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Title

Variations in phosphorus acquisition from sparingly soluble forms by maize and soybean in low- and medium-P soils using P-32

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Introduction

Soils characterized by low phosphorus (P) availability are widespread globally (Raghothama and Karthikeyan 2005) and for these soils to be agriculturally productive, they require regular applications of water-soluble superphosphate or ammonium phosphate fertilizers. These soluble phosphates applied to P deficient soils are retained by iron (Fe), aluminum (Al) and calcium (Ca) ions and are virtually unavailable to most plant species. Plants differ greatly in their ability to grow on low P soils because they have developed specific physico-chemical mechanisms/processes to utilize P compounds in these low P fertility soils. (Hiradate et al. 2007) Evaluating and identifying crop plants for genotype variation in their ability to access and utilize sparingly soluble forms of soil P (Ca-P, Al-P and Fe-P) has been proposed as a possible means for overcoming P deficiency stress in soils and optimize P fertiliser use in cropping systems where P is poorly available (Narruzzaman et al. 2006).

Radio-isotopic P techniques using the principle of isotopic exchange allow measurement of the amount of orthophosphate that can be transferred from the soil solid to the solution in a given time and thus provide a powerful alternative means for characterizing soil P availability and sources of P pools to plants in soil-plant systems (Buhler et al. 2003). The study aimed to evaluate the differential ability of maize and cowpea to access and utilize phosphorus from different soil P pools using a low-P (Hungarian) and a medium-P (Waldviertel) soil.

Materials and methods

Two experiments were simultaneously set up for non-labelled (without ^{32}P) and labelled (with ^{32}P) on 2 different soils using maize and soybean. The experiment was a factorial design with 2 crop species (maize and soybean), 2 soil (low- and medium available P and 4 replications.. A low-P soil from Hungary (total P 302, available P 21 [Bray PII], 13.3 $mg.kg^{-1}$ [Olsen P], pH 5.6 (KCl) classified as Dystric Eutrocrepts, and a medium-P soil (Waldviertel) from Austria (total P 513, available P 46 (Bray P2), 13.3 $mg.kg^{-1}$ Olsen P, pH 5.6 (KCl) was used (see **Table 1** for detailed physical and chemical characteristics of the two soils). Each pot received basal fertilizer equivalent to 200 kg N ha^{-1} as ammonium sulphate and 50 kg K ha^{-1} as potassium chloride. To ensure uniform labelling of the soil, 5 portions of dilute $K_2H^{32}PO_4$ solution (50 ml for Waldviertel and 30 mL for Hungarian soils were applied to a layer of 200 g soil) to achieve 70% of the water holding capacity of each soil. A total of 250 ml for the Waldviertel and 150 ml for the Hungarian containing 12.4 MBq (335μCi) of a $K_2H^{32}PO_4$ solution was applied to each of the 20 pots containing 1 kg soil.

A maize variety (DK 315 from Austria) and a soybean variety (TGX 1910-4F from IITA, Nigeria) were sown at one per pot immediately after the addition of ^{32}P and inoculated with a *Bradyrhizobium japonicum* solution in a glasshouse with a temperature regime of 34/21^{o}C day/night and relative humidity of 40-70%. Plants were sampled at 42 days after sowing (42 DAS) from the labelled and at 60DAS from the non-labelled pots. Soil samples (10-12 g) was taken at 0, 1, 5, 42 and 60 DAS, oven-dried at 70^{o}C for 18 h, milled and a portion was used for analysis. The harvested plant samples were separated into shoots (labelled) and shoot and roots (labelled), chopped into small pieces, oven dried, weighed, and grinded. The root dry weight of

the radioactive plants was estimated using the root/shoot ratio of the non-radioisotope plants. Total P in soils was determined using the colorimetric method after acid digestion, and available P (Bray P II and Olsen) was determined by colorimetric method after extraction. The inorganic soil P fractions were measured according to a fractionation scheme based on the Sekiya method (Otani T and Ae N 1997). Briefly the fractionation involved a sequential extraction of Ca-P (300 mg of soil extracted with acetic acid), Al-P (extracted with ammonium fluoride after the extraction of Ca-P) and Fe-P (soil after extraction of Al-P was washed twice with saturated sodium chloride and discarded and then extracted with and sodium hydroxide) and the P in extracts were determined by colorimetric method. The ^{32}P radioactivity in all the fractions (total-P, available-P, Ca-P, Al-P and Fe-P) was measured by liquid scintillation spectrometry (Packward 2000) using a flour solution consisting of 1mL solution and 9 mL of Aquasol-2 (NEN research product). Similarly, phosphorus in the ground plant materials were wet digested in a 4 ml H_2SO_4 and 3 ml H_2O_2 for 2 min till digest is colourless, and aliquots of the samples were diluted and the total P measured by the method of Murphy and Riley (1962) and ^{32}P was determined by the Liquid Scintillation Counter. The P in seed of maize and soybean was determined after five seed samples each of 100 mg were grinded and acid digested.

Table 1 Selected physical and chemical properties of soils used for the experiment

Properties	**Hungarian**	**Waldviertel**	**Units**
Sand	830	273	$g\ kg^{-1}$
Silt	88	582	$g\ kg^{-1}$
Clay	82	145	$g\ kg^{-1}$
Bulk Density	ND	1.29	$g.cm^{-3}$
Particle Density	ND	2.65	$g.cm^{-3}$
Pore volume	ND	0.51	%
Water content (saturation)	ND	47	%
pH (H_20/KCl)	5.5/4.6	6.5/6.0	
Total P	302	502	$mg\ kg^{-1}$
Available P (Bray/Olsen)	21/13.5	44/12.8	$mg\ kg^{-1}$
Inorganic P (Ca/Al/Fe)	36/85/65	56/144/68	$mg\ kg^{-1}$
EC (25°C)	ND	166	$\mu S.cm^{-1}$
Total N	0.83	1.21	$g\ kg^{-1}$
Organic carbon	7.91	20	$g\ kg^{-1}$
Ca (cobalthexamine)	1.82	13.69	$cMol.kg^{-1}$
Mg(cobalthexamine)	0.61	3.13	$cMol.kg^{-1}$
K(cobalthexamine)	0.09	0.15	$cMol.kg^{-1}$
Na(cobalthexamine)	0.04	0.06	$cMol.kg^{-1}$
CEC(cobalthexamine)	2.66	23.59	$cMol.kg^{-1}$

ND-- Not determined

Results and discussions

Plant growth and P uptake

The shoot and root biomass of both maize and soybean were significantly greater when grown on the Waldviertel soil than on the Hungarian soil, and there was a significant increase in shoot dry weight from 42 to 60 days after sowing **(Fig.1)**. Maize and soybean grown in the Hungarian soil had low shoot P concentrations (<1mg g^{-1} P) suggesting a strong P limitation. Plants showed symptoms of P deficiency after 42 DAS and produced three time less biomass than when grown in the Walviertel soil. The P concentration in shoot tissue of maize (1.1-1.5 mg g^{-1} P) was low compared to that in soybean (1.8-2.2 mg g^{-1} P) and decreased with plant age for maize and not for soybean (**Fig 2**). In the low available P soil, total P accumulation in shoot was higher in maize than in soybean suggesting that maize has higher ability to take more P from low available P soils that soybean. The Hungarian soil used had 21 mg kg^{-1} (Bray II) and 13.5 mg kg^{-1} Olsen) P, and for 1 kg of soil, more than 21 mg of P is expected to be available to the plant. suggesting that the available P extraction using Bray II may contain other P that is not easily available to maize and soybean for growth (Tiessen and Moir 1993). No nodules were observed on the roots of soybean grown in the Hungarian soil despite the rhizobium inoculation, whereas a lot of nodules (0.6 m $plant^{-1}$ DW) were observed on soybean roots grown on the Waldviertel, suggesting that nodule formation in the soybean was severely impaired by P deficiency (Adu-Gyamfi et al. 1989).

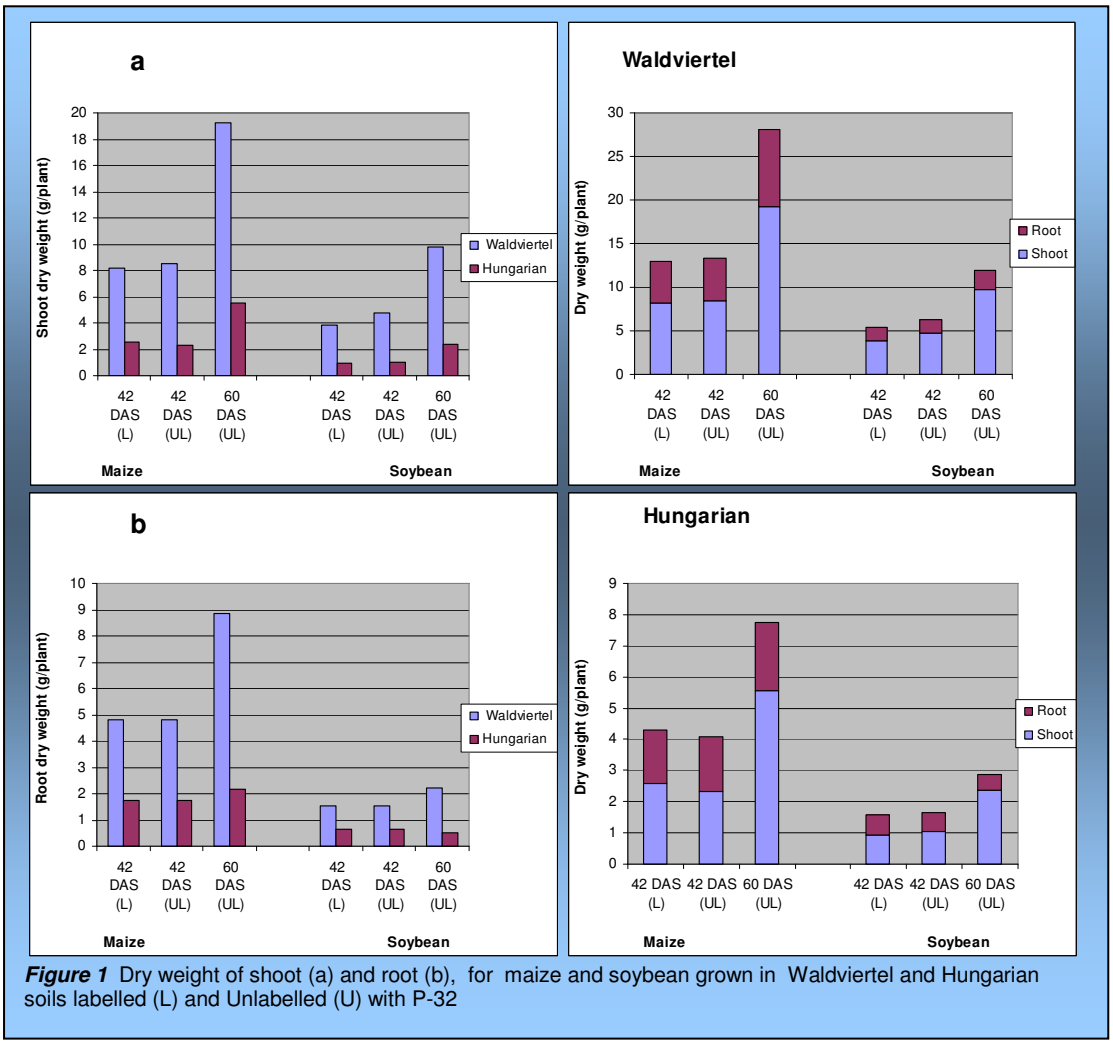


Figure 1 Dry weight of shoot (a) and root (b), for maize and soybean grown in Waldviertel and Hungarian soils labelled (L) and Unlabelled (U) with P-32

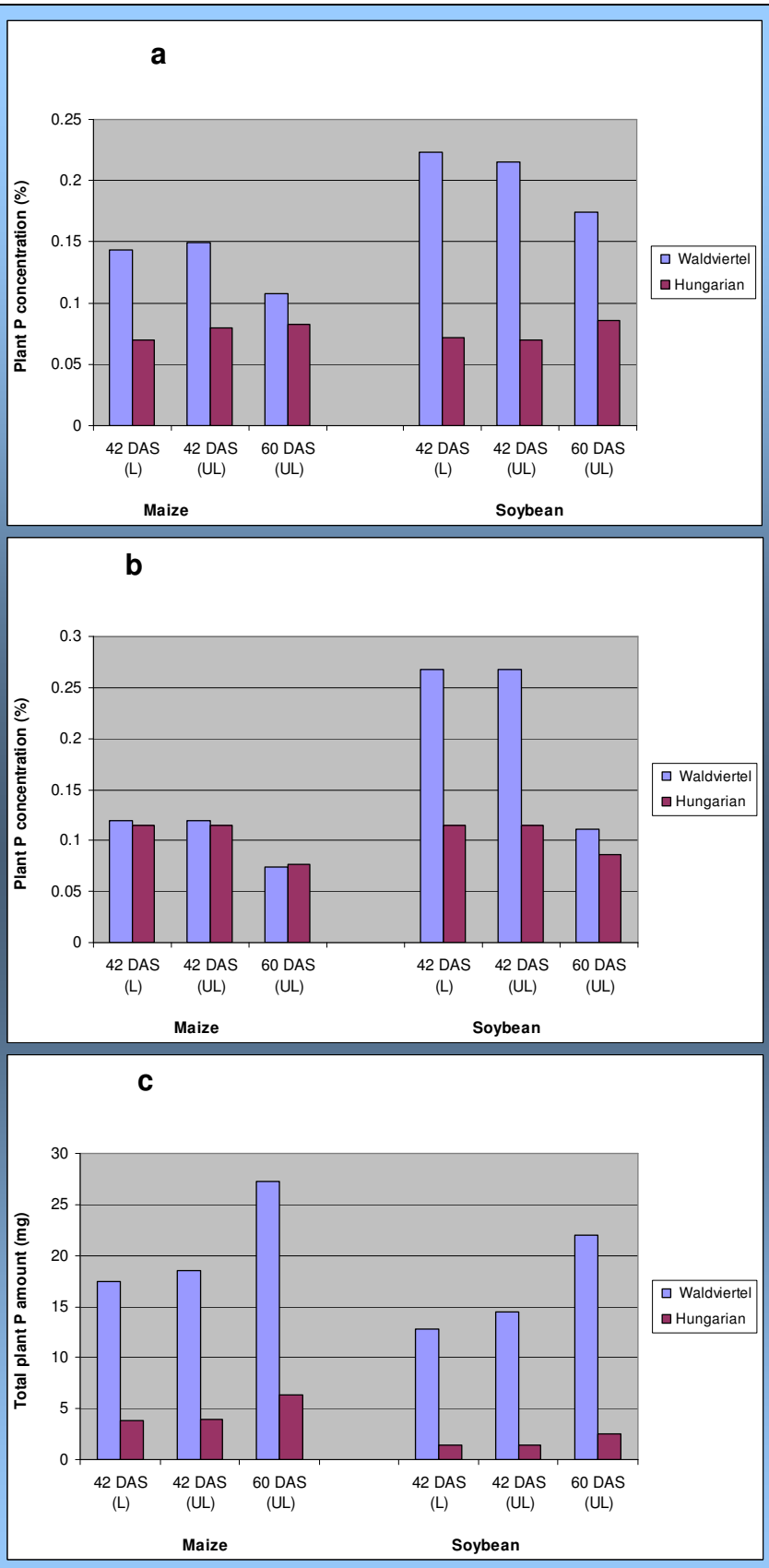


Figure 2 P concentration of shoot (**a**) and root (**b**) and plant P amount (**c**) for maize and soybean grown in 2 different soils. For legend see Figure 1

Dynamics of P fractions

Available P decreased significantly in Hungarian soil from 33 at 0 DAS to 27 at 60 DAS and for Waldviertel from 56 to 37.5 (mg kg^{-1}). All the soil P fractions (except Al-P than increased slightly) decreased from 0 DAS to 60 DAS in the two soils used. Aluminum-P and Fe-P were the highest inorganic fractions extracted by the Sekiya method, and in the Waldviertel soil the Al-P fraction was more than 2 times the amount in the Ca-P and Fe-P fractions at the beginning of the experiment. The Bray II and the Ca-P were the fractions depleted most by plants followed by the Fe-P fractions in the two soils, and differences observed between the crops were not significant **(Fig 3)**. Our results suggest that more P was released in the soil solution (labile) from the available and the Ca-P fractions for plant uptake than from the Fe- and Al-P fractions.(van Ray and van Diest 1979). Available P defined as the quantity of P which will come into solution for plant uptake during its life cycle, is both time- and plant-specific. Therefore there is a high probability that the depletion of P from the Bray II P-fraction may also include soil Ca- and Fe-P fractions. Neither maize nor soybean did deplete significant amount of P from the Al-P fractions **(Fig 3)**, although it has been reported that some crops such as groundnut, pigeonpea (Ae et al. 1990, Fujita et al. 1995) has the ability to take up P from Fe- and Al-P fractions through the released of carboxylic anions release from roots.

Among the inorganic pools, Al-P, Fe-P and Ca-P are the three major fractions in the soil. Our results imply that when P supply is limited, maize and soybean are able to access mainly Fe- and Ca-P sparingly soluble pools and not Al-P from the soil. Alternatively, the plant P uptake resulted in a considerable decrease in Bray II P with time, which would enhance dissolution of the sparingly soluble P. This is supported by the fact that the changes over time in available P were more sensitive than changes in total P as observed by Song et al. (2007)

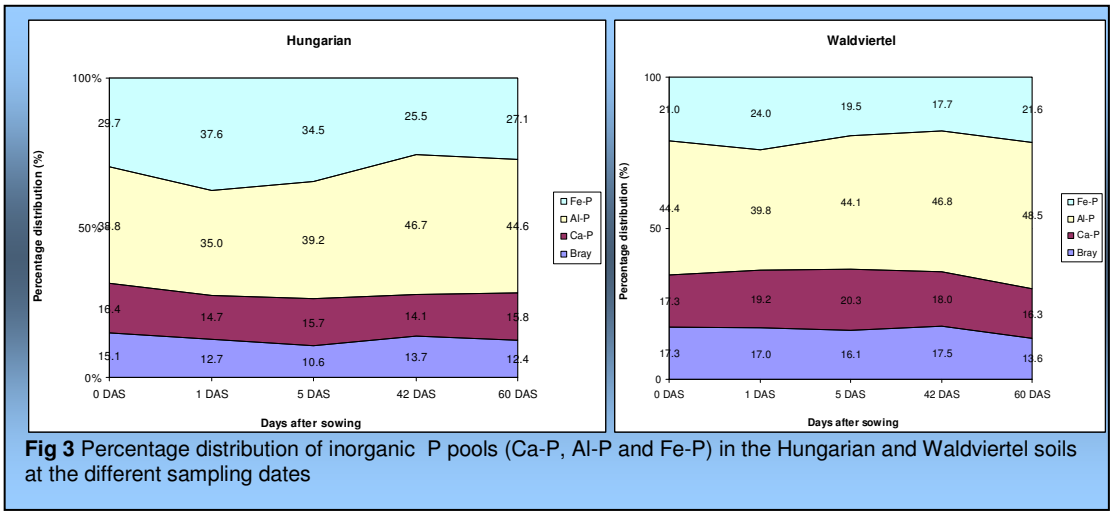


Fig 3 Percentage distribution of inorganic P pools (Ca-P, Al-P and Fe-P) in the Hungarian and Waldviertel soils at the different sampling dates

Isotopic ally exchangeable parameters and P uptake by maize and soybean in low- and medium-P soils

The high total radioactivity in maize compared to soybean suggests that plant P uptake from soil was higher by maize than by soybean irrespective of the soil used. There was a 1.4 times increase in radioactivity in maize over soybean in the

Waldviertel soil, but a 4.3 times increase by maize over soybean in the Hungarian soil **(Fig 4)**. Our data suggests that maize could take up more P at low-available P conditions than soybean. This is supported by the fact that the L value (with seed P uptake correction factor) of maize was two times higher than that of soybean in the low-P Hungarian soil even though Buhler et al. (2003) and Fossard (1994) observed that determination of E and L values is not precise enough to identify plant species or cultivars able to take up P from slowly or non-exchangeable p pools, or to quantify precisely the rate at which P in the soil organic matter is mineralized. The differential ability of cotton, wheat and white lupin to utilize P from $NaHCO_3$-Pi and HCl-Pi fractionations in the rhizosphere under P deficient conditions has been reported (Wang et al 2008).

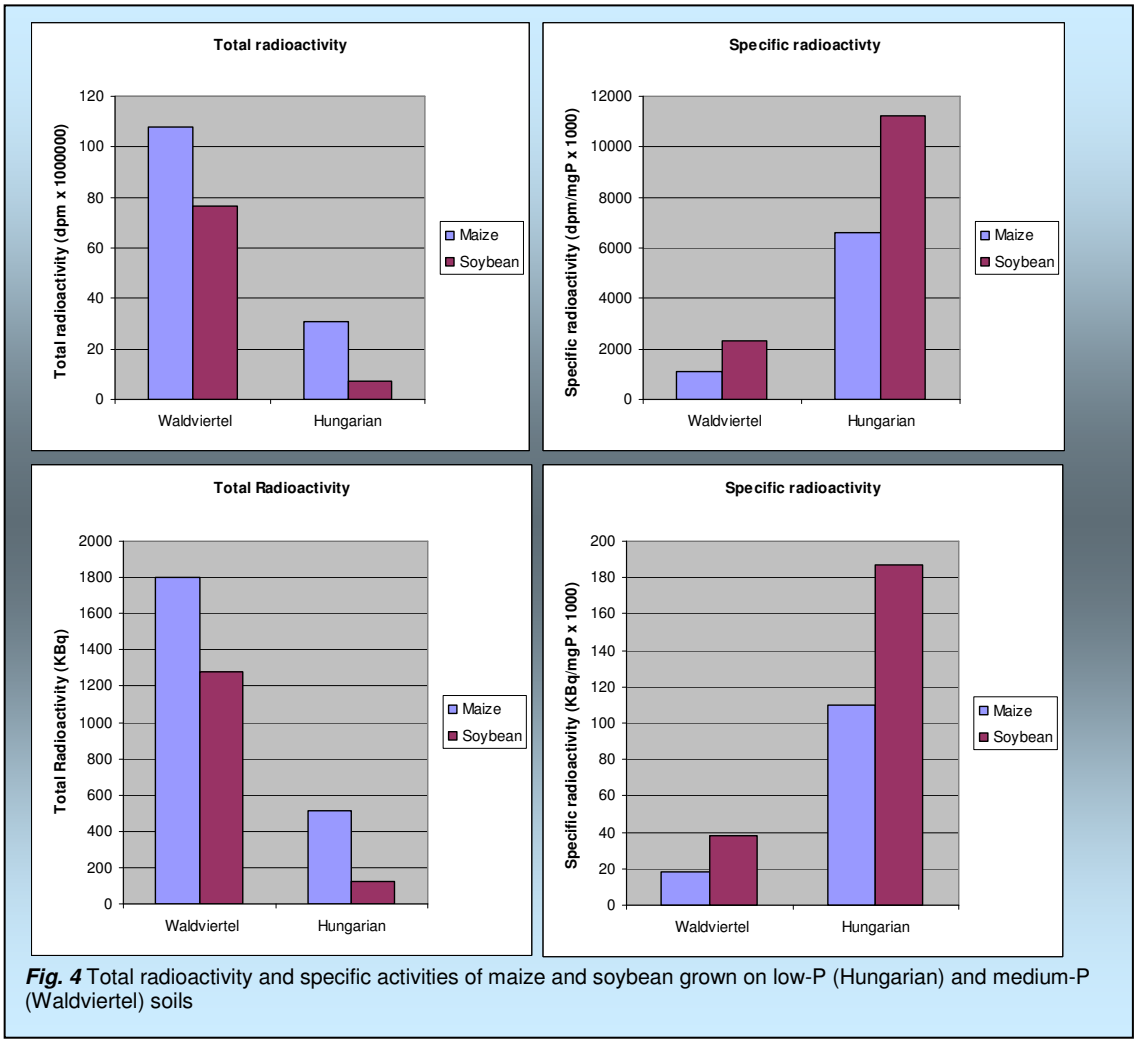


Fig. 4 Total radioactivity and specific activities of maize and soybean grown on low-P (Hungarian) and medium-P (Waldviertel) soils

Maize and soybean grown in the medium-P Waldviertel soil had lower specific radioactivity (dpm x 10^3. mgP^{-1} or KBq 10^3. mgP^{-1}) in shoot than those grown on the low-P Waldviertel soil and the values were lower in maize than in soybean **(Fig.4)**. Low specific radioactivity indicate that plants were using otherwise unavailable P sources. Our results therefore suggest that maize was more efficient to take up P from sparingly soluble inorganic-P sources than soybean.

Conclusions

The main finding from this study is that maize was more efficient to take up P from sparingly soluble inorganic-P sources than soybean in the medium-P Waldviertel soil as indicated by the low specific radioactivity (dpm x 10^3. mgP^{-1} or KBq 10^3. mgP^{-1}) in shoot. The L-values (with seed P uptake correction factor) of maize was two times higher than that of soybean in the low-P Hungarian soil suggesting the superiority of maize to access sparingly soluble P from soils compared with soybean. When soil P is limited, maize and soybean are able to access P mainly from the available P (Bray II), Fe- and Ca-P sparingly soluble fractions and not Al-P from the soil. Maize and soybean showed severe deficiency when grown on the Hungarian soil and the P concentration in plant was below (<1mg g^{-1}) suggesting that Bray II overestimated the available/labile P fractions in the soil.

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