Assessing the effect of indigenous soil bacteria on growth and phosphorus acquisition of three rice cultivars (Azucena, IAC 25 and Lemont)

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Abstract*:*

This study is aimed at understanding interactions between indigenous soil microorganisms and rice cultivars (*Oryza sativa* L.) in P limited conditions. And to investigate whether plant growth promoting microbes are transferable with water. Three rice cultivars (Azucena IAC 25 and Lemont) were grown in four treatments: The first was when non-autoclaved (live) wash was added to non-autoclaved (live) topsoil and the second treatment when the wash was autoclaved (sterile). The live wash / autoclaved topsoil and autoclaved wash / autoclaved topsoil are the third and fourth treatment respectively. 5% topsoil with 95% P-limited sterile subsoil was used for all four treatments plus P free Yoshida's nutrient solution). Plant growth, P-uptake and P use efficiency (PUE) were assayed at harvest. Root scan and element in shoot were also determined. Results revealed significant differences ($P<0.001$) for plant height and shoot dry weight (SDW), ($P=0.005$) for root dry weight (RDW) and root surface area, ($P<0.033$) for root/shoot ratio, ($P<006$) for average diameter, ($P<0.042$) for volume and tips and ($P=0.002$) for N concentration among cultivars due to the topsoil treatment. Plants grown with live topsoil had an average SDW of approximately 305.6 mg while those with sterile topsoil were half the size at 148.3 mg. Shoot N concentration, C/N ratio, Mn concentration were found to be effected by cultivar ($P<0.001$)in a highly significant manner. In this study result showed that Growth promoting microbes in soil were not transferrable with wash.

Keywords: soil microbes, rice cultivars, Phosphorus acquisition.

تقييم تأثير البكتريا المستوطنة في التربة على نمو وامتصاص الفوسفور في ثلاث اصناف من الرز (Azucena و IAC 25 و Lemont)

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المستخلص:

تهدف هذه الدراسة إلى فهم التأثيرات المتداخلة بين الكائنات الدقيقة المستوطنة في التربة واصناف الرز (*Oryza sativa* L) في ظروف تربة محدودة الفسفور. كذلك للتحقق من امكانية نقل مايكروبات التربة الداعمة للنمو بواسطة الماء. تم زراعة هذه الاصناف الثلاثة في اربعة معاملات هي : الاولى ماء غسل التربة (ماء مقطر اضيف الى التربة ثم تم ترشيحه) غير معقم (حي) اضيف الى نفس التربة (هذه التربة مأخوذة من الطبقة السطحية) غير معقمة . المعاملة الثانية عندما عقم ما غسل التربة. والمعاملة الثالثة كانت ماء غسل التربة حي . تربة$^{-1}$ معقمة مأخوذة من الطبقة السطحية للحقل اما العاملة الرابعة فكانت ماء غسل التربة معقم. تربة$^{-1}$ معقمة مأخوذة من الطبقة السطحية للحقل. تم تشكيل المعاملات بخلط 5% تربة مأخوذة من الطبقة السطحية للحقل في كل معاملة من المعاملات الاربعة مع 95% تربة معقمة مأخوذة من الحقل بعمق دون 20سم. تم ارواء المعاملات بمحلول يوشيدا المغذي. اما الصفات المدروسة التي تم قياسها عند الحصاد فهي نمو النبات وامتصاص الفسفور وكفاءة استخدام الفسفور(PUE) .كذلك تم مسح الجذور وحسبت تراكيز بعض المعادن في المجموع الخضري. كشفت النتائج عن وجود فرق معنوي عالي ($P<0.001$) لطول النبات و الوزن الجاف للمجموع الخضري و($P<0.005$) للوزن الجاف للجذور والمساحة السطحية للجذور و ($P<0.033$) لنسبة المجموع الخضري الى المجموع الجذري و ($P<0.006$) لمعدل قطر الجذر و ($P<0.042$) لحجم الجذور وعدد النهايات للجذور و ($P<0.002$) لتركيز النيتروجين بين الاصناف الذي يعود الى تأثير معاملة التربة المأخوذة من الطبقة السطحية. ان معدل الوزن الجاف للمجموع الخضري للنباتات النامية في التربة الحية كان 305.6 ملغم بينما 148.3 ملغم لتلك النامية في التربة المعقمة. تركيز النايتروجين ونسبة االكاربون الى النايتروجين وتركيز المنغنيز تأثرت بشكل معنوي بالاصناف ($P<0.001$). كما اظهرت هذه الدراسة يظاً ان مايكروبات التربة الداعمة للنمو لايمكن نقلها بواسة الماء.

Introduction:

P deficiency is a major abiotic stress that limits crop productivity on 30 − 40% of the World's arable land (26). 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. 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. These 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 (10). Other factors such as soil type (22), 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 (6,16,27). 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 (17,18) and mycorrhizal fungi (*Glomus etunicatum*) have been shown to enhance plant growth (15,24) through their positive interaction with plants by helping the plant to access sparingly available nutrients. Plants take up most mineral nutrients through the rhizosphere where micro-organisms interact with plant root exudates (8). 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 (11). Therefore root exudation can also stimulate soil micro-organisms that could solubilise inorganic P (8). Richardson (21) reported the important role of soil micro-organisms in soil P dynamics and subsequent availability of P to plants. A study by Wissuwa (18) 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. Soil sterilization by autoclaving is commonly used as an abiotic control in experimental studies. Autoclaving is often the preferred method for soil sterilisation as it can be used for the experimental containers on site is cost effective and does not create more contamination for the soil (12,25).This study examines the ability of different rice cultivars to interact with soil micro-organisms 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.

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 character-

ized 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 (17). 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.

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
Available K in soil (µg g^{-1})	402.3 ± 8.62	144.1 ± 4.68
Water holding capacity (%)	24.29 ± 0.24	29.16 ± 0.37
Electrical conductivity (µS cm^{-1})	83.16 ± 6.86	94.08 ± 6.43

Treatment:

The water-extractable soil micro-organisms were extracted by shaking two kg of Insch topsoil (based on dry weight) on a rotary shaker for one hour with deionised water at a 1:2 (w/v) soil: distilled water ratio and allow settling overnight. About 3.6 litres of the recovered supernatant was then filtered through a Whatman No. 40 paper. Henceforth, this soil wash extract will be referring to as "wash" in this study. Half of the washed topsoil and the wash were triple autoclaved and the rest was not.

To ensure that the autoclaved wash and topsoil were free of soil micro-organisms, culturable heterotrophic bacteria and fungi numbers were assessed by serial dilution of autoclaved and non-autoclaved topsoils and washes. Bacterial heterophic counts in autoclaved and non-autoclaved wash and topsoil was achieved by using Luria Bertani (LB) plates for bacteria. The procedure was modified from methods that were described by Jorgensen *et al*. (14). LB agar (1.5% w/v) was prepared and 100 µg L^{-1} of cyclohexamide antibiotic was added to the medium then poured into Petri dishes. As for fungal heterophic count, **Potato Dextrose Agar (PDA)** medium was prepared following the same procedure as with LB agar except for the use of 20 µg L^{-1} of the tetracycline instead of cyclohexamide antibiotic. One gram of each topsoil and 10 ml from each wash were used to estimate the microbial population size. Five serial dilutions of sterilized and unsterilized of both wash and topsoil were prepared using sterile, ¼ strength Ringers' solution. A 100 µL aliquot of each suitable dilution, in triplicate, was spread onto the top of each medium using a sterile loop. The plates were

then incubated at 25 °C for 48 hours. The number of colony forming units (cfu) was expressed on a per gram oven dry weight for soil sample and per 10 ml for wash.

The moisture content in three types of soils (autoclaved subsoil, autoclaved topsoil, and non-autoclaved topsoil) was determined using Ohaus Moisture Analyzer Balance (Moisture Analyzer MB45). The quantity of topsoil and subsoil mix used for each treatment was determined based on dry weight of soil so that 95% autoclaved subsoil with 5% topsoil either live or sterile were mixed for each treatment plus appropriate wash, which was either sterile or live then distributed into the pots of each treatment. Four treatments were used in this experiment (Figure 1). The first was when non-autoclaved (live) wash was added to non-autoclaved (live) topsoil and the second treatment when the wash was autoclaved (sterile). The live wash/autoclaved topsoil and autoclaved wash/ autoclaved topsoil are the third and fourth treatment respectively.

The results (Table 3) demonstrate the values of calculated average for microbial population size expressed as colony forming units (cfu). The numbers of colonies per plate observed in live topsoil and wash ranged from 223.3 to 283.3 cfu for fungi and from 235,000 to 2,710,000 for bacteria while there were no bacteria or fungi in autoclaved topsoil and wash.

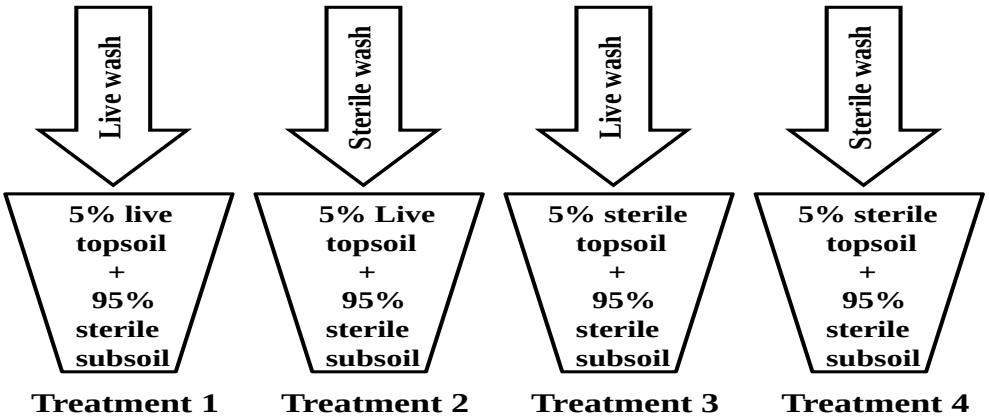

Figure 1: four treatments used for experiment three.

Table 3: Number of heterotrophic bacterial (LB) and fungal (PDA) colony forming units (cfu) in autoclaved and non-autoclaved topsoils and washes expressed on a per gram oven dry weight basis for topsoil sample and per 10 ml for wash. Mean = 3 and appropriate standard deviation.

Source of inocula	Status	cfu of heterotrophic fungi	cfu of heterotrophic bacteria
Topsoil	Non autoclaved	223.3 ± 66.5	2706666.6 ± 547844.2
	Autoclaved	0	0
Wash	Non autoclaved	283.3 ± 61.1	235333.3 ±15011.1
	Autoclaved	0	0

Soil sterilization by autoclaving breaks down organic matter content and induces chemical and physical changes in soil properties (9,23). 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 P, N 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 P, N and K in both subsoil and topsoil before and after autoclaving have been conducted. So all soil mixes (live wash/live topsoil, autoclaved wash/live topsoil, live wash/ autoclaved topsoil and autoclaved wash/autoclaved topsoil) used in treatments before sowing and after harvesting had been assessed for total P and N and electrical conductivity in soil; all data are shown in Table 2.

Soil analysis

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 Flow injection analysis (FIA) (4). The available P was measured by using acetic acid extraction as described by Allen (1) and available N was determined using the procedure described by Allen (3). 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 (2). 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 ^{o}C for at least 60 minutes. Results are demonstrated in Table 1.

Plant available N (Ammonium – N, Nitrate – N and Nitrite – N) was determined using procedure described by Allen (3). Available P in the soil was estimated by using acetic acid extraction as described by Allen (1). To measure plant available K, Elmer AAS (atomic absorption spectrophotometer) was used. For determination of total N and P in soil, the procedure described by Allen (2) was used. Results are shown in Table 2.

Table 2: Total P and N content in four treatment soils before sowing and after harvest. Mean = 3 and appropriate standard deviation

Parameter (unit)		Live wash live topsoil	Sterile wash live topsoil	Live wash sterile topsoil	Sterile wash sterile topsoil
Before sowing	Total P (µg g^{-1} soil)	763.9±83.5	827.8±122.4	929.3±290.6	795.5±86.7
	Total N (µg g^{-1} soil)	484.6±42.1	537.2±16.2	482.4±115.5	489.3±35.4
	EC (µS cm^{-1})	83 ±3.28	84 ±2.96	83 ±6.11	81 ±5.23
After harvest	Total P (µg g^{-1} soil)	620.5±210.8	621.6±186.5	776.3±30.6	791.3±97.9
	Total N (µg g^{-1} soil)	681.1±230.5	684.1±40.4	792.5±67.1	1240.2±117.6
	EC (µS cm^{-1})	272 ±12.50	324 ±12.52	276 ±2.60	517 ±1.85

Results presented in Table 2 showed that after harvest, total N and electrical conductivity in soil increased for all treatments. Looking at total P in soil, the average of total P in soil after harvest decreased approximately 143.4 µg g^{-1} soil for live wash/live topsoil treatment, 206.2 µg g^{-1} soil for sterile wash/live topsoil treatment and 153 µg g^{-1} soil for live wash/sterile topsoil treatment but for sterile wash/sterile topsoil treatment, P content decrease only 4.2 µg g^{-1}. Note, however, that the standard errors for this measurement are high so no conclusion can be drawn from this without further data.

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 (5). IAC 25 is known to be superior in P acquisition as identified by Wissuwa and Ae (30). While Azucena is recognized to grow well in P limiting conditions (7). But Anderson (5) 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* (32). 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 (29).

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 ºC for two days.

Growing plants in the growth room and procedures after harvest

A 500 ml pot experiment was conducted with three rice cultivars (Azucena, IAC 25, and Lemont) between January and February 2010 and continued for 28 days. The soil used for treatment was a mix of 95% triple autoclaved Insch subsoil amended with 5% Insch topsoil either live or triple autoclaved. A total of 60 polyethylene pots were filled with approximately 650 g topsoil/subsoil mix based on soil dry weight with about 60 ml of appropriate soil wash either live or autoclaved for each pot to form four treatments (Figure 2.1). The pots were arranged in a RCBD with 5 replications. The plants watered with P free Yoshida's nutrient solution (30). Shoot growth was monitored on a weekly basis. When the experiment was harvested, roots and shoots were separated and soil samples were retained for chemical analysis. Roots were washed carefully to remove adhering soil and kept in 50% ethanol solution. All root systems were scanned by WinRhizo (Pro 2009 A) and pictures of this were recorded. The numerical output of root screens (root length, surface area, average diameter, volume and tips) were analysed to determine significant effects of topsoil and wash treatments and whether the cultivars interact differently to these treatments. All

plant samples were oven dried at 70 °C for two days prior to measuring shoot and root dry weight. Shoots were ground for elemental analyses using a ball mill. The powdered material of shoot was subjected to analysis for total P and Mn after acid digest as described in experiment one. The percentage and total N and C in shoot were also determined using the same procedure explained in experiment one. Root/shoot ratio was determined using dry weight of the root and shoot. Statistical analysis was conducted using Minitab 16. Wherever measured data were not normally distributed the log of the data were calculated. For analysis of plant growth and nutrient uptake a general linear model in Minitab 16 software package was conducted in a three-way ANOVA of topsoil treatment (live or autoclaved topsoil), soil wash (live or autoclaved wash) and cultivar (Azucena, IAC 25 and Lemont).

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 (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

For determination of total phosphorus and manganese in shoot, acid digest and Flow injection analysis (FIA) (21) was used. To determine the percentage of C and N in shoot, thermal conductivity detection following combustion at 1650 °C on a CE Instruments NA2500 elemental analyzer (ThermaQuest Italia S.p.A., Rodano, Italy) was used.

Results:

Plant height

The plant height at harvest was taller in live than in autoclaved soil grown plants (Figure 2). ANOVA output revealed significant differences ($P<0.001$) with both topsoil treatment and cultivar but no evidence of any interaction on final plant height was found.

Plant biomass and growth

Figure 3 demonstrates representative plants of each individual cultivar (Azucena, IAC 25 and Lemont) as they show differences in plant performance due to four different topsoil treatments (Live wash/live topsoil, sterile wash/live topsoil, live wash/sterile topsoil and sterile wash/sterile topsoil). For all cultivars, plants in live topsoil had better growth compared to those in sterile topsoil treatment.

Results of plant growth are presented in Table 4. Analysis of variance revealed significant ($P<0.001$) differences for SDW among cultivars due to the topsoil treatment, while wash and cultivar were found to be not significant and there was no evi-

dence of any interaction. Plants grown with live topsoil had an average SDW of approximately 305.6 mg while those with sterile topsoil were half the size at 148.3 mg. ANOVA revealed the same pattern for RDW, with only a topsoil treatment effect (P=0.005). This clearly can be seen in Figure 4 where RDW reduced by 36.5% for Azucena, 40.4% for IAC 25 and 23.3% for Lemont in the sterile topsoil compared to the live topsoil.

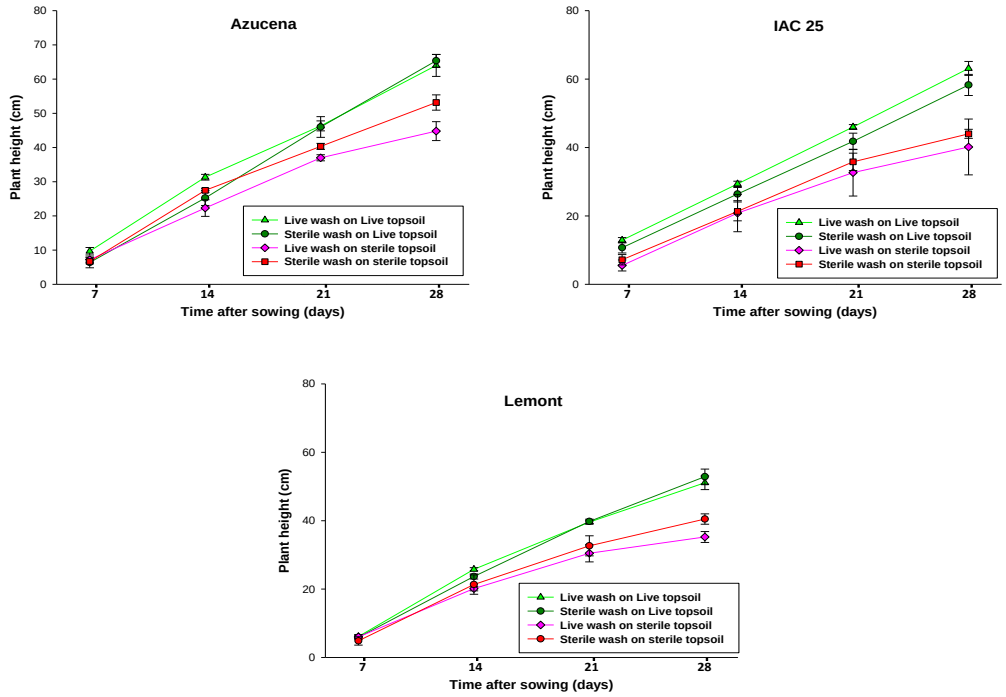

Figure 2: Plant heights of Azucena, Lemont and IAC 25 grown in four different treatments.

Wash and cultivar were shown to have no effect on RDW and no interaction was observed. This effect of topsoil was reflected on root/shoot ratio and plant dry weight, which were found to be significantly affected by topsoil treatment at *P* value of 0.033 and <0.001 respectively but not by wash and no significant interaction was found. Upon further analysis on the numerical output of root screening by WinRhizo, it was found that topsoil treatment was shown to have a significant effect upon root length, surface area**,** average diameter, volume and tips (P=0.015, 0.005, 0.006, 0.004 and 0.042 respectively) and no other significance or interaction was found (Table 4).

Elements in shoot

The topsoil and wash treatments and cultivar were found to have no significant effect on P concentration in the shoot and no interaction was found (Table 5). All cultivars accumulated more total P in shoot in live than in sterile soil. For example, the increase in total P in shoot was 222.8% for Azucena, 162.5% for IAC 25 and 93% for Lemont. For total shoot P, 32.6% of variation was attributable to the experimental factors but only the topsoil was significant (more P per shoot in live than sterile soil). No factor was shown to have an effect on C concentration in shoot and no evidence of any interaction was present.

For the shoot N status results, plant 26 and 53 (Azucena in live wash/sterile topsoil and IAC 25 in sterile wash/sterile topsoil treatment respectively) were identified as being possible outliers by means of box plot. They were statistically confirmed as being true outliers by plotting the data with a box plot. Subsequently all data collected for plants 26 and 53 were removed from all analysis of shoot N concentration. Shoot N concentration was found to be effected by topsoil treatment (P=0.002) and cultivar (P<0.001) in a highly significant manner, while for wash treatment, no significant effect and no interaction was found (Table 5). When total N in shoot was investigated, it was found that the difference between topsoil treatments was highly significant at *P* value of <0.001 and no other significance or interaction for total N in shoot was observed. The topsoil treatment (P=0.001) and cultivar (P<0.001) were found to have a highly significant effect upon C/N ratio in the shoot and there was no evidence of any interaction. Cultivar (P<0.001) was found to have a significant effect upon Mn concentration in shoot while topsoil and wash treatments had no significant effect and no evidence of any interaction was present. Rice cultivar IAC 25 appears to take up more Mn than the other two cultivars.

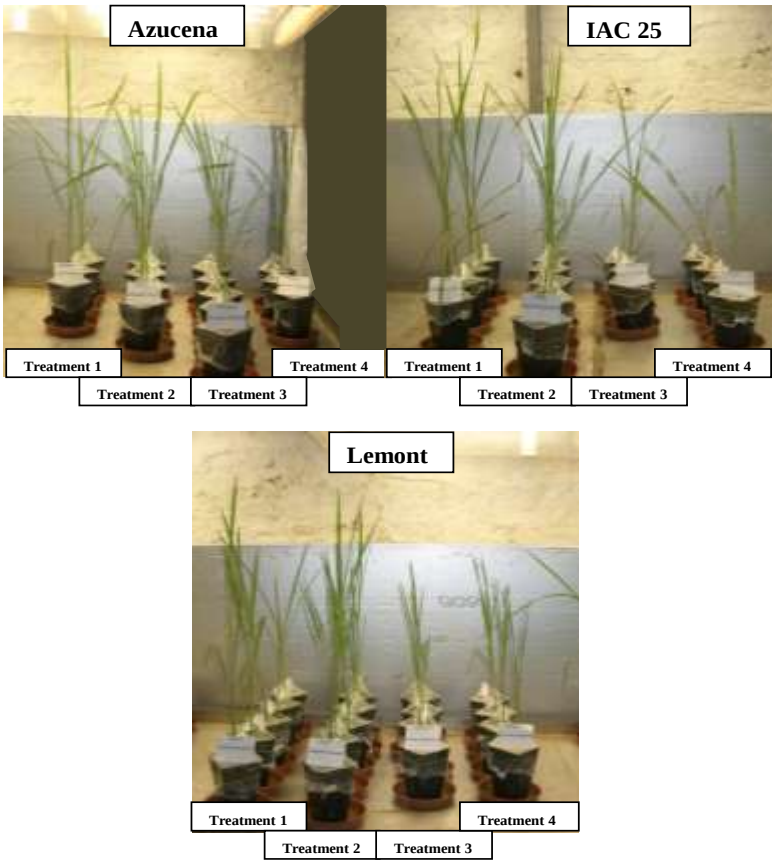

Figure 3: Differences in plant growth of each individual cultivar (Azucena, IAC 25 and Lemont) grown in a half litre pots (8 x 8 cm at the top, 5.5 x 5.5 cm at the bottom, 12.3 cm deep) due to four different topsoil treatments.

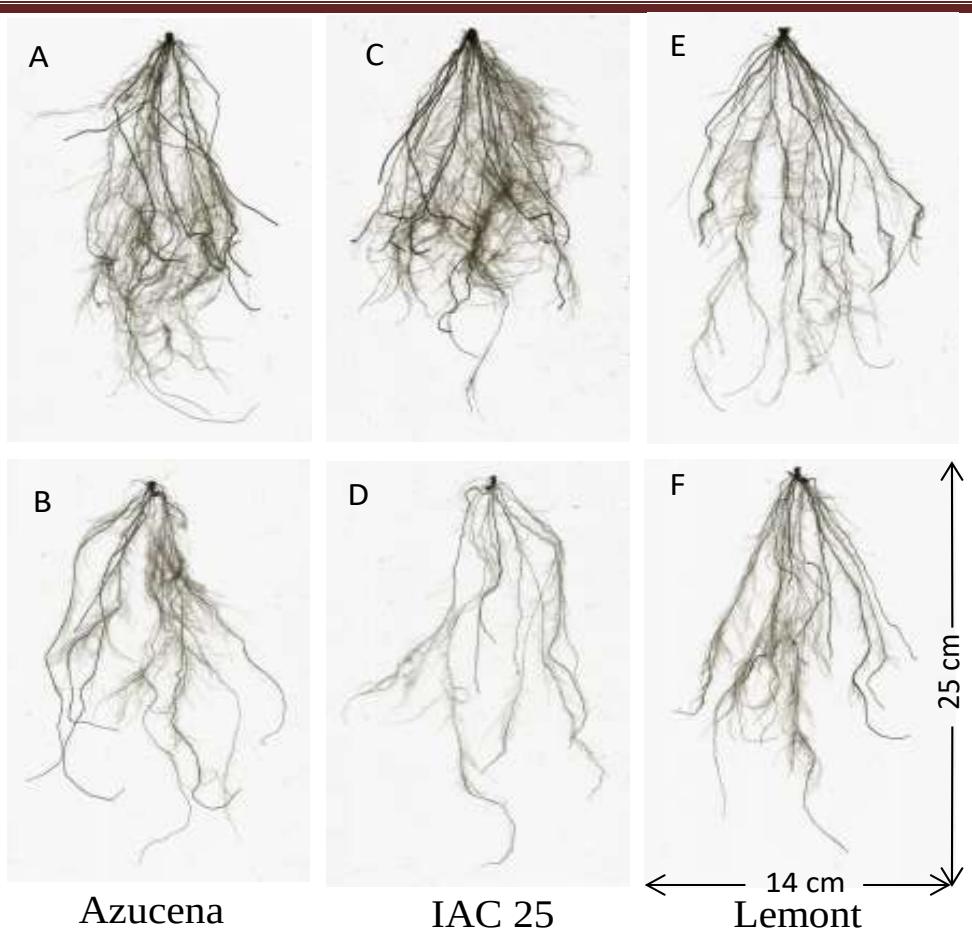

Figure 4: Scanned images of roots for Azucena, IAC 25 and Lemont. A, C and E grown in live wash/live topsoil treatment; B, D and F in sterile wash/sterile topsoil treatment.

Discussion:

The possibility suggested by Henry *et al*. (13) that soil dynamics may be changed due to sterilization in such a way that could create toxic effects proves not to be relevant in the experiment conducted here. Observations on the germination of cultivars used in the experiment reported and additional experiment not presented in this paper support this observation. In all experiments, the germination of the three cultivars (Azucena, IAC 25 and Lemont) in pots with live or sterile topsoil was not affected by treatment. This experiment aimed at investigating plant/soil microbes' interactions in P deficient soil on three rice cultivars and testing whether or not plant growth promoting soil microbes are transferable and can be inoculated with water. Results revealed that almost all plant growth parameters and elements in shoot were shown to be affected by topsoil treatment where the growth and element uptake in live were higher compared to autoclaved topsoil. This may support the hypothesis that the presence of soil micro-organisms stimulates the growth of rice plants. Cultivar was found to have a significant effect on final plant height and the recorded data of element status in shoot. Importantly, there was no significant effect for wash on any growth parameters or element uptake observed and no evidence of any interaction was found.

Table 4: Analysis of variance and average parameters of plant growth for three rice cultivars grown with four different inocula treatments. Mean = 5 and appropriate standard deviation

		Live topsoil		Autoclaved topsoil		ANOVA#							
Parameters	**Cultivar**	**Live wash**	**Autoclaved wash**	**Live wash**	**Autoclaved wash**	**T (1)**	**W (1)**	**C (2)**	**TxW (1)**	**TxC (2)**	**WxC (2)**	**TxWxC (2)**	**R^2**
Shoot dry weight (mg)	**Azucena**	298 ±65	329 ±75	149.1±47	177.4±38	67.86* **0.000**	0.05 0.821	3.06 0.060	1.38 0.248	2.28 0.117	1.58 0.220	1.08 0.351	62.43%
	IAC 25	406 ±82	299.6±118	133.6±75	151.8±5.0								
	Lemont	240 ±47	260.9±36	121.9±25	155.8±38								
Root dry weight (mg)	**Azucena**	79 ±24	51.8 ±22	45 ±34	38 ±11	9.22 **0.005**	0.93 0.341	0.05 0.948	2.27 0.141	0.30 0.739	1.09 0.346	0.95 0.395	10.81%
	IAC 25	88 ±20	52.9 ±21	36 ±32	48 ±35								
	Lemont	60 ±22	67.8 ±23	46 ±23	52 ±10								
Root/Shoot Ratio	**Azucena**	0.26±0.03	0.17 ±0.09	0.30 ±0.19	0.21 ±0.03	4.95 **0.033**	0.66 0.423	1.84 0.174	0.02 0.902	0.45 0.643	0.76 0.475	0.60 0.554	4.94%
	IAC 25	0.22±0.03	0.19 ±0.07	0.24 ±0.12	0.32 ±0.24								
	Lemont	0.24±0.06	0.26 ±0.08	0.41 ±0.24	0.34 ±0.05								
Final plant height (cm)	**Azucena**	64.0±5.6	65.4 ±3.7	44.8 ±6.2	53.2 ±3.9	87.69 **0.000**	2.33 0.136	16.31 **0.000**	3.41 0.073	0.59 0.559	0.84 0.438	0.21 0.816	71.59%
	IAC 25	63.1±4.1	58.3 ±6.9	40.2 ±14.1	44.0 ±2.6								
	Lemont	51.1±4.5	52.9 ±4.9	35.3 ±3.2	40.5 ±3.0								
Plant dry weight (mg)	**Azucena**	377 ±89	381 ±65	195 ±68	216 ±48	63.98 **0.000**	0.01 0.907	2.13 0.134	2.22 0.145	2.12 0.135	1.46 0.245	1.34 0.274	60.51%
	IAC 25	494 ±97	352 ±124	170 ±103	200 ±31								
	Lemont	300 ±68	329 ±50	168 ±6.3	208 ±46								
Root length (m)	**Azucena**	19.4±1.99	16.8 ±6.95	13.6 ±4.79	12.6 ±2.32	6.55 **0.015**	0.04 0.838	1.69 0.199	2.45 0.127	1.08 0.351	0.89 0.421	1.09 0.346	12.51%
	IAC 25	21.9±2.90	14.4 ±5.37	9.9 ±6.99	14.0 ±6.93								
	Lemont	17.9±3.78	19.6 ±5.41	16.1 ±7.73	19.5 ±4.00								
Root surface area (cm^2)	**Azucena**	171 ±37	124 ±57	101 ±50	92 ±29	9.01 **0.005**	0.57 0.455	0.31 0.732	3.01 0.092	0.85 0.434	1.21 0.311	1.15 0.330	14.30%
	IAC 25	185 ±25	109 ±44	74 ±62	103 ±70								
	Lemont	131 ±41	149 ±45	112 ±58	133 ±27								
Root average diameter (µm)	**Azucena**	279 ±32	232 ±13	230 ±43	230 ±35	8.52 **0.006**	1.53 0.224	1.39 0.262	1.56 0.221	0.18 0.834	1.20 0.313	1.13 0.335	12.44%
	IAC 25	269 ±10	238 ±21	222 ±48	222 ±40								
	Lemont	228 ±29	242 ±25	216 ±14	217 ±14								
Root Volume (cm^3)	**Azucena**	1.21±0.39	0.73 ±0.37	0.61 ±0.44	0.55 ±0.23	9.80 **0.004**	1.39 0.246	0.03 0.973	3.28 0.079	0.62 0.543	1.44 0.250	1.23 0.304	16.09%
	IAC 25	1.25±0.19	0.66 ±0.29	0.46 ±0.45	0.62 ±0.52								
	Lemont	0.77±0.32	0.90 ±0.32	0.62 ±0.34	0.72 ±0.16								
Root tips (divided by1000)	**Azucena**	14 ±1.7	14 ±4.6	12 ±4.8	11 ±0.87	4.47 **0.042**	0.04 0.838	3.14 0.056	2.27 0.141	0.29 0.754	0.25 0.779	1.09 0.346	10.45%
	IAC 25	18 ±2.7	13 ±4.3	9.4 ±6.02	14 ±4.9								
	Lemont	17 ±1.9	17 ±4.9	14 ±5.5	17 ±2.8								

ANOVA output and R^2; T, topsoil treatment; W, soil wash treatment; C, cultivar (Azucena, IAC 25 and Lemont); degrees of freedom between brackets;* F ratio above and probability value below. The factors and interactions in bold are significant.

Table 5: Analysis of variance and average elemental (P, C, N and Mn) concentration and content present in shoot of three rice cultivars grown with four different inocula treatments. Mean = 5 and appropriate standard deviation

		Live topsoil		Autoclaved topsoil		ANOVA#							
Parameters	**Cultivar**	**Live was**	**Autoclaved wash**	**Live Wash**	**Autoclaved wash**	**T (1)**	**W(1)**	**C(2)**	**Tx (1)**	**TxC (2)**	**WxC (2)**	**TxWxC (2)**	**R^2**
Shoot N conc. (mg g^{-1})	**Azucena**	38.4 ±3.2	40.8 ±1.95	34.7±10.1	34.2±1.4	10.64*	0.22	9.74	0.01	0.07	0.75	2.14	34.77%
	IAC 25	32.5 ±5.1	32.1 ±2.3	30.9±5.5	25.3±0.23	**0.002**	0.645	**0.000**	0.934	0.933	0.478	0.132	
	Lemont	40.0 ±2.1	36.4 ±5.2	32.2±0.73	36.2±0.52								
Total N in shoot (mg)	**Azucena**	11.5 ±2.9	13.4 ±2.6	4.9 ±1.4	6.1 ±1.6	75.95	0.00	2.08	1.00	1.23	2.40	0.62	63.20%
	IAC 25	13.4 ±4.1	9.5 ±3.6	4.4 ±2.8	3.8 ±0.12	**0.000**	0.958	0.140	0.325	0.304	0.105	0.542	
	Lemont	9.6 ±2.1	9.5 ±2.1	3.9 ±0.88	5.6 ±1.5								
Shoot C conc. (mg g^{-1})	**Azucena**	397 ±13	403 ±20	373 ±15	382 ±6.05	3.04	0.02	1.15	1.25	2.35	0.49	2.27	12.01%
	IAC 25	387 ±13	387 ±24	384 ±10	374 ±11	0.090	0.894	0.328	0.271	0.110	0.615	0.118	
	Lemont	400 ±7.1	380 ±26	384 ±13	404 ±10								
Total C in shoot (mg)	**Azucena**	119 ±28	132 ±24	56 ±18	68 ±16	68.96	0.03	2.64	1.72	2.29	1.70	0.86	62.52%
	IAC 25	158 ±34	115 ±45	52 ±30	57 ±0.7	**0.000**	0.862	0.086	0.199	0.116	0.198	0.433	
	Lemont	96 ±20	100 ±18	47 ±9.1	63 ±17								
C/N Ratio	**Azucena**	10.4 ±0.52	9.9 ±0.09	11.4±2.89	11.2±0.33	12.56	0.24	12.95	0.24	0.15	1.21	1.67	40.66%
	IAC 25	12.1 ±1.57	12.1 ±0.44	12.7±2.23	14.8±0.37	**0.001**	0.629	**0.000**	0.628	0.859	0.311	0.203	
	Lemont	10.0 ±0.49	10.6 ±1.09	11.9±0.49	11.2±0.11								
Shoot P conc. (µg g^{-1})	**Azucena**	976 ±374	1274±268	911 ±264	648 ±241	2.43	0.00	0.29	1.13	1.04	0.04	1.00	0.00%
	IAC 25	863 ±282	944 ±423	918 ±578	851 ±207	0.128	0.946	0.749	0.295	0.365	0.960	0.379	
	Lemont	1071±396	980 ±291	938 ±100	940 ±142								
Total P in shoot (µg)	**Azucena**	306 ±163	418 ±135	139 ±76	120 ±70	29.07	0.17	0.76	0.00	0.85	0.21	0.87	32.60%
	IAC 25	346 ±132	294 ±232	94 ±16	130 ±34	**0.000**	0.680	0.475	1.000	0.438	0.811	0.429	
	Lemont	265 ±119	250 ±52	116 ±37	143 ±14								
Shoot Mn conc. (mg g^{-1})	**Azucena**	5.75 ±0.21	4.78 ±0.28	6.71±3.15	6.65±0.98	0.28	0.24	12.75	0.01	2.05	0.20	0.50	28.17%
	IAC 25	8.13 ±3.03	8.12 ±2.94	8.82±2.24	9.32±2.23	0.601	0.625	**0.000**	0.911	0.143	0.818	0.610	
	Lemont	5.46 ±0.71	5.74 ±1.99	5.00±1.27	3.44±0.86								
Total Mn in shoot (mg)	**Azucena**	1.72 ±0.43	1.57 ±0.38	1.06±0.66	1.19±0.35	17.92	0.06	7.65	0.46	1.75	0.14	0.69	39.95%
	IAC 25	3.21 ±1.16	2.49 ±1.66	1.15±0.69	1.42±0.37	**0.000**	0.813	**0.002**	0.500	0.189	0.872	0.509	
	Lemont	1.29 ±0.15	1.51 ±0.61	0.61±0.23	0.52±0.07								

ANOVA output and R^2; T, topsoil treatment; W, soil wash treatment; C, cultivar (Azucena, IAC 25 and Lemont); degrees of freedom between brackets; *F ratio above and probability value below. The factors and interactions in bold are significant. Element content in shoot = element concentration in shoot (mg g^{-1}) x shoot dry mass (g).

The strong influence of topsoil treatment and the absence of wash effect on the measured variables of plant growth and shoot elements suggest that it may be microbes in the soil that stimulate P dynamics and not in the wash used in this study. The wash was filtered so that it might be possible to conclude, if it was shown to stimulate growth in sterile soil, that bacteria were the component with positive effect. This relied on an assumption that filtering removed fungi, not bacteria. However, the wash had no effect. It is not possible therefore to state that either bacteria or fungi are the microbes responsible for the positive effect of live soil on rice growth. It is expected that the wash will contain very little mycorrhizal inocula because it is likely that root fragments, mycelium and even the majority of mycorrhzal spores will be removed by filtering (David Johnson, personal communication). It was anticipated that the wash would remove fungi but not bacteria. However, when tested on PDA plates, it contained fungi. It clearly is not possible, therefore to conclude that mycorrhizae are the predominant organism responsible for the growth promotion, although this possibility does exist. More experiments would be required to determine the details of the microbical community active in these experiments. In summary, two sources of incula (topsoil and wash) were used and it was found that plant growth and elemental status in shoot were significantly affected by topsoil treatment and cultivar but there was no significant effect of the wash and there was a weak cultivar effect with no evidence of any interaction suggesting that the microbes involved in the response are not easily transferred in water. Several observations relating to cultivars are clear. First, IAC 25 seems to take up more Mn than Azucena. Second, IAC 25 seems to have a higher PUE than Azucena. Third, it seems Azucena has a higher root to shoot ratio than IAC 25. It is known that IAC 25 has a higher internal PUE than Azucena (29) and can accumulate more biomass than Azucena with less P cost due to its less tissue P concentration. Moreover, rice plants are differing in root growth traits (20). So it is imperative therefore to explore the genetic traits associated with P uptake and PUE. Hence, a survey to discriminate the differences between large numbers of rice cultivars may be useful to understand how rice plants interact with soil P in P starving condition. This should contribute to further our understanding on how to enhance P efficiency of an elite rice cultivar for sustainable agricultural production.

It was found that topsoil treatment affected plant growth of all cultivars and they grew better in the presence of inocula compared to autoclaved soil. Genetic variation between cultivars participated in the differences observed in element uptake and final plant height. Most importantly, wash was found to have no significant effect upon all measured variables, which implies that growth promoting microbes in soil were not transferrable with wash in this study.

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