DIFFERENTIAL EFFECTS OF SOIL MICROBES ON THE GROWTH AND PHOSPHORUS ACQUISITION OF TWO RICE CULTIVARS (AZUCENA AND IAC 25)

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ABSTRACT:

Phosphorus (P) is a finite resource and is a major limiting factor for rice yield on a large area of World's arable land. P is limited to plants because of its chemical fixation and low solubility leading to P deficiency in most soils. Soil microbial communities exert important control over soil processes and their functions are central to nutrient cycling that determines plant productivity. This study is aimed at understanding interactions between indigenous soil micro-organisms and rice cultivars (*Oryza sativa* L.) in P limited conditions. Two rice cultivars (Azucena and IAC 25) were grown in either live or sterile soil (5% live or sterile topsoil with 95% P-limited sterile subsoil, plus P free Yoshida's nutrient solution). Plant growth, P-uptake and P use efficiency (PUE) were assayed at harvest. Results revealed a highly significant (P<0.001) interaction on shoot dry weight (SDW) where Azucena grew better in sterile soil than its live topsoil counterpart. The opposite was observed for IAC 25. This effect was even more pronounced for root growth with the root system of sterile topsoil grown IAC 25 being reduced by more than 60%. This translated into an effect on root/shoot ratio where topsoil sterilisation (no inocula) had little effect on Azucena but in IAC 25 it reduced from 0.35 to 0.21 (P values for both treatment and cultivar effect were less than 0.001). Significant interaction between cultivar and treatment for shoot P concentration and P use efficiency (g shoot dry weight/mg P in shoot) were found. These results suggest that IAC 25 requires the presence of soil microbes to access soil-bound P under P-limiting condition while Azucena does not.

Keywords: *Oryza sativa*, Phosphorus acquisition, soil microbes, shoot dry weight

التأثيرات المختلفة لميكروبات التربة في نمو وامتصاص الفوسفور لصنفين من الرز (Azucena و IAC25)

المستخلص:

يعد الفوسفور عنصر غذائي مهم وعاملا محدداً كبيراً لانتاج محصول الرز على مساحة واسعة من الأراضي الصالحة للزراعة في العالم. ان جاهزية عنصر الفوسفور للنبات محدودة بسبب عمليات التثبيت الكيميائي له وقلة ذوبان هذا العنصر مما يؤدي إلى نقصه في معظم أنواع الترب. أن المجتمعات الميكروبية الموجودة في التربة لها دور مهم وفعال في السيطرة على التحولات الحيوية المختلفة في التربة وان دور تلك المجتمعات الميكروبية يعد اساساً في تدوير المغذيات الموجودة في التربة والتي تحدد إنتاجية النبات. تهدف هذه الدراسة إلى فهم التأثيرات المتداخلة بين الكائنات الدقيقة المستوطنة في التربة وصنفين من أصناف الرز (Azucena و IAC 25) في ظروف تربة محدودة الفسفور. تم زراعة هذين الصنفين في معاملتين الاولى تربة حية (5% تربة حية (بدون تعقيم) مأخوذة من الطبقة السطحية للحقل مع 95% تربة معقمة مأخوذة من الحقل بعمق دون 20سم) والمعاملة الثانية تربة ميتة (5% تربة معقمة مأخوذة من الطبقة السطحية للحقل مع 95% تربة معقمة مأخوذة من الحقل بعمق دون20سم). تم ارواء المعاملتين بمحلول يوشيدا المغذي. اما الصفات المدروسة التي تم قياسها عند الحصاد فهي نمو النبات وامتصاص الفسفور وكفاءة استخدام الفسفور(PUE) . كشفت النتائج عن وجود فرق معنوي عالي (P <0.001) في الوزن الجاف للنبات حيث ان الصنف Azucena نما أفضل في التربة المعقمة من نظيره المزروع في التربة الحية. ولوحظ العكس بالنسبة للصنف IAC 25 . كان هذا التأثير أكثر وضوحا بالنسبة لنمو الجذور حيث ان المجموع الجذري للصنف IAC 25 المزروع في التربة المعقمة كان منخفض بنسبة تزيد عن 60٪. وان هذا التأثير على الجذر انعكس على نسبة الجذر/المجموع الخضري حيث كان لتعقيم التربة (تربة بدون مكروبات) تأثير قليل على الصنف Azucena لكن بالنسبة للصنف IAC 25 فقد انخفضت نسبة الجذر/المجموع الخضري من 0.35 الى 0.21 (علماً ان تأثير كلا العاملين (الاصناف والمعاملات كان معنوي اي ان P <0.001). ان تأثير كلا العاملين الاصناف والمعاملات كان معنوياً على تركيز الفسفور في المجموع الخضري وكفاءة استخدام الفسفور(الوزن الجاف للمجموع الخضري بالغرام/وزن الفسفور في المجموع الخضري بالملغرام). وتشير هذه النتائج إلى أن الصنف IAC 25 في ظروف نقص الفسفور يحتاج الى وجود ميكروبات التربة للوصول إلى الفسفور الممسوك بالتربة في حين أن هذة الحالة لا تنطبق على الصنف Azucena.

* Part of PhD dissertation of first author.

Introduction

P deficiency is a major abiotic stress that limits crop productivity on 30 − 40% of the World's arable land (von Uexküll and Mutert, 1995). P is an immobile element and is readily bound with soil particles. In most soils, P availability is therefore suboptimal and inadequate for high yield production. P is also expensive and the majority of farmers especially in developing countries cannot afford the rising prices of P fertilizers. So P availability in soil is a matter of concern and invites research attention to find an alternative way for sustainable production and food security for the world's growing population. Interactions between soil micro-organisms and plants occur ubiquitously and have important effects on several biological processes. In terrestrial ecosystems, soil microorganisms are an important component due to their functions as decomposers, mutualists and pathogens. Hence, those interactions may be beneficial (e.g. symbiotic associations) or pathogenic in their nature. There are intermixed, complex interactions in the root-soil-microbe interface that shape the soil ecosystem. Interactions between plants and the belowground micro-organisms are influenced by various edaphic variables particularly soil pH that has a major influence on soil microbial activity and biogeography (Fierer and Jackson, 2006). Other factors such as soil type (Salles *et al*., 2004), moisture, aeration and many other soil abiotic factors in addition to the climatic conditions that prevail in a given region also can shape the function and structure of the soil microbial community and ultimately influence interaction processes in soil. In addition to this, plant species play a major role in the structure and function of soil microbial communities especially in the rhizosphere (Wieland *et al*., 2001). The latter, in turn, can also influence the density and distribution of plant species on terrestrial ecosystems (Bray *et al*., 2003). Naturally, there exists negative interaction as a result of active, deleterious soil-borne pathogens, which are often plant-specific and thereby can determine the survival and density of aboveground plant species. The beneficial effects of soil micro-organisms such as nitrogen fixing bacteria (*Rhizobium*), P solubilising bacteria, which have been used as biofertilizer since the 1950s (Kudashev, 1956) and mycorrhizal fungi (*Glomus etunicatum*) have been shown to enhance plant growth (Sieverding, 1991) through their positive interaction with plants by helping the plant to access sparingly available nutrients. These mutualistic bacteria and fungi interact with their hosts through acting as a biological control of pathogens and cycling of nutrients and thereby positively affecting plant health. The plant, in return, sustains their number and activity through the flow of photosynthetically fixed carbon to the surrounding soil and influences the soil environment in the rhizosphere. Plants take up most mineral nutrients through the rhizosphere where micro-organisms interact with plant root exudates (Felix and Donald, 2002). Root exudation of various chemical molecules into the rhizosphere is largely dependent on the nutritional status of the plant. In P limiting conditions, the released compounds can cause some nutrient elements to be relatively more available for uptake by the plants. The solubilisation of immobile P can be achieved by changing the pH in the rhizosphere through the excretion of organic acid anions. Similarly, root exudates can be increased by an effect of microbial activity in the rhizosphere, which leads ultimately to increase nutrient availability (Gardner *et al*., 1983). Therefore root exudation can also stimulate soil micro-organisms that could solubilise inorganic P (Felix and Donald, 2002). Richardson (2001) reported the important role of soil micro-organisms in soil P dynamics and subsequent availability of P to plants. It is conventional that culture-based techniques are often used for the identification and isolation of soil micro-organisms, but it is also understood that only a small proportion of soil micro-organisms (approximately <1%) can be cultured (Atlas and Bartha, 1998). This has made it difficult to investigate the role of each soil micro-organism in an artificial environment, so to study the effect of plant/soil micro-organism interactions on plant growth, it is logical and reasonable to investigate the outcome effect of the whole community of indigenous soil biota as an integrated bio system in untreated soil in order to represent the soil conditions in natural ecosystems in the field and to understand how beneficial micro-organisms within the whole soil microbial community in a given soil behave to affect the nutritional status of a certain plant. Two preliminary experiments have been conducted in Aberdeen by Anderson (2009).

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The first investigated how different rice cultivars grow under P limiting conditions and how they interact with soil. Four rice cultivars were grown in 95% Insch subsoil and 5% topsoil (as a source of inocula) amended with 0, 34 or 100 mg of P per kg soil as KH_2P0_4. Result indicated that there were significant cultivar differences in plant mass, total P concentration and P acquisition only in the 0 P-treatment. The results suggested that the system was P-deficient and thus this Insch subsoil is suitable to be used in our study. In the second experiment the subsoil was triple autoclaved and re-inoculated with 5% topsoil (live) or triple autoclaved (sterile) topsoil as a control. Results demonstrated a strong treatment effect on plant growth and most importantly there was a very strong cultivar x treatment interaction observed. This indicates that there was variation in P acquisition and strong cultivar-specific interactions with the rhizosphere microbial community. Furthermore, a study by Wissuwa (2001) indicated that there is a genotypic variation between rice cultivars in their ability to take up fixed P from the soil. From a plant breeding viewpoint, investigating the reasons behind the differences between cultivars in plant performance that are caused by the presence or absence of soil micro-organisms will further our understanding about key traits, which could be used in breeding programs for sustaining food production. Moreover, developing cultivars capable of performing well in low-P conditions will aid farmers in increasing rice production with less dependence on P-fertilizer. The symbiotic interactions between soil micro-organisms and rice cultivars and their effects on plant growth are largely unexplored. Soil micro-organisms play a key role in nutrient uptake and turnover. Through the use of sterile and non-sterile soil inocula, this study examines the ability of different rice cultivars to interact with soil microorganisms to access soil P. This research will therefore address the symbiotic relationship between some rice cultivars and soil indigenous microbes looking at how the presence of inocula in soil enhances the growth of rice in P deficient conditions. It is vital to use more efficient cultivars that could interact with soil micro-organisms to promote growth with a minimum dependence on chemical fertilizers for sustainable yield production. Therefore the objectives of study were:

1. To evaluate the response of rice cultivars to indigenous soil micro-organisms in acquiring/utilizing P in P-limiting conditions.
2. To investigate whether different rice cultivars react differently to the presence of inocula through understanding how plants and soil microbes interact to promote plant growth.

Materials and methods

Soil selection

Two Insch soils were chosen to be used in this study. The first soil utilized throughout was Insch subsoil, which was sourced from a cultivated field in northeast Scotland (Insch series, Inschfield Farm, Aberdeenshire, UK). This soil is characterized as a freely draining sandy loam with particle size distribution of 70% from 2 mm to 60 μm; 16% from 60 to 20 μm; 7% from 20 to 2 μm and 7% < 2 μm (MacMillan *et al*., 2006). This soil is also suitable for root studies as it displayed low-adhesion with root system during washing. The Insch subsoil had 0.81 mg g^{-1} total P on a dry weight basis, which indicates that it is deficient in P and a suitable growth medium for the purposes of this study. The second soil used in this experiment was the arable Insch topsoil also from Inschfield Farm, Aberdeenshire, UK. Samples for Insch topsoil were collected from (0 − 20 cm) depth to be used as a source of inocula.

Soil analysis

Physical-chemical characteristics of Insch subsoil and topsoil were measured and summarised in Table 1. Soil sterilization by autoclaving breaks down organic matter content and induces chemical and physical changes in soil properties (Eno and Popenoe, 1964). So using such autoclaved soils in experiments designed to test factors related to nutrient availability, it is necessary to determine soil available nutrients especially the macronutrients N, P and K in soil to evaluate the macronutrient status in soil and most importantly to ensure that soil being used as a growth medium is deficient in P. Therefore to characterise the soil that is being used, measurement of plant available N, P and K in both subsoil and topsoil before and after autoclaving have been conducted. Total P and N content in both subsoil and topsoil before and after autoclaving were determined as well; all data are shown in Table 2.

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Characterization of subsoil and topsoil

The soil pH was measured at a soil: distilled water ratio of 1:2.5 (w/v) using pH meter (HI8424 microcomputer pH meter, HANNA Instruments). The total P and N were determined by acid digest and the automated spectrophotometric flow injection analyser (FIA) (Allen *et al.*, 1974). The available P was measured by using acetic acid extraction as described by Allen (1989a) and available N was determined using the procedure described by Allen (1989b). Using an electric muffle furnace, organic matter content in the soil was estimated by loss on ignition at 550 ^{o}C. The method was modified from that described by Allen (1989c). Electrical conductivity (EC) was measured at a 1:5 soil: de-ionised distilled water suspension using microprocessor conductivity meter (PRIMO 5). Sterilizing the soil was done by triple autoclaving the soil at 115 kPa and 121 °C for at least 60 minutes. Results are demonstrated in Table 1.

Determination of plant available N ($NH4^{+}$, NO3 - and $NO2^{-}$) in soil

Plant available N (Ammonium – N, Nitrate – N and Nitrite – N) was determined using procedure described by Allen (1989b). 100 ml of 1.0 M KCl (74.56 g L^{-1} de-ionised water) was added to a 10 g sample, in four replicates, of each four fresh soils (subsoil and topsoil before and after autoclaving) and shaken for 30 minutes then allowed to settle. An aliquot of the clear supernatant was transferred and analysed using FIA (ISO/FDIS 15681). Results are shown in Table 2.

Determination of plant available P in soil

Available P in the soil was estimated by using acetic acid extraction as described by Allen (1989a). Five g, in four replicates, of each four air-dried soils were weighed into 250 ml conical flasks then 150 ml of extractant (2.5% v/v acetic acid) was added. The soil samples and blank flasks were shaken for two hours on a rotary shaker, and then allowed to settle overnight. The clear supernatant was then filtered through Whatman No. 40 paper into centrifuge tubes and the first 5 – 10 ml of filtrate was rejected. P was determined in the remaining filtrate of all samples. P concentration was measured by FIA. P content was calculated by multiplying soil dry weights with P concentrations. Results are demonstrated in Table 2.

Determination of plant available K in soil

To measure plant available K, 125 ml of 1.0 M ammonium acetate solution (77.08 g ammonium acetate salt in one litre de-ionised water) adjusted to pH 7.0 was added to a 5 g sample, in four replicates, of each four fresh soil and shaken for 1hr then allowed to settle. An aliquot of the clear supernatant was transferred and analysed using PerkinElmer AAS (atomic absorption spectrophotometer). Results are shown in Table 2.

Determination of total N and P in soil

To determine total N and P in soil, the procedure described by Allen (1989c) was used. Four and half ml of digest reagent (2.8 ml of concentrated sulphuric acid, 0.08 g of lithium sulphate and 2.33 ml hydrogen peroxide) was added to approximately 0.2 g of oven dried, finely ground sample of each soil, in four replicates, and heated to 360 °C for 2 hr to allow digestion. After this time 1 ml of hydrogen peroxide was again added and further digested for an hour. The diluted digest then underwent total N and P analysis using FIA. Results are demonstrated in Table 2.

Plant material

Seeds of two rice cultivars Azucena and IAC 25 were generated from seeds originally obtained from the International Rice Research Institute. These rice varieties, which belong to the cultivated species (*Oryza sativa* L.), were selected for the study to grow because they are known to be different in P acquisition and interact differently with rhizosphere microbial community in P limited conditions (Anderson, 2009). IAC 25 is known to be superior in P acquisition as identified by Wissuwa and Ae (2001). While Azucena is recognized to grow well in P limiting conditions (Chin *et al*., 2011). But Anderson (2009) suggested that Azucena and IAC 25 might be different in taking up P from the soil in which they are grown. Both of these cultivars are *tropical Japonicas* (Zhao *et al*., 2010). Azucena is from the Philippines, while IAC 25 has been bred in Brazil where soils are particularly P-deficient. These cultivars belong to upland rice (Wissuwa and Ae, 2001).

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Preparation of rice seeds for germination

Seed of rice cultivars were surface sterilised in 1% sodium hypochlorite for two minutes then washed under running tap water before being soaked in a beaker filled with tap water for 5 minutes. The seeds were placed on wet filter paper in a Petri dish, which was sealed with Para film (Pechiney Plastic Packaging, Chicago) then kept in an incubator at a temperature of 30 °C for two days.

Growing rice cultivars in the growth room

A half litre pot experiment was set up with two rice cultivars (Azucena and IAC 25) in the Cruickshank controlled growth room at the School of Biological Sciences, University of Aberdeen from January to February 2010 for 28 days. The growth room was supplied with two automatic vents for intake of fresh air and control temperature. The fresh supply of air was continuously circulated by two fans. The light in the room was supplied by fluorescent grow light. The experiment was performed using a randomised complete block design (RCBD) with four replications forming 16 pots in the experiment. Both subsoil and topsoil samples were air-dried and sieved on a 3 mm mesh. The soil was a mixture of 5% topsoil and 95% P-limited subsoil (MacMillan *et al*., 2006) with an initial pH of 5.0. All subsoil was triple autoclaved while the topsoil was either live or triple autoclaved forming two treatment levels. The topsoil and subsoil was thoroughly mixed for each treatment, which was 5% live (as a source of inocula) or sterile topsoil. About 650 g soil mix based on soil dry weight was distributed into each pot and the pots were brought to approximate field capacity using distilled water prior to seeding. The pots were placed on individual saucers to avoid any contamination to the sterile treatment pots. When the plumule of pre-germinated seeds was just visible (about one millimetre), two seeds of similar plumule length were transplanted into each pot containing the different soil treatment at the depth of 1.5 – 2.0 cm. To avoid air contamination, the pots were covered with cling film and when seedlings emerged a small hole was introduced to allow plant growth through, and the pots were thinned to one seedling. Rice plants were grown in controlled conditions under a 12 hr light regime with a light intensity of approximately 350 − 400 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR) with 25 ± 2 °C at night and 28 ± 2 °C in the day. Relative humidity was maintained between 55 and 70% throughout. Plants were fed with Yoshida's nutrient solution which lacked the $NaH_2PO_4.2H_2O$ fraction (YNS-P) (after Yoshida *et al*., 1976). Each plant received 1000 ml (YNS-P) to achieve a calculated optimal plant nitrogen requirement. Each pot was initially fed with 100 ml twice a week for the first two weeks and then three times a week for the last two weeks and soil moisture was maintained at approximately field capacity throughout the growing period using distilled water. Shoot growth was monitored on a weekly basis as height of the plant (length from the soil to the tip of the longest leaf). Plants were harvested after 28 days by removing the shoots. Root systems were recovered using a water jet then carefully washing several times to remove adhering soil. Roots were kept in 50% ethanol to be scanned and analysed using WinRhizo (Pro 2009 A) software package. Soil samples were retained for chemical analysis. Plant material (roots and tops) was dried to constant weight in an oven at about 70 °C for two days prior to measuring shoot and root dry weight. Root/shoot ratio was calculated by dividing root dry weight by shoot dry weight. Shoots were ground for elemental analyses using a stainless steel ball mill model (Retsch MM200). Data were analysed using Minitab 16 software package. If data were not normally distributed, it was first log transformed. A general linear model (GLM) was used to test for a block effect, but for all described results there was found to be no significant effect due to the block. The GLM was then used to investigate cultivar differences for growth parameters and element status in shoot and to identify whether the presence of inocula in topsoil had a significant effect upon the recorded measurements.

Root scanning

Each root sample was placed on a Perspex tray containing water and separated to be scanned using the WinRhizo Pro 2009 A (Regent Instruments, Canada) software package and a picture for root system was recorded. The numerical output of root scans (root length, surface area, average diameter, volume and tips) was recorded. Specific root length was calculated using root length (m) divided by root dry weight

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(g). Specific root surface area was also measured through root surface area (m^2) divided by root dry weight (kg). The output of root screens, specific root length and specific root surface area were analysed to determine significant effects of treatment and cultivar.

Element analysis

Determination of total phosphorus and manganese in shoot

The shoots were ground in stainless steel ball mill (Retsch MM200), after being oven dried at 70 °C for 48 hours. Plant material was digested using acid digestion (Allen, 1989c) as described above for total P in soil and the concentration of P in shoot was conducted by colorimetric analysis using the automated spectrophotometric flow injection analyser (FIA analysis), while atomic absorption spectrometry (AAS) was used to determine manganese (Mn) concentration in shoot. Based on the concentration of P present in the shoot, total P in shoot was determined by multiplying P concentration (mg g^{-1}) in shoot with shoot dry weight (g). This was used to determine whether or not the plant was P deficient. P use efficiency was calculated by dividing shoot dry weight (g) by total P in shoot (mg). Total Mn in shoot was measured by multiplying Mn concentration (mg g^{-1}) in shoot with shoot dry weight (g).

Determination of carbon and nitrogen present in shoot

Approximately 0.2 mg of shoot material per sample was accurately weighed and placed in tin capsules (8 x 5 mm) to determine the percentage of C and N in shoot by thermal conductivity detection following combustion at 1650 °C on a CE Instruments NA2500 elemental analyzer (Therma Quest Italia S.p.A., Rodano, Italy). Reference samples were run every eight samples. The mass of C and N present in the shoot was calculated, using the C and N percentage present in the shoot and dry weight of the shoot.

Results

Soil

Insch soil used in this experiment is suitable for investigating plant and soil microbes' interaction in P-limiting soil due to its low content of about 0.81 mg g^{-1} soil (Table 2). Comparing the autoclave effect on available and total element content in soil using Two-Sample T-test revealed that autoclaving was found to have a significant effect upon available N and K in both topsoil and subsoil. However, total P and N measured before and after autoclaving in both topsoil and subsoil were found to be not significantly different (Table 2). Autoclaving was shown to cause a highly significant (P<0001) increase on the available P in topsoil while in subsoil it did not (Table 2).

Table 1: Chemical and physical properties of Insch subsoil and topsoil. Mean of 4 replicates ± appropriate standard deviation

Parameter (unit)	Insch subsoil	Insch topsoil
pH (H_2O)	5.02 ± 0.02	5.1 ± 0.1
Total P content (µg g^{-1})	813.9 ± 59.1	1438.6 ± 169.8
Available P (µg g^{-1})	12.24 ± 0.01	27.61 ± 0.01
Total N content (µg g^{-1})	1054.8 ± 83.1	4583.6 ± 359.1
Available N (µg g^{-1})	15.70 ± 0.01	17.73 ± 0.01
Organic matter (%)	0.59 ± 0.12	1.87 ± 0.15
Water holding capacity (%)	24.29 ± 0.24	29.16 ± 0.37
Electrical conductivity (µS cm^{-1})	83.16 ± 6.86	94.08 ± 6.43

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Table 2: Plant available N, P and K and total N and P in topsoil and subsoil before and after autoclaving. Mean of 4 replicates ± standard deviation

Soil type		Available N in soil (µg g^{-1})	Available P in soil (µg g^{-1})	Available K in soil (µg g^{-1})	Total P in soil (µg g^{-1})	Total N in soil (µg g^{-1})
Topsoil	**Live**	17.73a±1.48	27.61a±0.68	402.3a±8.62	1439a±170	4584a±359
	Sterile	51.02b±0.87	51.94b±2.18	434.3b±5.48	1644a±105	5056a±137
	***P* value**	**0.000**	**0.000**	**0.002**	0.109	0.091
Subsoil	**Live**	15.70a±0.55	12.24a±0.63	144.1a ±4.68	814a±59	1055a±83
	Sterile	13.94b±0.60	10.52a±1.40	122.2b±11.64	848a±52	1057a±80
	***P* value**	**0.008**	0.090	**0.040**	0.421	0.973

For each parameter, values followed by the same letter are not significantly different.
The factors in bold are significant.

Plant height

The mean heights of four live and autoclaved soil-grown plants are shown in Figure 1. ANOVA analysis of plant height revealed a highly significant interaction (P<0.001) between soil treatment and cultivar. In Azucena, autoclaved soil-grown plants were significantly (P=0.047) taller at harvest (Table 3). But in IAC 25, live soil-grown plants were taller than plants grown in autoclaved soil at harvest (P=0.001).

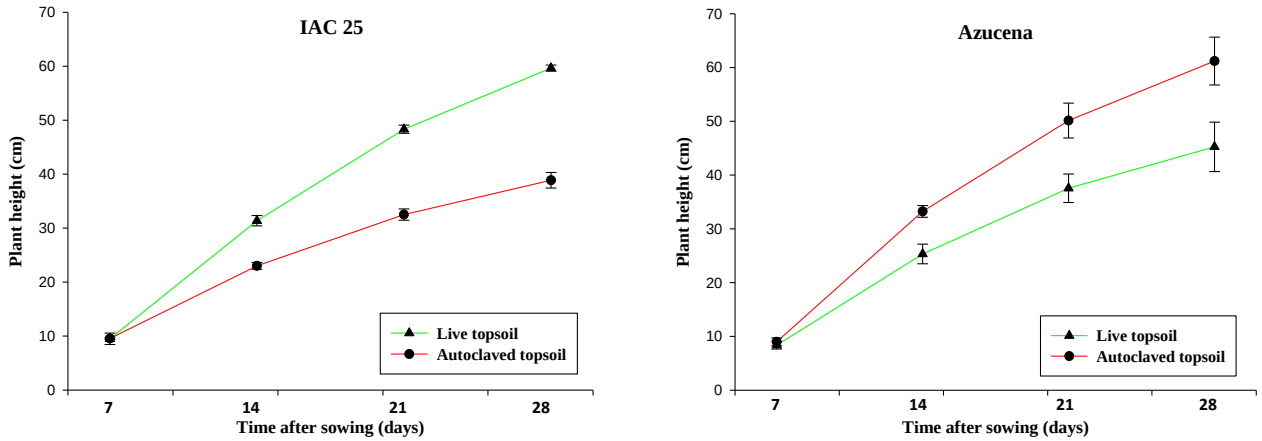

Figure 1: Plant heights of Azucena and IAC 25 grown in live and sterile soil. Mean of 4 replicates ± appropriate standard error.

Plant biomass and root scans

Figure 2 shows representative plants of two cultivars displaying different plant growth due to two different topsoil treatments. Analysis of variance revealed a highly significant (P>0.001) interaction on shoot dry weight (SDW) (Table 3). Azucena in sterile soil grew significantly (P=0.046) better and accumulated 35% more shoot biomass compared to its live soil counterpart. The opposite was observed for IAC 25 where SDW of sterile soil grown IAC 25 declined by 34.8% in a significant manner (P<0.001). This variation in SDW was caused by difference between cultivars in P uptake where Azucena plants grown in sterile treatment accumulated approximately 59% more total P in the shoot than its live treatment-grown counterpart (P=0.022), while the P uptake of IAC 25 in sterile treatment was reduced significantly (P=0.001) by 48.1% compared to live treatment (Table 4).

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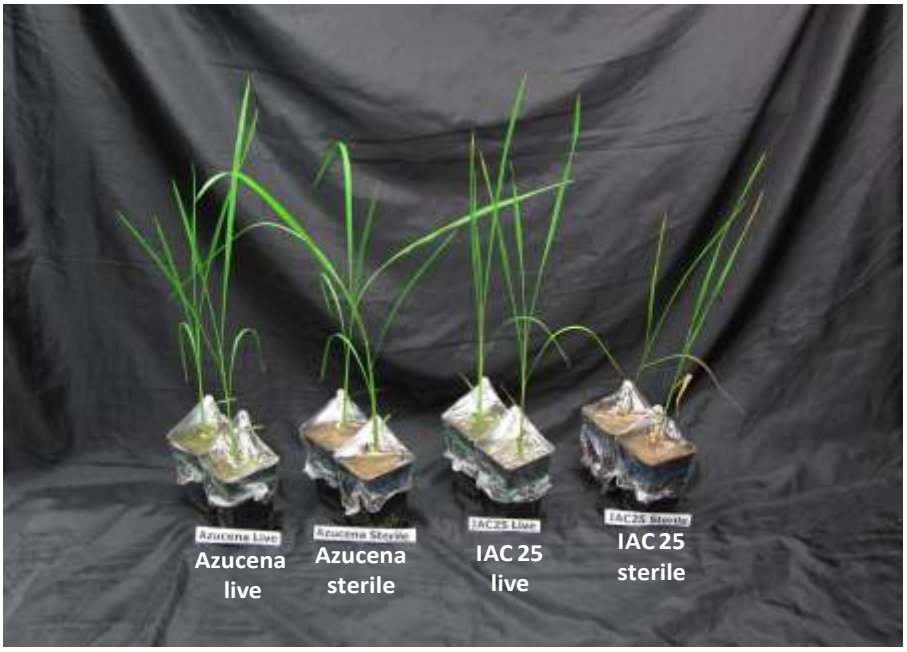

Figure 2: Differences in plant growth of each individual cultivar (Azucena and IAC 25) grown in a half litre pots due to two different topsoil treatments.

The reduction in plant biomass of IAC 25 in sterile soil was always accompanied by the symptoms (Figure 3) seen predominantly on the old leaves. It started first on the tips of the old leaves as paleness with yellowish brown or brownish purple necrotic spots, then the same along the leaf edge, and finally on the leaf base. The highly significant (P<0.001) interaction was also evident for root dry weight (RDW). There was also a significant soil treatment (P=0.003) and cultivar (P=0.001) effect on RDW. The root system of sterile soil grown IAC 25 was reduced by more than 61% (P=0.001), while the RDW for Azucena grown in sterile soil treatment was little affected and increased only by 14% compared to its live soil counterpart. This remarkable effect of soil treatment on RDW of both cultivars can clearly be seen in Figure 4 showing images recorded by scanning.

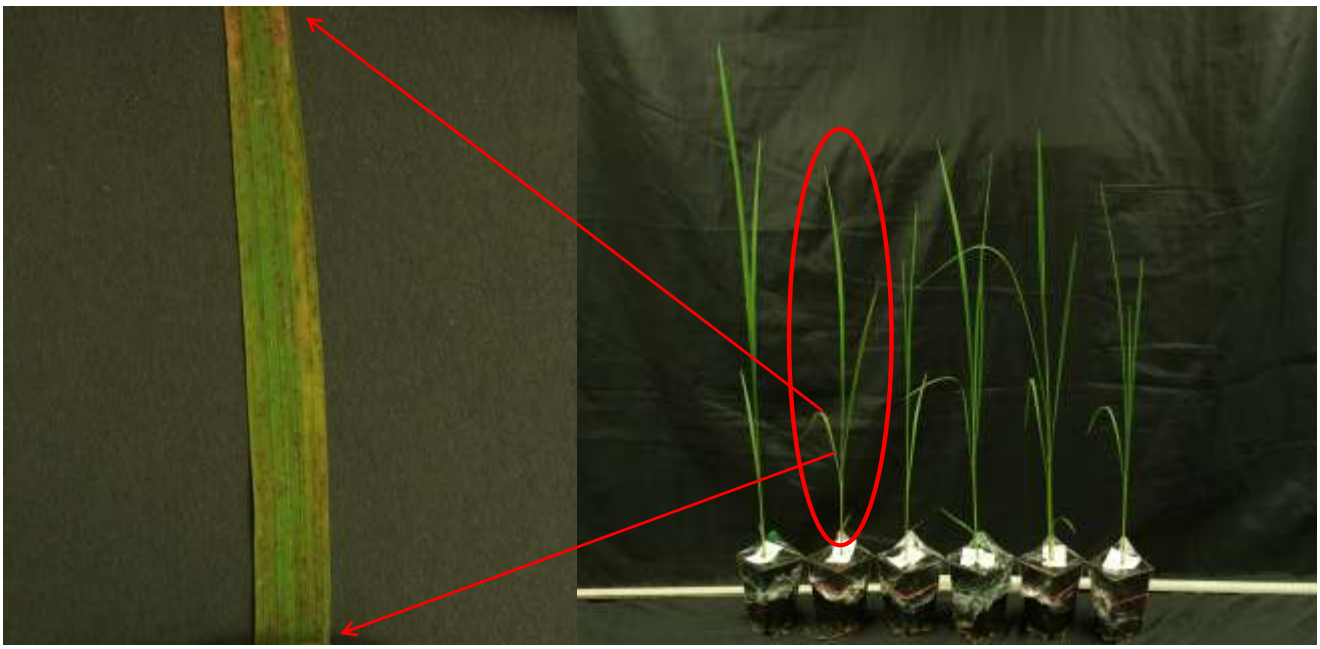

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Figure 3: Symptoms seen on the old leaves of rice cultivar IAC 25 grown in sterile soil.

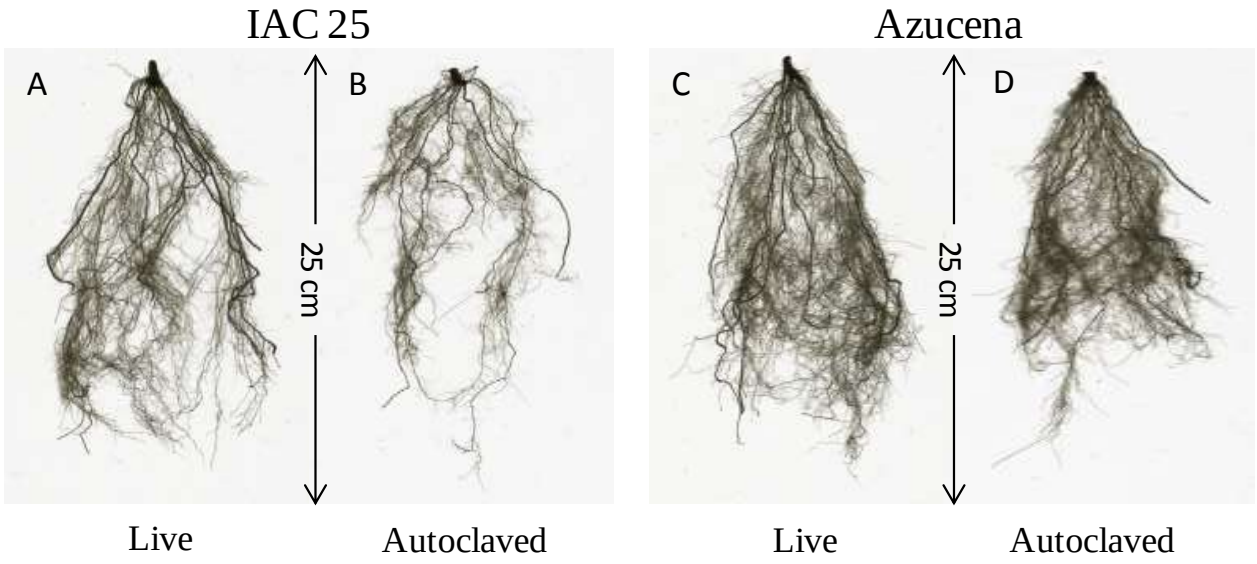

Figure 4: Scanned images of IAC 25 and Azucena roots grown with live topsoil (A and C) and with sterile topsoil (B and D).

This was translated into an effect on root/shoot ratio where a significant (P=0.005) interaction was found and autoclaving the soil (no inocula) reduced the ratio but to a greater extent in IAC 25 (P values for both soil treatment and cultivar effect were less than 0.001). A strong soil treatment x cultivar interaction was found for both plant dry weight (PDW) and final plant height (P<0.001). Differences between cultivars in PDW were found due to cultivar effect at a P value of 0.048 (Table 3).

Analysis of variance on root traits recorded from root scanning revealed that soil treatment (P<0.001) was found to have a highly significant effect upon the root length, where root length for Azucena and IAC 25 increased significantly (P<0.05) in live compared to its sterile soil treatment by 23.1% and 70% respectively. Cultivar was shown to have no effect on root length, while a strong soil treatment x cultivar interaction (P=0.005) was found. This effect was reflected on specific root length, where both cultivar and interaction with soil treatment were found to have a highly significant (P>0.001) effect (Table 3).

The root surface area (RSA) was found to be highly significantly affected by soil treatment (the presence of inocula) and cultivar at a P value of 0.002 and 0.001 respectively. In addition to this there was found to be a highly significant interaction (P=0.001) between these two factors on RSA. R^2 for RSA was 77.5%. RSA for IAC 25 was significantly (P=0.001) higher (at about two times) in live soil treatment than its sterile soil compartment, while RSA for Azucena was little affected and decreased only by 1.4%. This effect on root surface area was translated into an effect on specific RSA where strong soil treatment x cultivar interaction (P<0.001) was found. Further to this, cultivar (P=0.035) was shown to have a significant effect on specific RSA (Table 3).

A significant soil treatment x cultivar interaction (P=0.002) was found for root volume. Cultivar (P<0.001) was also shown to have a significant effect indicating Azucena had a larger root volume. Treatment alone had no significant effect. The treatment x cultivar interaction therefore reveals that root volume was unaffected by treatment in Azucena but it was very significantly reduced in IAC 25 where root volume of sterile soil grown IAC 25 was significantly (P=0.001) lower by 58.2% compared to that grown in live soil treatment. Soil treatment (P=0.002), cultivar (P=0.029) were found to have a highly significant

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effect upon the number of root tips, further to this a significant soil treatment x cultivar interaction (*P*=0.011) was found. A significant (*P*=0.001) increase at 12% on root tips in live compared to sterile soil treatment was found for IAC 25 while for Azucena, it increased only 2% (Table 3).

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Table 3: ANOVA output and average of growth parameters of two rice cultivars (Azucena and IAC 25) grown in two different soil treatments. Mean of 4 replicates ± appropriate standard deviation

	Azucena		**IAC 25**		**#ANOVA for treatment of Azucena**		**#ANOVA for treatment of IAC 25**		**#ANOVA for soil treatment x cultivar interaction**			
	Live topsoil	**Autoclaved topsoil**	**Live topsoil**	**Autoclaved topsoil**	**T (1)**	**R^2**	**T (1)**	**R^2**	**T (1)**	**C (1)**	**TxC (1)**	**R^2**
Shoot dry weight (mg)	240b ±48	324a ±47	316a ±5.9	206b ±15	6.27* **0.046**	42.94%	183.6* **0.000**	96.31%	0.53* 0.481	1.45 0.252	31.32 **0.000**	66.88%
Root dry weight (mg)	101a ±20	115a ±18	109a ±4.0	42b ±7.4	1.00 0.356	0.00%	252.39 **0.000**	97.29%	13.61 **0.003**	19.87 **0.001**	31.36 **0.000**	80.48%
Root/shoot ratio	0.42a ±0.01	0.35b ±0.03	0.35b ±0.02	0.21c ±0.02	18.72 **0.005**	71.68%	87.24 **0.000**	92.49%	92.25 **0.000**	107.81 **0.000**	11.47 **0.005**	93.29%
Plant dry weight (mg)	341ab ±84	439a ±63	426a ±4.9	249b ±21	4.43 0.080	32.89%	251.04 **0.000**	97.28%	2.66 0.129	4.86 **0.048**	32.77 **0.000**	71.31%
Final plant height (cm)	45.3b ±9.2	61.2a ±8.9	59.7a ±1.1	38.9b ±2.9	6.23 **0.047**	42.78%	179.72 **0.000**	96.23%	0.54 0.477	1.45 0.251	31.21 **0.000**	66.82%
Root length (cm)	1739a ±124	1412b±188	1936a±131	1133b±92	8.37 **0.028**	51.29%	99.93 **0.000**	93.39%	66.48 **0.000**	0.35 0.565	11.83 **0.005**	83.45%
Specific root length	176b ±29	124c ±21	176b ±10	269a ±30	8.27 **0.028**	50.94%	33.32 **0.001**	82.20%	2.88 0.115	36.62 **0.000**	35.86 **0.000**	82.83%
Root surface area (cm^2)	199a ±29	202a ±31	196a ±17	97b ±10	0.02 0.898	0.00%	95.60 **0.000**	93.11%	16.18 **0.002**	20.58 **0.001**	18.19 **0.001**	77.59%
Specific root surface area	199b ±15	176b ±5.7	179b ±11	230a ±20	8.24 **0.028**	50.83%	19.02 **0.005**	72.03%	3.83 0.074	5.68 **0.035**	27.24 **0.000**	69.23%
Root volume (cm^3)	1.83a ±0.60	2.34a ±0.97	1.58a ±0.58	0.66b ±0.62	2.15 0.193	14.14%	78.78 **0.000**	91.74%	1.28 0.280	28.11 **0.000**	15.56 **0.002**	73.66%
Root tips	9395b±1920	9179b±1146	9622b±1635	8478a±1297	0.43 0.537	0.00%	35.99 **0.001**	83.33%	16.43 **0.002**	6.18 **0.029**	8.96 **0.011**	65.58%

ANOVA output and R^2; T, topsoil treatment; C, cultivar (Azucena and IAC 25); degrees of freedom between brackets;* denotes F ratio above and probability value below. The factors and interactions in bold are significant. For each cultivar, values followed by the same letter are not significantly different as determined by Tukey's test. Specific root length = (root length m/ root dry weight g). Specific root surface area = (root surface area m^2/ root dry weight kg).

Elements in shoot

There was a highly significant cultivar x soil treatment interaction (*P*=0.004) for shoot N concentration where Azucena was higher than IAC 25 (3.3% vs 2.6% respectively) and while Azucena was not affected by treatment, in IAC 25 the [N] dropped from 2.8 to 2.4% due to sterile soil. This pattern of interaction effect reflected on total N in shoot but no significant effect for the treatment on total N in shoot was found (Table 4). For shoot C concentration, a weakly significant (*P*=0.042) effect for the treatment was found, where both cultivars had slightly higher [C] in live compared to sterile treatment. This effect was reflected in the C/N ratio, where cultivar (*P*<0.001) showed highly significant effects and there was a significant cultivar x treatment interaction (*P*=0.001). The GLM analysis showed that soil treatment was found to have no effect upon the concentration and total Mn in shoot. Further to this no evidence of any interaction effect was observed but there was a significant (*P*<0.05) difference between cultivars for total and concentration of Mn in shoot where [Mn] in IAC 25 was 1.9 times more than Azucena. Strong cultivar x soil treatment interaction (*P*=0.004) was present for shoot P concentration where Azucena was higher than IAC 25 (0.11% vs 0.09% respectively). Comparing the [P] in sterile treatment to live treatment, one-way ANOVA for soil treatment revealed that the [P] in Azucena grown in the sterile increased significantly (P=0.039) from 0.10 to 0.12% relative to live treatment, while in IAC 25 dropped from 0.10 to 0.08% in a significant (P=0.050) manner. This trend of cultivar behaviour for [P] was also true for total P in shoot where Azucena accumulated approximately 1.6 times more P in sterile than live treatment, while the sterile soil grown IAC 25 accumulated half as much than live soil grown counterpart (Table 4). ANOVA revealed that there was a significant (*P*<0.001) cultivar x soil treatment interaction for total P in shoot. The percentage of P in all plants was almost more than 0.1 except for IAC 25 grown in sterile soil with P percent of about 0.08% (Figure 5). In this experiment, 71.4% of the variation in total P per shoot was explained by both soil treatment and cultivar. The PUE was found to be significantly highly affected by cultivar (*P*=0.006). Further to this a significant interaction (*P*=0.006) was present. PUE was higher in IAC 25 grown in sterile than in live soil treatment while the opposite was true for Azucena.

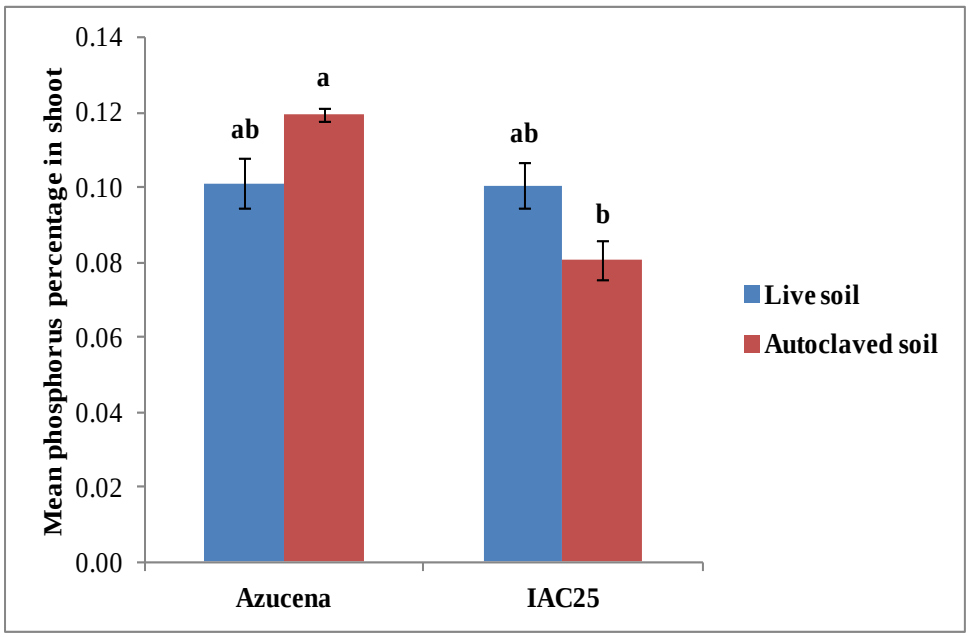

Figure 5: Phosphorus percentage found in Azucena and IAC 25 grown in two different soil treatments (live and sterile). Mean value of four replicates of each cultivar ± standard error. For each cultivar, values followed by the same letter are not significantly different as determined by Tukey's test.

Table 4: ANOVA output and average of elements (P, C, N and Mn) status in shoot of two rice cultivars (Azucena and IAC 25) grown in two different soil treatments. Mean of 4 replicates ± appropriate standard deviation

	Azucena		**IAC 25**		**#ANOVA for treatment of Azucena**		**#ANOVA for treatment of IAC 25**		**#ANOVA for soil treatment x cultivar interaction**			
	Live topsoil	**Autoclaved topsoil**	**Live topsoil**	**Autoclaved topsoil**	**T (1)**	**R^2**	**T (1)**	**R^2**	**T (1)**	**C (1)**	**TxC (1)**	**R^2**
Shoot N conc. (mg g^{-1})	33.04a±1.24	33.49a±1.34	27.78b±0.91	24.44c±0.58	0.24* 0.641	0.00%	38.13* **0.001**	84.14%	7.42* **0.018**	181.72 **0.000**	12.73 **0.004**	92.99%
Total N in shoot (mg)	7.91b ±1.51	10.91a±1.91	8.79ab±0.15	5.04c ±0.30	6.10 **0.049**	42.13%	489.11 **0.000**	98.59%	0.36 0.560	16.46 **0.002**	30.15 **0.000**	74.57%
Shoot C conc. (mg g^{-1})	388a ±15	372a ±10	386a ±18	366a ±17	2.78 0.146	20.28%	2.53 0.163	17.97%	5.18 **0.042**	0.32 0.581	0.07 0.795	14.62%
Total C in shoot (mg)	92.8b ±16.5	121.3a±20.0	122.2a±4.1	75.5b ±4.0	4.85 0.070	35.46%	266.43 **0.000**	97.43%	1.90 0.194	1.55 0.237	32.22 **0.000**	68.53%
C/N ratio	11.77c±1.35	11.13c±1.82	13.90b±1.81	14.98a±0.37	27.19 **0.002**	78.91%	8.60 **0.026**	52.07%	1.32 0.273	239.90 **0.000**	19.54 **0.001**	94.50%
Shoot Mn conc. (mg g^{-1})	4.67b ±1.76	4.54b ±1.94	8.44a ±1.46	9.28a ±1.15	0.01 0.921	0.00%	0.81 0.403	0.00%	0.19 0.670	27.92 **0.000**	0.37 0.556	62.94%
Total Mn in shoot (mg)	1.17b ±0.55	1.43b ±0.59	2.67a ±0.48	1.92ab±0.34	0.41 0.544	0.00%	6.53 **0.043**	44.14%	0.96 0.347	15.81 **0.002**	4.08 0.066	54.34%
Shoot P conc. (µg g^{-1})	1010ab±134	1193a±36	1005ab±124	805b ±105	6.89 **0.039**	45.71%	6.02 **0.050**	41.76%	0.03 0.875	13.39 **0.003**	12.75 **0.004**	60.70%
Total P in shoot (µg)	244bc ±70	388a ±61	318ab ±43	165c ±13	9.38 **0.022**	54.50%	44.64 **0.001**	86.18%	0.03 0.857	8.10 **0.015**	32.35 **0.000**	71.42%
PUE	1.00ab±0.15	0.84b ±0.03	1.01ab±0.13	1.26a ±0.15	4.90 0.069	35.77%	6.21 **0.047**	42.67%	0.46 0.512	11.22 **0.006**	11.03 **0.006**	56.78%

ANOVA output and R^2; T, topsoil treatment; C, cultivar (Azucena and IAC 25); degrees of freedom between brackets;* F ratio above and probability value below. The factors and interactions in bold are significant. For each cultivar, values followed by the same letter are not significantly different as determined by Tukey's test. Element content in shoot = element concentration in shoot (mg g^{-1}) x shoot dry mass (g). P use efficiency (PUE) = shoot dry weight (g)/P in shoot (mg).

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