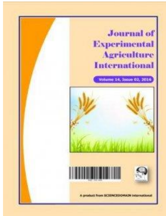

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Phosphorus Efficiency in Brazilian Soybean Cultivars

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Authors' contributions

This work was carried out in collaboration between all authors. Author IPB carried out the experiment, managed the analyses of the study, performed calculations, managed the literature searches, interpreted the results, wrote the first draft of the manuscript and finished the paper with the others authors suggestions. Author AS performed calculations, managed the literature searches, helped with results interpretation and writing the paper. Author VIF helped in designing the study, performed the statistical analysis and helped with the discussion. Author NCM helped to carry out the experiment, managing the analyses and discussion. Author TM designed the study, helped in carrying out the experiment, managed the analyses and performed calculations. All authors read and approved the final manuscript.

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ABSTRACT

The primary goal of this study was to evaluate the phosphorus uptake and use efficiency in Brazilian soybean cultivars, besides root morphology and architecture characteristics related to phosphorus uptake, carrying out two greenhouse experiments. The experiment 1 was completely

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randomised, with 56 treatments (soybean cultivars) and 3 replicates. Experiment 2 was completely randomised design with three replicates, and the ten cultivars (greater and lower efficiency) were selected for this assay according to their ability to absorb phosphorus less available determined in experiment 1.
The study was carried out at Center of Nuclear Energy in Agriculture, University of São Paulo, Brazil, between February 2011 and August 2012. The isotopic dilution technique was used in the first experiment to assess the phosphorus availability in the soil and to determine the ability of plants to access labile phosphorus by measuring the specific activity of plants grown in soil labeled with radioactive phosphorus. Nine cultivars showed greater phosphorus uptake and used efficiency. The second experiment evaluated the root morphology and architecture. The cultivars with greater uptake efficiency have root morphology and architecture characteristics that favour acquisition of phosphorus from soil compartments that are inaccessible to other cultivars. Phosphorus uptake by plants was not affected by soybean seeds phosphorus content. Identification of these cultivars is very important because it could enable soybean farming in low fertility soils, reducing fertiliser dependence.

Keywords: L value-seed; ^{32}P; root architecture; root morphology.

1. INTRODUCTION

Soybean (*Glycine max* L.) occupies a prominent place in human diet and animal feed. Phosphorus (P) availability is one of the limiting factors for attaining high yields in soybean. In general, tropical soils have low P content; therefore, P supply through fertilisation is imperative. However, even soils that have received phosphate fertilisation for successive years may present low P content in the soil solution due to P fixation in clays, which occurs mainly in weathered soils [1]. Phosphate fertilisers are produced from phosphate rocks, which are finite and unsubstituted resources, making their depletion inevitable. In this scenario, the search for solutions that guarantee the sustainable supply of P for agricultural crops is urgent [2]. A very promising approach to address P scarcity in agriculture is the selection of efficient genotypes in P uptake and utilisation.

Different plant species, or even different cultivars of the same species, have different P uptake abilities, which is due to: the physiological capacity to absorb P in diluted solutions; exudation of organic acids that are able to solubilise P adsorbed in the soil solid phase; greater or lesser root system development [3]; or morphology and root architecture characteristics [4]. Studies on the selection of P-uptake efficient species and cultivars have already been conducted using different methodologies and agricultural crops [3,5-8].

Phosphorus uptake efficiency can be evaluated by using the isotopic dilution technique, which is a direct measure [3,5] and is related to the amount of available P in the soil. Plants absorb P from the soil solution, which presents low concentrations of this element, necessitating its constant and rapid replacement through the dissolution of the labile phosphate. The labile P is determined with greater precision by using isotopic exchange with the ^{32}P radioactive phosphate. Among the established procedures to determine the labile P is the *L value* calculation from the specific activity [9,10], which the plant itself acts as an extractor instead of a chemical reagent. The *L value* represents the amount of P in the soil and the soil solution accessible to isotopic equilibrium, and is, therefore, an estimate of the total amount of P that is available to the plant (isotopically exchangeable P). Therefore, the *L value* can be used to identify the more efficient cultivars in P uptake from soils compartment with less readily available P. In experiments using the ^{32}P radioisotope, if all plants extracted P from the same compartment, the shoot specific activity of all plants should be compatible, although the accumulated amount of P in shoots could be different [3]. As the *L value* is derived from the specific activity we can apply the same logic to this parameter.

Some authors consider that the P content of seed can also influence plant P uptake efficiency. Gunes et al. [6] suggested that the difference in responses to P deficiency in soil between different wheat genotypes was due to the unequal P content of the seeds. In contrast, Ozturk et al. [7] and Silva et al. [8] concluded that although there were differences among cultivars in P reserves of wheat seeds, this variation did not influence the P uptake efficiency. Therefore, another advantage of isotopic dilution technique is the possibility to account for the plant P derived from the seed in the *L value* calculation, obtaining the so-called *L value-seed*. Since the P uptake efficiency is directly proportional to the *L value* and *L value-seed* thus, the greater the *L value-seed*, the more efficient the plant is to absorb P from soil compartments with less available P.

Change in the root architecture, which determines root system spatial configuration, is among the many mechanisms employed by plants to access the soil compartments with less available P [4,11]. The root system presents a wide variation in its architecture, varying with plant species, genotypes in the same species, or even different parts of the same root system. Differences in root characteristics are a consequence of domestication and plant breeding, which have often led to contrasting spatial arrangements of roots [12]. The importance of root architecture stems from the fact that many soil resources are unevenly distributed, making the root configuration vital for the plant to explore these resources with greater or lesser effectiveness. In general, the soil surface layer of agricultural or natural soils is where greater P content is concentrated. Consequently, root characteristics that improve soil exploration in this layer are important for P acquisition. Thus, the most efficient plants in P uptake are those that can detect and make modifications in the root system as the nutrient availability spots change [4].

Optimisation of P use in agricultural systems is not only achieved via greater plant P uptake efficiency but also via greater P use efficiency. There is little benefit to a plant that is very efficient in P uptake if the yield (fruits, vegetative part or roots) is low. Phosphorus use efficiency in plants can be achieved via several ways [13-16]. From a physiological perspective, Rose et al. [17] state that plants with greater P use efficiency operate with lower shoot P concentration. Therefore, this approach is quite appropriate when it comes to selecting genotypes more adapted to access the soil compartment with less available P.

Considering the importance of soybean to the world economy, the requirement for adequate P supply and the future shortage prospect of this element, knowledge of cultivars with greater ability to access the less available soil P compartments is certainly a path to agricultural sustainability. The objectives of this work were: to select Brazilian soybean cultivars with greater ability to access the less available P compartments in the soil and with greater P use efficiency; to verify if the seed P influences P uptake by the plant; and to identify the root morphological and architecture characteristics related to greater efficiency in accessing less available P soil compartments.

2. MATERIALS AND METHODS

Two greenhouse experiments were carried out at the Center of Nuclear Energy in Agriculture, University of São Paulo, Piracicaba - SP. The soil used in both experiments was collected from a soil layer of 0-20 cm, is classified as Typic Haplustox [18]. The soil had a clayey average textural class (706 g kg^{-1} of sand, 43 g kg^{-1} of silt, and 251 g kg^{-1} of clay), of which the chemical characteristics are showed in Table 1. Soil liming was performed using calcined limestone to increase the base saturation to 60% [20]. Soil moisture was maintained at around 70% of the maximum water holding capacity throughout the cultivation period in both experiments. To procedure the soil moisture maintenance, firstly the soil was thoroughly watered to saturation. The weight was taken when there was no sign of water dropping at the bottom of the pots (maximum water holding capacity). The pots were weighed every three days and the reduction of pot weight was used to calculate relative water losses to be replaced.

2.1 Experiment 1 - P Uptake and Utilisation Efficiency

This experiment was carried out to select Brazilian soybean cultivars that are able to access and use P from soil compartments with less available P, and to verify if the P content of the seeds influences the absorption of P by the plant. The experimental design was completely randomised, with 56 treatments (soybean cultivars recommended for south/southeast and center-west Brazilian regions) and 3 replicates. Plastic pots (3.0 L) were coated with plastic bags and filled with 2 kg of air-dried soil. To guarantee some P reserves, as the soil has a low content of this nutrient [20], 20 mg kg^{-1} of P as triple superphosphate and 150 mg kg^{-1} of P as Patos natural phosphate were applied. The soil was incubated for 30 days, with moisture maintained at 70% water holding capacity.

Table 1. Soil chemical characteristics used in the cultivation of soybeans under greenhouse conditions in Piracicaba – SP

pH[a]	OM[b]	P[c]	S[d]	K[c]	Ca[c]	Mg[c]	Al[e]	H+Al[f]	SB[g]	CEC[h]	V[i]	m[j]	Cu[k]	Fe[k]	Mn[k]	Zn[k]
	$g\ dm^{-3}$	$mg\ dm^{-3}$		----------------$mmol_c\ dm^{-3}$-----------------							---- %----		---- $mg\ dm^{-3}$------			
4,5	32	7	3	0,9	12	6	2	47	19,1	66	29	11	0,9	109	4,7	1,6

[a]Active acidity $CaCl_2$ (0,01 mol L^{-1}); [b]Organic matter determined by using the colorimetric method; [c]Phosphorus, potassium, calcium, and magnesium measured by ion exchange resin method; [d]Sulfur measured by using the turbidimetry method; [e]Exchangeable aluminum determined by using the titrimetric method (1 mol L^{-1}); [f]Potential acidity determined by pH measurement using the Shoemaker-McLean-Pratt (SMP) method; [g]Sum of the bases; [h]Cation exchange capacity; [i]Base Saturation (100 × SB/CEC); [j]Aluminum saturation (100 × Al^{3+}/effective CEC); [k]Copper, iron, manganese, and zinc: Method of extraction with DTPA and analysis using an atomic absorption spectrophotometer [19]

At the end of the incubation period, a phosphoric acid labeled with 9.62 MBq of ^{32}P solution and 2 mg kg^{-1} P as a carrier, previously added in 20 g of sand, was applied in each pot. The soil was homogenised with the sand inside the plastic bags, and then the pots were irrigated with 0.5 L of deionised water. The plastic bags remained closed for three days in order to achieve isotopic balance between ^{32}P and ^{31}P.

Three days after labelling the soil with the radioisotope, four soybean seeds of each cultivar were sown per pot. Six days after sowing, the emergence of seedlings occurred in most of the pots, and the seedlings were thinned in the 1st trifoliate stage, leaving two plants per pot. Nitrogen supply was guaranteed by the inoculation of the soybean seeds, which was carried out on the same day of sowing, with the bacteria *Bradyrhizobium japonicum* (SEMIA 5079 e 5080) strain. Three weeks after sowing, top dressing with macro and micronutrients was performed based on the soil analysis and recommendation for soybean cultivation [20].

The following cultivars recommended for different Brazilian regions were evaluated in this work:

- Brazilian South/Southeast region: BRS 183, BRS 212, BRS 230, BRS 7860RR, BRS Macota, IAC Foscarin 31, CD 212RR; BR 16, BRS 245 RR, CD 201, BRS 260, BRS 268, BRS Invernada, BRS Raiana, IAC-24, BRSMG 750SRR; BRS 233, BRS 256RR, BRS Pala, BRS Cambona, BRS Candiero, CD 211, BRS Favorita RR, BRSMG 68 [Vencedora], BRSGO 204 [Goiânia], BRSMG 250 [Nobreza], Valiosa RR, and BRSGO Edéia.
- Brazilian Midwest region: BRS 217 [Flora], BRS 218 [Nina], BRS Milena, BRS Rosa, BRSGO Caiapônia, BRSGO Santa Cruz, BRSMG Segurança, CD 219RR, CD 227, BRS 252 [Serena], BRS Jiripoca, BRS Piraíba, BRSGO Bela Vista, BRSGO Luziânia, BRSMT Pintado, BR/Emgopa 314 [Garça Branca], BRS Aurora, BRS Celeste, BRS Gralha, BRS Nova Savana, BRS Sambaíba, BRS Tianá, BRS Raimunda, BRSGO Ipameri, BRSGO Jataí, BRSGO Paraíso, BRSMG 251 [Robusta], and BRSMG Garantia.

The stems, leaves, and petioles of the plants (shoots) were collected 40 days after emergence, with all plants still at the vegetative stage. The collected material was washed in deionised water, dried at 60 °C until constant weight, and the shoot dry mater was determined (g^{-1} pot). Each sample was milled separately in a Willey-type mill to perform the nitric-perchloric digestion [21]. Then the ^{32}P activity of the vegetative shoot was assessed in a liquid scintillation counter using the Cerenkov effect (Betaplate, Wallac, Finland), as described by Vose [22]. The shoot P concentration was determined by colorimetric analysis of the metavanadate [21]. Soybean seeds were analysed for total P content [23] to be discounted in the *L value* calculations, generating the *L value-seed*. In the calculation of the *L value-seed,* it was considered that about 65% of the total P stored in seeds is used for plant development [24].

The specific activity and the *L value* were calculated according to the following equations, based on Hocking et al. [3] and Larsen [10]:

$$SA = {}^{32}P/{}^{31}P \quad (1)$$

where: SA = specific activity (CPM µg P $plant^{-1}$); ^{32}P = radioisotope activity in the plant (CPM); ^{31}P = plant phosphorus content (µg P $plant^{-1}$)

$$L\ value = X\ (SA_0/SA_p - 1) \quad (2)$$

where: *L value* = *L value* (mg kg^{-1} of P); SA_0 = specific activity of the applied solution (CPM µg P^{-1}); SA_p = specific activity of the plant (CPM µg P^{-1}); X = amount of phosphorus applied as a carrier (mg)

The *L value-seed* was calculated according to Silva et al. [8], as follows:

$$L\ value\text{-}seed = [Y\ ((X_T - Z)/\ Y_T) - X] \quad (3)$$

where: *L value-seed* = plant phosphorus derived from the seed subtracted from the *L value* (mg kg^{-1} of P); Y = ^{32}P activity in the applied solution (CPM); X_T = plant phosphorus content (mg); Z = total phosphorus content of the seed (mg); Y_T = ^{32}P activity in the plant shoot dry matter (CPM); X = ^{31}P applied per pot as a carrier (mg).

The calculation of P utilisation efficiency was adapted from Rose and Wissuwa [14], as follows:

$$PUE = (SDM/TP).100 \quad (4)$$

where: PUE = phosphorus use efficiency (%); SDM = shoot dry matter (g); TP = total phosphorus content in shoot biomass (mg).

The results were submitted to analysis of variance (F Test, $P < .05$). When there were differences between averages, the Scott-Knott test was performed. To establish the degree of linear relationship between two variables, correlation matrices were made using Pearson correlation coefficients ($P < .05$). A graphical representation in the Cartesian plane was used to group the cultivars in terms of P uptake and utilisation efficiency. The axis of the abscissa (x) corresponds to the P utilisation efficiency, while the axis of ordinates (y) corresponds to P uptake efficiency. The cultivars with 'greater P uptake and utilisation efficiency' are presented in the first quadrant, while cultivars with 'lower efficiency in P uptake and greater efficiency in P use' are presented in the second quadrant. Cultivars with 'lower efficiency in the P uptake and utilisation' and those with 'greater efficiency in P uptake and lower efficiency in P use' are presented in the third and fourth quadrants, respectively.

2.2 Experiment 2 - Root System Evaluation

The second experiment was carried out to identify the root morphology and architecture characteristics related with efficient P uptake from soil compartments with less available P. The cultivars were selected for this assay according to their ability to absorb less available P (*L value-seed*), determined in Experiment 1.

Polyethylene pots containing 7.5 L of air-dried soil (Table 1) were used, distributed in a completely randomised design with three replicates. Four soybean seeds were sown per pot, and thinning was performed six days after emergence of the plants, leaving one plant per pot. Three weeks after sowing, top dressing with macro and micronutrients was performed, except P, according to the results of the soil analysis and the recommendation for soybean plants [20].

Twenty days after seedling emergence, the root system was collected with the aid of a water jet and a 2 mm sieve. The roots were preserved in ethanol solution (25% v/v), scanned on a scanner (EPSON Perfection V700 PHOTO, DPI 23.6 pixels), and the images were analysed using WinRhizo Pro software (Regent Instruments, Quebec, Canada), according to Silva et al. [8]. The parameters analysed from the scanned images were: total root length, total root surface area, total projected area of the roots, total root volume, and mean root diameter. All the results were submitted to analysis of variance (F Test). When differences between means were found, the Scott-Knott test was performed ($P < .05$).

The root angles were evaluated according to Trachsel et al. [25] and Silva et al. [8]. The angle of first and second ramifications of basal roots, originating near the base of the hypocotyl relative to the horizontal plane, were determined by visual evaluation, and then assigned scores from one to nine, where 'one' (1) indicates angles smaller than 10 ° (shallower roots) and 'nine' (9) indicates angles greater than 90 ° (deeper roots). Only the mean value of the root angle notes was determined, and no statistical analysis was performed.

3. RESULTS AND DISCUSSION

3.1 Experiment 1 - P Uptake and Utilisation Efficiency

No cultivar presented P deficiency visual signs. The mean shoot dry mass, total P, and *L value* are presented in Table 2. The shoot dry mass was significantly different among cultivars and ranged from 3.2 g pot^{-1} (IAC-24) to 8.2 g pot^{-1} (BRSMG Segurança). As all plants were grown under the same environment, the almost threefold difference in shoot dry matter accumulation suggests that there are differences among soybean cultivars in acquisition and use of P, which was the only limiting factor for all plants. There were no significant differences in shoot total P among cultivars, although the cultivar that presented the greater value (19.6 mg pot^{-1} for BRS Jiripoca) was almost triple the one with the lowest value (7.0 mg pot^{-1} for IAC-24), which is probably due to the large variation in the results (CV = 24%). There were significant differences in *L value* among cultivars. The cultivar BRS Nova Savana showed a greater *L value* (28.7 mg of P kg^{-1} of soil), while the cultivar BRS Sambaíba presented the lowest *L value* (16.1 mg P kg^{-1} soil). In a study using *L value* in maize, Yang et al. [26] also observed significant differences among the five evaluated genotypes regarding tolerance to low available P, including the mechanisms used to access it.

Fig. 1 shows average *L value-seed*, which represents P uptake efficiency. There were significant differences among soybean cultivars for *L value-seed*, suggesting that different soil P compartments were accessed. The cultivars BRS Nova Savana and BRS Sambaíba had the greater (28.3 mg P kg^{-1} soil) and the lowest (15.9 mg P kg^{-1} soil) *L value-seed,* respectively. The greater *L value-seed* of the BRS Nova Savana cultivar - as well as those cultivars that did not statistically differ from it - indicates that it was able to access the soil P compartments that were not available for other cultivars. Less than half of the cultivars were classified as having greater efficiency in P uptake, which shows a variation in P uptake mechanisms among these soybean cultivars (Fig. 1). This difference in cultivar ability to acquire P from soil has already been observed in other plant species. Fernandes and Muraoka [5] used the specific activity to select seven maize hybrids that are very efficient to absorb P in Brazilian savanna soil. The *L value-seed* was used by Silva et al. [8] to identify wheat cultivars with greater efficiency in the P uptake, and by Marcante et al. [27] who selected cotton cultivars using this characteristic. The *L value-seed* was also used [28,29] to classify rice and bean cultivars based on P uptake efficiency. The authors observed significant differences among cultivars in both species. The cultivar differences in P uptake efficiency probably is due to different strategies that plants have developed to extract P from soil compartments with less available P. The strategies are very diverse and can vary

Table 2. Mean values of shoot dry mass (SDM), accumulation of total phosphorus (TP) in shoots, and *L values* (*L value*) of Brazilian soybean cultivars

Cultivar	SDM*	TP*	*L value**
	g $pot^{-1(a)}$	mg $pot^{-1(a)}$	P mg soil kg^{-1}
BRS 183	5,4 b	12,0a	26,4 a
BRS 212	6,2 b	13,5 a	21,5 b
BRS 230	6,3 a	14,2 a	20,1 b
BRS 7860RR	5,2 b	13,1 a	20,4 b
BRS Macota	5,2 b	10,0 a	17,0 b
IAC Foscarin 31	5,5 b	12,7 a	24,1 a
CD 212RR	5,8 b	14,0 a	19,7 b
BR 16	7,2 a	13,7 a	19,5 b
BRS 245RR	5,3 b	11,4 a	21,3 b
CD 201	7,6 a	16,1 a	23,9 a
BRS 260	6,1 b	14,8 a	22,2 a
BRS 268	5,5 b	11,3 a	19,8 b
BRS Invernada	7,3 a	16,5 a	21,4 b
BRS Raiana	4,9 b	10,2 a	20,1 b
IAC-24	3,2 b	7,0 a	19,7 b
BRSMG 750SRR	6,5 a	14,4 a	25,6 a
BRS 233	7,6 a	14,7 a	27,1 a
BRS 256RR	5,6 b	14,3 a	22,9 a
BRS Pala	5,0 b	11,9 a	22,4 a
BRS Cambona	6,7 a	13,4 a	20,0 b

** Means followed by the same vertical letter do not differ from each other based on the Scott-Knott test (* P < .05); [(a)] Pot containing two soybean plants*

Table 2. Mean values of shoot dry mass (SDM), accumulation of total phosphorus (TP) in shoots, and *L values* (*L value*) of Brazilian soybean cultivars

(Continued)

Cultivar	SDM*	TP*	*L value**
	g pot$^{-1(a)}$	mg pot$^{-1(a)}$	P mg soil kg^{-1}
BRS Candiero	7,2 a	14,4 a	20,3 b
CD 211	5,6 b	15,0 a	18,1 b
BRS Favorita RR	5,1 b	11,6 a	22,7 a
BRS MG 68 [Vencedora]	5,9 b	12,9 a	20,1 b
BRS GO 204 [Goiânia]	6,6 a	13,6 a	21,7 b
BRS MG 250 [Nobreza]	7,0 a	12,7 a	19,2 b
Valiosa RR	6,0 b	13,6 a	19,0 b
BRSGO Edéia	6,9 a	12,4 a	24,1 a
BRS 217 [Flora]	6,6 a	14,3 a	23,4 a
BRS 218 [Nina]	6,0 b	12,7 a	21,1 b
BRS Milena	7,4 a	17,1 a	20,1 b
BRS Rosa	5,7 b	12,0 a	19,7 b
BRSGO Caiapônia	7,8 a	17,2 a	20,6 b
BRSGO Santa Cruz	6,2 b	12,9 a	21,2 b
BRSMG Segurança	8,2 a	15,0 a	20,6 b
CD 219RR	6,5 a	14,9 a	17,6 b
CD 227	4,1b	10,2 a	18,0 b
BRS 252 [Serena]	6,9 a	15,2 a	25,6 a
BRS Jiripoca	7,9 a	19,6 a	18,2 b
BRS Piraíba	6,9 a	14,7 a	19,9 b
BRSGO Bela Vista	5,2 b	10,3 a	28,3 a
BRSGO Luziânia	6,2 b	15,9 a	22,4 a
BRSMT Pintado	7,1 a	13,3 a	23,3 a
BR/Emgopa 314 [Garça Branca]	4,6 b	11,3 a	18,4 b
BRS Aurora	4,5 b	7,6 a	22,9 a
BRS Celeste	5,0 b	11,7 a	24,5 a
BRS Gralha	5,6 b	13,6 a	21,2 b
BRS Nova Savana	4,8 b	10,3 a	28,7 a
BRS Sambaíba	6,2 b	11,7 a	16,1 b
BRS Tianá	6,0 b	12,5 a	18,2 b
BRS Raimunda	6,6 a	17,1 a	20,8 b
BRSGO Ipameri	5,7 b	15,0 a	23,3 a
BRSGO Jataí	5,3 b	10,2 a	22,2 a
BRSGO Paraíso	5,5 b	11,1 a	22,5 a
BRSMG 251 [Robusta]	7,7 a	13,2 a	20,0 b
BRS MG Garantia	7,6 a	14,6 a	22,6 a
CV (%)	21	24	16

** Means followed by the same vertical letter do not differ from each other based on the Scott-Knott test (* $P < .05$); [(a)] Pot containing two soybean plants*

from symbiosis with mycorrhiza to modifications in root architecture [30].

Selection of cultivars with greater P uptake efficiency makes agricultural production using less phosphate fertiliser possible. This is particularly important in tropical regions that, in general, have weathered and low fertility soils.

Average P use efficiency of soybean cultivars varied between 38% (cultivar CD211) and 60% (cultivar BRS Aurora) (Fig. 2). Less than half of the cultivars had greater efficiency in using the P absorbed to the plant for growth and development. Among the 56 evaluated cultivars, only 26 cultivars showed greater efficiency in P use, of which only 13 cultivars showed averages higher than 50% of P use efficiency. Although few cultivars have presented greater P use efficiency, they represent a possibility of using P that is not contained in the soil solution, but in compartments with less available P. In addition, this is an important contribution to efforts aimed at reducing the exploitation of phosphate reserves, as it will be possible to reduce the doses of fertilisers applied when very efficient cultivars are planted.

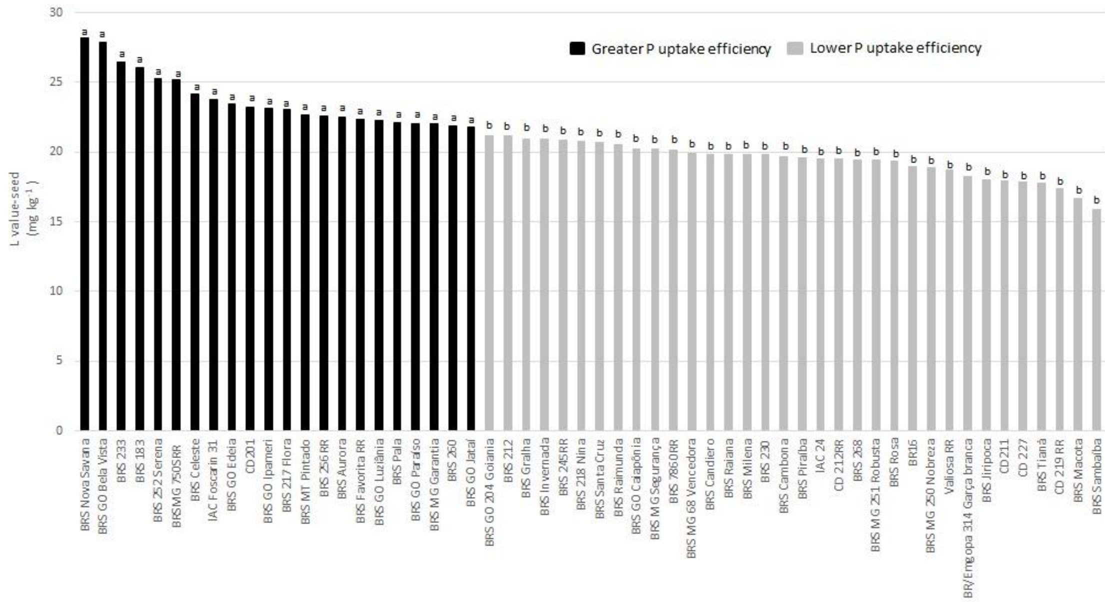

Fig. 1. Average phosphorus uptake efficiencies of Brazilian soybean cultivars (*L value-seed*, mg P kg^{-1} soil)

Means followed by the same letter do not differ from each other, according to the Scott-Knott test (P < .05); coefficient of variation = 16%*

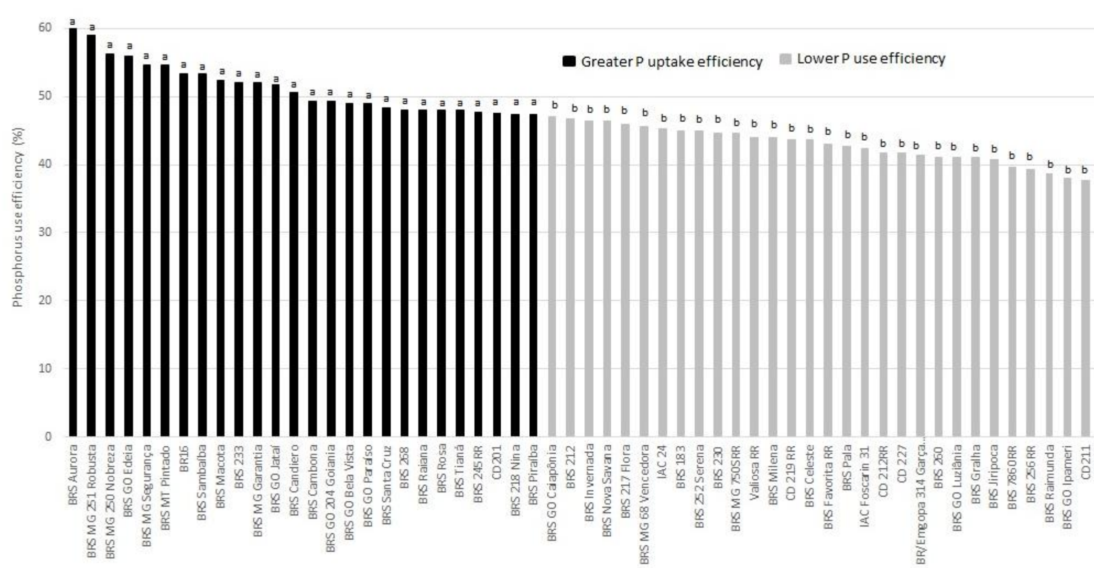

Fig. 2. Average phosphorus utilisation efficiencies (PUE, %) of soybean cultivars recommended for Brazilian conditions

Means followed by the same letter do not differ from each other, according to the Scott-Knott test (P < .05); coefficient of variation = 12%*

Phosphorus content of the soybean seeds did not influence P uptake by plants, as there was no significant difference between the *L value* and the *L value-seed* for the soybean cultivars, according to the F Test (* P < .05) (data not shown). This result indicates that the P uptake efficiency is intrinsic to each cultivar, and that the P content of the seed does not interfere with genotype selection for P uptake efficiency. In a wheat experiment, Silva et al. [8] also observed that the seed P content did not interfere in P absorption by the plant, because there was no difference between the *L value* and the *L value-seed*. However, Franzini et al. [29] observed an opposite result and concluded that P content of bean seeds affected the P uptake efficiencies of the plants. Thus, the seed P content is not a parameter that should be taken into account in research for the uptake efficiency of this element in Brazilian soybean cultivars.

The Pearson correlation coefficients for shoot dry matter, total P, *L value*, *L value-seed,* and P use efficiency are shown in Table 3. There was no correlation between shoot dry matter and *L value-seed*, which shows that shoot production in soybean is influenced by factors besides the efficiency in P absorption. Among such factors are total P and P use efficiency, which presented a positive and significant correlation, respectively, with the shoot dry matter. A different result was found in the selection of Chinese soybean genotypes, in which no positive correlation between shoot dry matter and total P was observed [31]. There was no significant correlation between *L value-seed* and total P, which is probably because of the fact that under the condition of low available P, the plants invest more intensely in the growth and development of the root system. As the root system was not analysed for the *L value* and total P in the present study, it was impossible to prove this assumption. Similar results were found among cotton cultivars [27], but not among wheat cultivars [8], suggesting that perhaps monocotyledonous and dicotyledonous plants may differ regarding the fate of the absorbed P. Total P had a significant and negative correlation with P use efficiency, indicating that greater P utilisation efficiency occurs with lower amount of P in the shoot. The fact that P use efficiency is positively correlated with shoot dry mass and negatively correlated with total P suggests that the genotypes with greater P use efficiency are most adapted to soil conditions with low P, as they are capable of producing biomass with a lower P amount in the shoot.

Fig. 3 shows Brazilian soybean cultivar classification according to P uptake and use efficiency. Nine soybean cultivars presented greater P uptake and use efficiency, but BRS Nova Savana (greater *L value-seed*), BRS Jiripoca (greater total P) or BRSMG Segurança (greater SDM) were not among them. This fact demonstrates that the choice of a soybean cultivar suited to low P environments cannot be made based on only one characteristic. In P nutrition, the ideal cultivar should be efficient both in absorbing P from soil compartments with less available P and in using the absorbed P. Thus, only 16% of the cultivars evaluated fit this ideal profile, while 30% of the cultivars were not efficient, neither in the uptake nor in the use of P. Therefore, the latter cultivars should not be cultivated in soils with low P content. Most of the cultivars that are efficient in absorbing and using P are those recommended for the Brazilian Midwest region, a Savanna region that has a natural low soil fertility. This imply that P absorption and utilisation efficiencies have influenced selection of genotypes to some extent.

Table 3. Pearson correlation coefficients for shoot dry mass (SDM, g pot^{-1}), shoot total phosphorus (TP, mg pot^{-1}), Value L (*L value*, mg P kg^{-1} soil), Value L discounting seed phosphorus (*L value-seed*, mg P kg^{-1} soil), and phosphorus utilisation efficiency (PUE, %) of Brazilian soybean cultivars recommended for planting in different Brazilian regions

	SDM	**TP**	***L value***	***L value-seed***	**PUE**
SDM	1	0,83*	-0,07	-0,09	0,24*
TP		1	-0,03	-0,03	-0,31*
L value			1	0,99*	-0,10
L value-seed				1	-0,12
PUE					1

*Correlation coefficients followed by * were significant (* P < .05)*

The explanation for some cultivars being more efficient in P uptake and use is mainly related to root traits for better topsoil exploitation, because this layer concentrates the available P. Thus, the characteristics of root architecture such as shallower growth angles of axial roots are essential for P efficiency [32-34]. Other characteristics such as root hairs density and length, phenology, phene interactions [34], evolution of high- and low-affinity P transports and processes such as exudation of organic acids (to solubilise P complexes) or phosphatases and phytases (to access organic P) regulating the response to P deprivation [1] also contribute to the differentiation among cultivars regarding P efficiency. Therefore, when a cultivar has some of these characteristics, there is certainly an increase in the exploitation of soil compartments that are not available for cultivars without these adaptations. Another explanation relates with the phenological cycle of different cultivars. Except for CD 201, all cultivars with great efficiency in P uptake and use are late-maturing. This is a strategy to improve P efficiency, because cultivars with larger phenological cycle remain in the soil for longer, extending the root foraging and therefore improving the P acquisition and use [34].

3.2 Experiment 2 - Root System Evaluation

From Experiment 1, the following cultivars were randomly selected for further study in Experiment 2, according to P uptake efficiency (*L value-seed*) classification: Greater efficiency - BRS GO Bela Vista, BRS 233, BRS 183, BRSMG 750SRR, and BRS 217 Flora; Lower efficiency -

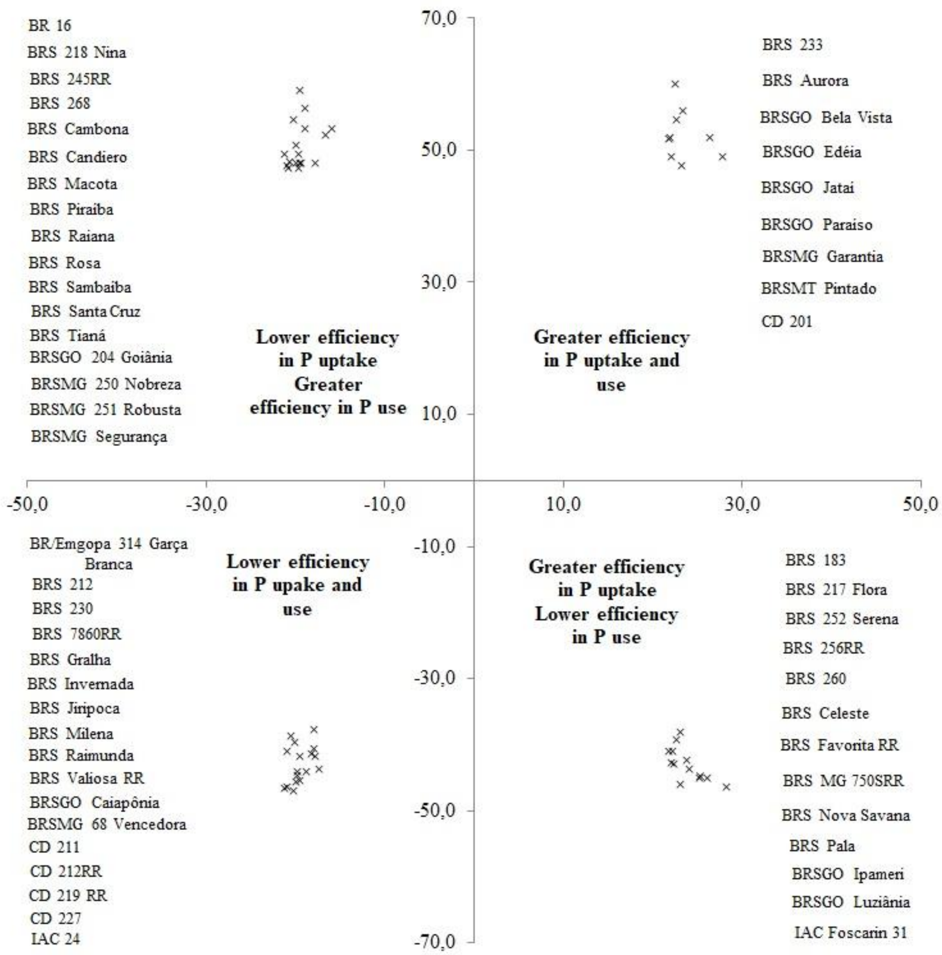

Fig. 3. Classification of soybean cultivars recommended for planting under Brazilian conditions based on phosphorus uptake efficiency (*L value-seed*, mg P kg soil^{-1}) and phosphorus use efficiency (PUE, %)

BRS 218 Nina, BRS Raiana, BRSMG 251 [Robusta], BRS Rosa, and BRS Jiripoca. The average values for cultivar group root characteristics with greater and lower P uptake efficiency are presented in Table 4. The group comprising cultivars with greater P uptake efficiency presented root length, total root surface area, projected root total area, and total root volume greater than those of the cultivars with less P uptake efficiency. These root morphology characteristics are very important because they enable the plant to increase the root-soil contact area. The largest root surface is a plant strategy to increase P acquisition, since this is an immobile nutrient and slowly replaced in the soil solution. P availability in soil regulates several aspects of root growth, including length. All these aspects vary greatly among species and genotypes, and these variations are due to environmental adaptations that occur through a complex genetic control which include not only morphological aspects but also root architecture [32].

Studies comparing contrasting bean genotypes in the context of adaptation to low P availability in the soil suggested that the root architecture parameters, including branching, length, and growth plasticity, were related to the genotype efficiency in P absorption from the soil [33,34]. In another study, lower P availability significantly affected most of the root characteristics in different rice cultivars, indicating substantial genetic variation for both root architecture and morphological characteristics [35]. The soybean cultivars with greatest P uptake efficiency also showed the lowest root angles, except for BRS 217 Flora (Table 4). This means that these cultivars have shallow root systems, which allow greater access and better exploitation of the topsoil. This root system characteristic is desirable considering that the upper layer of agricultural soil is the portion with greater nutrient content, due to plant residue deposition over time and the previous fertilisation. The root shallowness is critical to optimise P uptake mainly due to the immobility of P in soil, as mobility of P in soil depends on the diffusion to reach the roots, and that there is a substantial decline in P availability with soil depth. Consequently, root characteristics that increase the ability of the plant to acquire P from the topsoil are very important. These results corroborate previous work that show the importance of the topsoil layer exploration by the roots to acquire P [34,36,37,38]. In some common bean genotypes, the superficial exploration of the basal roots was regulated by the availability of P in the soil [33,39]. In maize, genotypes with superficial seminal and long roots showed greater growth and P accumulation in soils with low availability of this nutrient, both in the field and in greenhouse [40]. Therefore, basal roots with smaller angles in relation to the horizontal plane is a key feature for greater efficiency in P acquisition for annual species, as emphasised by Lynch [38].

Table 4. Mean values of root length (RL), total root surface area (STA), projected root total area (PTA), total root volume (TV), mean root diameter (AD), and values assigned to the angle (RA) of roots of Brazilian soybean cultivars with greater or lower efficiency in phosphorus absorption

Efficiency	Cultivar	RL	STA	PTA	TV	AD	RA
		cm	cm^2	cm^3	cm^3	mm	-
Greater efficiency	BRS GO Bela Vista	2497,0 a	419,1 a	133,4 a	5,9 a	0,37 b	1
	BRS 233	1399,6 b	226,6 b	105,5 a	2,0 b	0,33 c	1
	BRS 183	2251,2 a	305,2 b	116,3 a	4,7 a	0,44 a	1
	BRSMG 750SRR	1388,5 b	143,3 c	85,8 a	1,2 b	0,34 c	1
	BRS 217 [Flora]	1530,1 b	159,4 c	98,1 a	1,6 b	0,33 c	2
Lower efficiency	BRS 218 [Nina]	829,3 c	101,7 c	35,9 b	0,8 b	0,38 b	2
	BRS Raiana	987,6 c	105,7 c	36,4 b	0,9 b	0,37 b	2
	BRSMG 251 [Robusta]	1044,0 c	114,1 c	39,3 b	1,0 b	0,35 c	2
	BRS Rosa	1095,9 c	101,0 c	43,5 b	1,3 b	0,38 b	2
	BRS Jiripoca	809,9 c	78,3 c	27,5 b	0,7 b	0,34 c	2
	CV (%)	14	35	45	61	6	

**Means followed by the same vertical letter do not differ from each other based on the Scott-Knott test (* $P < .05$)*

4. CONCLUSION

Nine cultivars of soybean with higher efficiency in P uptake and use, without the influence of seed P content, were identified. The Brazilian soybean cultivars with greater efficiency in P uptake had the following root morphological characteristics: greater length, area, and volume. The lower root angles were also present in the most efficient cultivars in P uptake, which favours exploration of the superficial soil layer, which is richer in this nutrient. These cultivars were able to explore the soil compartments with less available P, were more adapted to soil conditions with low fertility, and thus could be planted in marginal soils with less dependence on phosphate fertilisers.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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