern region of India, which has tremendous ethnopharmacological significance (Meghvansi et al., 2010). The soil was fertilized with vermicompost at 2.5 t ha^{-1} before the seed sowing in nursery. The vermicompost used had available N, P and K, 1.5–2.00%, 0.8–1.04% and 0.9–1.50%, respectively. The two-week old seedlings were transplanted to the main experimental field. The field trial was conducted on a plot of 15 × 20 m. The experimental design was randomized block design with three replicates. Standard agronomic and cultural practices were followed to raise a good crop. Sampling was done on 5 August 2011. Rhizospheric soil samples were collected from 15 to 23 cm depth from 10 random spots in each block and pooled. The leaves and roots of 10 plants from each block were collected and pooled and the samples were brought to the laboratory for further processing.

2.2. Physico-chemical analysis of leaves and soil

The air-dried leaves were finely ground to powder (<0.5 mm particle size) using a grinder. The air-dried rhizospheric soil samples were sieved through a 2-mm mesh screen and were used for physico-chemical analysis. In order to measure the total amounts of P, K, Na, and Ca in leaves, 1 g of the powdered leaves was wet-digested in a digestion block (Kelplus KES12L; Pelican Equipments, Chennai, India) at 200 °C in tri-acid mixture (HNO_3:H_2SO_4:$HClO_4$; 10:1:4). The digested samples were then diluted using ddH_2O for a final volume of 100 ml and filtered for further elemental analysis. The total P in the leaves was estimated by the vanadomolybdophosphoric acid colorimetric method (Tandon, 1993) using a spectrophotometer (Specord 200; Analytik Jena, Germany). The total amounts of Ca, Na, and K in the leaves were determined by the ammonium acetate method of Hanway and Heidel (1952) using a flame photometer (FP114; Thermo Scientific, USA). The total amounts of N and C in the leaves and the soil were estimated using an elemental analyser (EA3000, Eurovector, Italy).

The pH of the soil (diluted-water; 1:5) was measured using a digital pH meter (PT10; Sartorius AG, Germany). The total amounts of P and K in the soil were determined using the same methods as previously described for plant leaves. Organic carbon (OC) in the soil was determined using 1 N potassium dichromate and back-titrated using 0.5 N ferrous ammonium sulphate solution, as suggested by Walkley and Black (1934). The available nitrogen (i.e., NH_4-N) in