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JOINT FAO/IAEA DIVISION OF NUCLEAR TECHNIQUES IN FOOD AND AGRICULTURE

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SELECTION OF GREEN MANURE SPECIES FOR EFFICIENT ABSORBTION OF POORLY-AVAILABLE FORMS OF SOIL PHOSPHORUS

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Abstract

Green manuring is an agronomic practice in which plants or their residues are added to the soil, improving of the soil physical, chemical and biological attributes, and increasing organic matter and fertility levels through nutrient cycling. It is estimated that green manures can increase P bioavailability. The integration of plant species in crop rotations to immobilize P is one of the most promising agronomic measures to improve the availability of P for the main crop. This study aimed to assess 21 species of green manure and a standard plant species (*Lupinus albus*) on their ability to absorb the available forms of P by the ^{32}P isotopic dilution technique. It also aimed to determine if the isotopically exchangeable P, the L-values, differed when calculated with or without taking seed N into account. The results were statistically correlated and analyzed by hierarchical clustering (HCA) in order to group similar plant species. Jack bean was the most efficient species in P utilization while the *Stylosanthes* spp. were the most efficient in P uptake. The seed-derived P affected the P uptake efficiency evaluated by L-value technique.

1. INTRODUCTION

Green manuring is an agronomic practice, in which plant material or crop residues are added to soil, promoting the improvement of the soil physical, chemical and biological attributes, and increase the soil organic matter and its fertility level [1] due to the retention of nutrients [2]. Conditioner plants that have a greater ability to recycle phosphorus can recover the organic and inorganic fractions of low availability and reduce the high amounts of phosphate applied to soil [3], especially when grown in soils with low fertility.

Legumes, when used as green manures, have the ability to utilize insoluble phosphates [4–6] and to release P in available form after mineralization. Thus, in cultivated soil with green manure, the reservoir of available P is significantly enriched [7, 8]. Grasses have also been used, mainly in the Cerrado region of Brazil, due to higher resistance to drought, higher biomass production and lower seed cost. Moreover, the high temperature associated with high humidity in summer, promotes rapid decomposition of plant residues with low C: N ratios [9].

Isotopic labeling of plant tissue is widely used in studies of green manure nutrient dynamics, especially for N, S [10] and P [6, 7, 11], using ^{15}N, ^{35}S, ^{32}P and ^{33}P, respectively. This technique has the great advantage of being able to separate the nutrient derived from the soil from that derived from the applied green manure, making it possible to determine the exact green manure nutrient utilization by the crop. It also allows direct measurement of the differences between plants in their absorption capability of less available forms of soil P [12].

The aim of this study was to use the ^{32}P isotopic technique (L-value), to compare the P uptake efficiency of a range of green manure species, and to verify if the P content in the plant derived from the seeds of the green manure affects the L-value.

2. MATERIALS AND METHODS

2.1. Experimental

The experiment was conducted in the greenhouse at the Center for Nuclear Energy in Agriculture (CENA/USP), Piracicaba (22º42'30" S, 47º38'01" W), São Paulo State, Southeast Brazil. The dystrophic Typic Haplustox soil [13] used in this study was collected from the 0.0–0.2 m depth, dried, sieved in a 2 mm mesh sieve and homogenized. The soil had clay, silt and sand contents, respectively, of 280, 70 and 650 g kg^{-1}, and the following chemical characteristics: pH (0.01 mol l^{-1} $CaCl_2$), 4.5; organic matter, 22.5 g kg^{-1}; P extracted by resin, 6.25 kg^{-1}; K, 0.75 $mmol_c$ kg^{-1}; Ca, 14.4 $mmol_c$ kg^{-1}; Mg, 6.5 $mmol_c$ kg^{-1}; H + Al, 44.2 $mmol_c$ kg^{-1}; CEC, 65.9 $mmol_c$ kg^{-1}; sum of bases, 21.6 $mmol_c$ kg^{-1}; base saturation, 32.8%, according to methodology described by [14]; and P by Mehlich-1, 3.7 mg kg^{-1} [15].The soil was limed according to [14], to reach 70% base saturation, and incubated for 30 days prior to the beginning of the experiment, maintaining the moisture content at approximately 70% of water holding capacity.

The study was conducted in 3.0 l plastic pots, lined with polyethylene bags, containing 2.5 kg of air-dried soil. A ^{32}P radioisotope solution (74 MBq pot^{-1}, carrier free) diluted in 200 ml l^{-1} of distilled water was used to label the soil. After applying this solution, the soil was incubated for 20 days to reach isotopic equilibrium ($^{32}P/^{31}P$).

The experimental design was complete randomized with three replications. The treatment consisted of 21 green manure species: *Brachiaria brizantha* cv. Marandú, *B. brizantha* cv. Xaraés*, B. ruziziensis, Calopogonium muconoides,* sunhemp (*Crotalaria juncea*)*, and four other species of Crotalaria (C. breviflora, C. mucronata, C. ochroleuca and C. spectabilis), Stylosanthes guianensis* cv. Campo Grande*, S. guianensis* cv. Mineirão*,* jack bean *(Canavalia ensiformis),* pigeon pea cv. Fava larga *(Cajanus cajan)*, dwarf pigeon pea *(Cajanus cajan)*, sunflower cv. IAC-Iarama *(Helianthus annuus),* sunflower cv. IAC-Uruguai *(Helianthus annuus)*, lab lab (*Dolichos lablab),* millet *(Pennisetum glaucum),* dwarf velvet bean *(Mucuna deeringiana),* grey velvet bean *(Mucuna cinereum)* and black velvet bean *(Mucuna aterrima).* White lupin *(Lupinus albus)* was used as the standard species, described in the literature as efficient in P uptake [5, 12, 16, 17].

Six seeds of the bigger sized species (jack bean, pigeon pea, sunflowers, lab lab, velvet bean and white lupin) and twelve seeds of the others were sown in each pot and thinned to two plants pot^{-1} seven days after emergence (DAE). N and K were applied at rates of 100 mg N kg^{-1} as urea and 100 mg K kg^{-1} as KCl. The pots were watered daily with deionized water and were maintained at 70% of water retention capacity.

The plant shoots were harvested 50 DAE, oven-dried at 60°C for 72 hours, weighed and ground to pass a 20 mesh sieve. The plant samples were digested with a mixture of nitric and perchloric acids and analyzed for ^{32}P activity in a Liquid Scintillation Counter by the Cerenkov effect [18], and the total P concentration was determined by the method described by [19].

2.2. L-values

With plant accumulated P values and ^{32}P / ^{31}P specific activities (SA), the L-value and L-value discounting the P in the plant derived from seed [12, 20] were calculated from the following equations [21]:

SA = ^{32}P / ^{31}P; where SA is the specific activity (disintegration per minute, dpm µg P^{-1}); ^{32}P is radioisotope activity in the plant (dpm); ^{31}P is P content in the plant (µg P pot^{-1});

L-value = (A_0 / SA_f − 1); where L-value (mg P kg^{-1} soil); A_0 is ^{32}P activity of the applied carrier-free solution (dpm); SA_f is the specific activity of the plant (dpm µgP^{-1}).

L-s value = A_0 / SA_f; where L-s value is the L-value calculated considering the P in the plant subtracting the P derived from the seed (mg of P kg^{-1} soil); A_o is carrier free ^{32}P activity of the applied solution (dpm); SA_{fs} = $^{32}P/^{31}P$, where ^{32}P is the plant ^{32}P activity and ^{31}P is the P content of the plant. The plant P content subtracting the seed derived P was calculated, assuming that 60% of P contained in the seed is used in plant development [22].

The L-value is by definition the soil P availability obtained through the isotopic dilution technique. Under similar soil P conditions, the higher the L-value the more efficient is the plant species in obtaining P.

2.3. Statistical analysis

Data of shoot dry matter (SDM), P concentration and P uptake in SDM, L-value and L-s value of the green manure species were arranged in a matrix, which was statistically analyzed using Pearson linear correlation and hierarchical cluster analysis (HCA). The statistical method of HCA was used in order to verify the similarities among the species of green manure, by calculating the Euclidean distance among the samples.

The SAS 9.1 - "Statistical Analysis System" [23] and the SYSTAT version 10.2 software programs, using the UPGMA (un-weighted pair group arithmetic average clustering), were used to perform binary grouping to define groups according to the degree of similarity between the species [24]. The cluster analysis was preceded to the standardization of data before the Euclidian distances calculation, as the variables presented different scales. After standardization, all variables will be equally important in the determination of these distances. Final results of the groups were presented as dendrograms. The P uptake efficiency by plants is inversely proportional to SA and directly proportional to L-value and L-value discounting the P in the plant derived from the seed.

3. RESULTS AND DISCUSSION

The results obtained with the white lupin as the standard species were: SDM = 1.77 g pot^{-1}, P in SDM = 1.66 mg pot^{-1}, SA = 9.05 dpm g^{-1}, L-value = 43.20 mg P kg^{-1} soil and L-s value = 5.93 mg P kg^{-1} soil. White lupin was more efficient in absorbing P, resulting in the lowest SA, and the highest L and L-s values among the green manure species (Tables 1 and 2).

TABLE 1. MEANS AND STANDARD DEVIATIONS OF SHOOT DRY MATTER (SDM), P CONCENTRATION IN SDM (P CONC), P UPTAKE AND SEED P UPTAKE (P SEED) OF 21 SPECIES OF GREEN MANURE

Species	SDMM (g)	P conc (g kg^{-1})	P uptake (mg)	P seed (mg)
Brachiaria ruziziensis	3.37 ± 0.05	1.06 ± 0.05	3.57 ± 0.12	0.042
Brachiaria marandú	3.08 ± 0.23	1.05 ± 0.11	3.19 ± 0.22	0.042
Brachiaria xaraés	2.43 ± 0.33	1.18 ± 0.09	2.88 ± 0.52	0.042
Calopogonium	3.50 ± 0.17	1.27 ± 0.06	4.41 ± 0.03	0.102
Crotalaria breviflora	2.20 ± 0.13	1.61 ± 0.12	3.51 ± 0.07	0.132
Sunhemp	6.26 ± 0.11	0.80 ± 0.04	4.99 ± 0.31	0.372
Smooth rattlepod	2.10 ± 0.05	1.22 ± 0.04	2.57 ± 0.10	0.048
Crotalaria ochroleuca	3.43 ± 0.18	1.15 ± 0.07	3.95 ± 0.17	0.054
Showy rattlepod	3.55 ± 0.11	1.17 ± 0.04	4.16 ± 0.28	0.150
Stylosanthes cv. Campo Grande	0.99 ± 0.16	1.85 ± 0.04	1.82 ± 0.28	0.042
Stylosanthes cv. Mineirão	0.60 ± 0.13	1.74 ± 0.29	1.09 ± 0.33	0.042
Jack bean	16.10 ± 0.47	0.71 ± 0.02	11.46 ± 0.66	7.872
Pigeon pea	4.73 ± 0.13	1.03 ± 0.04	4.87 ± 0.11	0.804
Dwarf pigeon pea	4.73 ± 0.28	1.08 ± 0.04	5.07 ± 0.11	0.456
Sunflower cv. Iarama	5.17 ± 0.16	1.10 ± 0.03	5.70 ± 0.35	0.786
Sunflower cv. Uruguai	5.30 ± 0.12	1.06 ± 0.05	5.61 ± 0.32	0.576
Lab lab	5.24 ± 0.28	1.14 ± 0.05	5.94 ± 0.04	1.494
Millet	3.79 ± 0.24	0.87 ± 0.02	3.32 ± 0.28	0.042
Dwarf velvet bean	7.37 ± 0.61	0.86 ± 0.02	6.30 ± 0.37	3.726
Grey velvet bean	8.30 ± 0.59	0.86 ± 0.02	7.12 ± 0.41	5.634
Black velvet bean	10.08 ± 0.56	0.84 ± 0.01	8.45 ± 0.45	6.204

The values of plant SDM of 21 green manure species correlated significantly and negatively with P concentrations in SDM (–0.733***) and positively with P uptake in SDM (0.975***). The increase of SDM reduced the P concentration in SDM, while the total P uptake was higher.

The cluster analysis identified the following six groups of green manure species (Fig. 1):

1st: jack bean;
2nd: black velvet bean,
3rd: grey velvet bean and dwarf velvet bean;
4th: *Stylosanthes* cv. Minerão and *Stylosanthes* cv. Campo Grande;
5th: lab lab, sunflower cv. Uruguai, sunflower cv. Iarama, dwarf pigeon pea, pigeon pea and sunhemp;
6th: *Crotalaria breviflora*, *Crotalaria macronata* , *Brachiaria brizantha* cv. Xaraés, *Crotalaria spectabilis*, *Crotalaria ochroleuca*, *Brachiaria brizantha* cv. Marandú and *Brachiaria ruziziensi*s.

Among the five groups of green manure species (Fig. 1) and the values presented in Table 1, jack bean presented superior values of SDM and higher values of P uptake. The variables that showed higher Pearson correlations coefficients were: SA and L-value (–0.949***), SA and L-s value ($–0.095^{ns}$) and L-value and L-s value ($–0.0012^{ns}$).

TABLE 2. MEANS AND STANDARD DEVIATIONS OF SPECIFIC ACTIVITY (SA), L-VALUE AND L-VALUE DISCOUNTING THE P IN PLANT DERIVED FROM SEED (L-S) AND VARIATION (Δ) BETWEEN L-VALUE AND L-S VALUE OF 21 SPECIES OF GREEN MANURE

Species	SA (dpm μg^{-1} P)	L-value (mg P kg^{-1} soil)	L-s value (mg P kg^{-1} soil)	Δ (%)
Brachiaria ruziziensis	138.33 ± 14.83	2.79 ± 0.56	2.76 ± 0.55	1.18
Brachiaria marandú	172.52 ± 19.69	2.44 ± 0.16	2.41 ± 0.16	1.33
Brachiaria xaraés	241.22 ± 38.32	2.27 ± 0.21	2.24 ± 0.22	1.52
Calopogonium	140.23 ± 3.56	2.60 ± 0.16	2.54 ± 0.16	2.31
Crotalaria breviflora	192.37 ± 27.13	3.12 ± 0.68	3.00 ± 0.66	3.79
Sunhemp	80.56 ± 5.61	2.54 ± 0.22	2.35 ± 0.23	7.46
Smooth rattlepod	184.08 ± 6.96	3.29 ± 0.13	3.23 ± 0.12	1.88
Crotalaria ochroleuca	140.07 ± 7.35	2.65 ± 0.09	2.62 ± 0.08	1.38
Showy rattlepod	135.92 ±1.30	2.63 ± 0.14	2.54 ± 0.13	3.65
Stylosanthes cv. Campo Grande	300.54 ± 44.88	4.55 ± 0.75	4.45 ± 0.73	2.04
Stylosanthes cv. Mineirão	1047.05 ± 34.79	4.77 ± 0.79	4.61 ± 0.80	3.44
Jack bean	10.43 ± 0.55	7.59 ± 0.29	2.35 ± 0.62	68.98
Pigeon pea	88.11 ± 1.53	3.05 ± 0.23	2.55 ± 0.20	16.53
Dwarf pigeon pea	75.53 ± 3.79	3.60 ± 0.53	3.28 ± 0.48	9.03
Sunflower cv. Iarama	93.14 ± 6.45	2.65 ± 0.20	2.29 ± 0.21	13.82
Sunflower cv. Uruguai	84.44 ± 2.89	2.84 ± 0.17	2.55 ± 0.12	10.37
Lab lab	84.34 ± 4.34	2.89 ± 0.16	2.16 ± 0.12	25.17
Millet	150.92 ± 9.08	2.24 ± 0.18	2.21 ± 0.17	1.29
Dwarf velvet bean	46.36 ± 2.26	3.76 ± 0.40	1.51 ± 0.13	59.85
Grey velvet bean	31.38 ± 2.87	4.94 ± 0.15	1.01 ± 0.44	79.61
Black velvet bean	19.65 ± 1.44	6.47 ± 0.24	1.70 ± 0.48	73.68

Green manure species were classified with respect to P uptake efficiency in the following five groups by the hierarchical cluster analysis with both variables SA and L-value (Fig. 2).

1st: highly efficient (black velvet bean and jack bean);
2nd: very efficient (grey velvet bean, *Stylosanthes* cv. Minerão and *Stylosanthes* cv. Campo Grande);
3rd: efficient (dwarf velvet bean, dwarf pigeon pea and *Crotalaria macronata*);
4th: moderately efficient (millet and *Brachiaria brizantha* cv. Xaraés);
5th: less efficient (lab lab, sunflower cv. Uruguai, pigeon pea, *Crotalaria breviflora*, sunhemp, *Brachiaria brizantha* cv. Marandú, *Crotalaria spectablis*, sunflower cv. Iarama, *Crotalaria ochroleuca*, *Calopogonium* and *Brachiaria ruziziensis*).

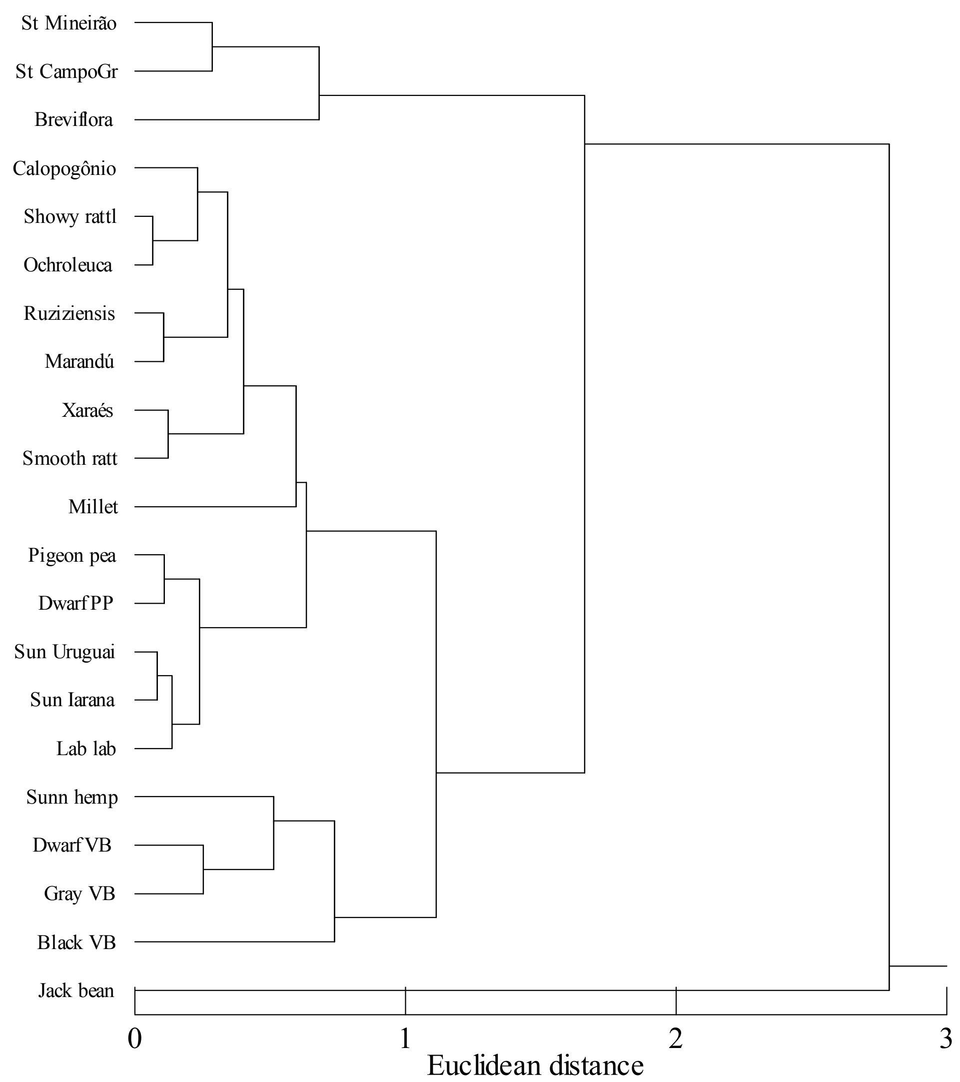

FIG. 1. Dendrogram obtained by hierarchical method of nearest neighbor based on distance of 0.5 for the green manure species. Hierarchical Cluster Analysis (HCA) - Average Method - for shoot dry matter (SDM), concentration and accumulation of P in SDM.

The cluster analysis with L-value discounting the P in plant derived from seed classified green manure species in five groups (Fig. 3).

1st: highly P uptake efficient (*Stylosanthes* cv. Minerão and *Stylosanthes* cv. Campo Grande);

2nd: very efficient (grey velvet bean);

3rd: efficient (black velvet bean and dwarf velvet bean);

4th: moderately efficient (dwarf pigeon pea, *Crotalaria macronata* and *Crotalaria breviflora*);

5th: less efficient (lab lab, millet, *Brachiaria brizantha* cv. Xaraés, sunflower cv. Iarama, jack bean, sunhemp, *Brachiaria brizantha* cv. Marandú, *Crotalaria ochroleuca*, sunflower cv. Uruguai, pigeon pea, *Crotalaria spectabilis, Calopogonium mucunoides* and *Brachiaria ruziziensis*).

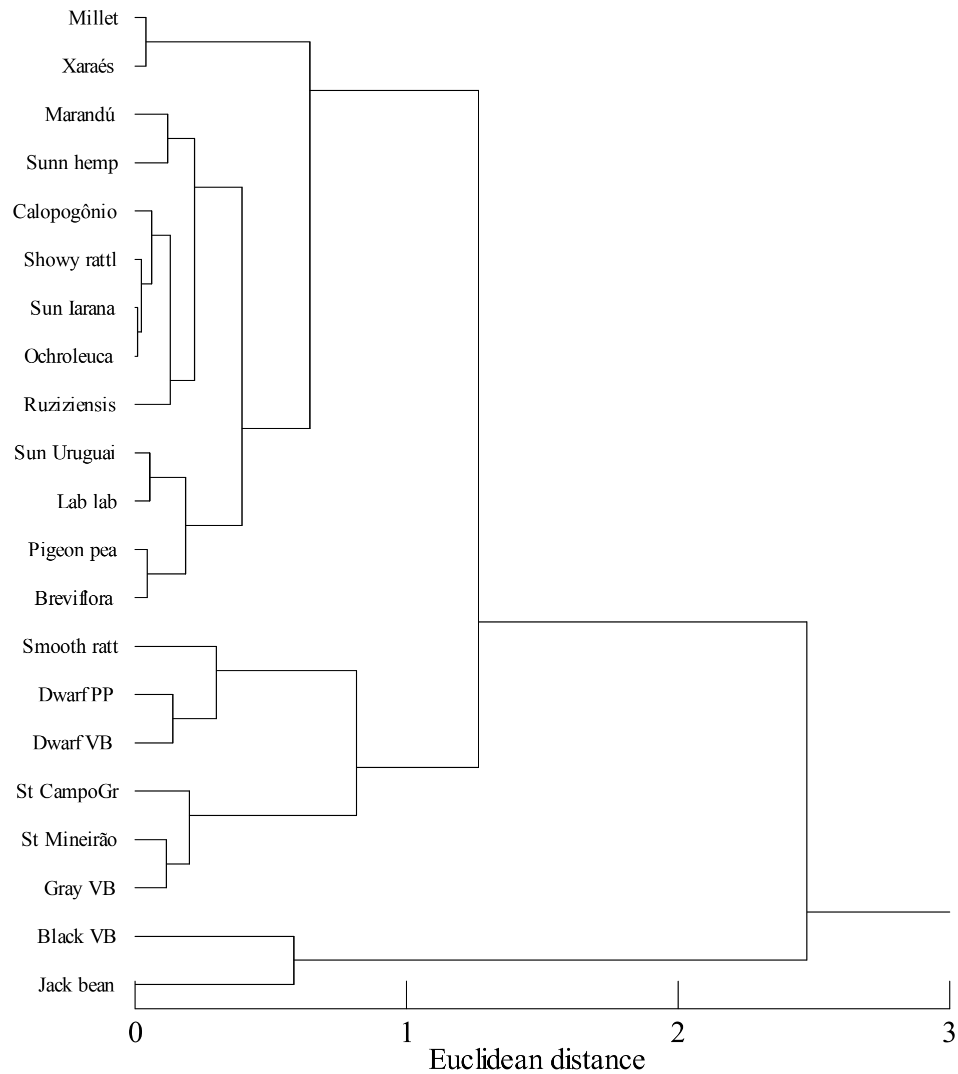

FIG. 2. Dendrogram obtained by hierarchical method of nearest neighbor based on distance of 0.5 for the green manure species. Hierarchical Cluster Analysis (HCA) - Average Method - for P specific activity (SA) and L-value.

The green manure plants may convert the relatively unavailable native P in the soil or residual fertilizer to more available chemical forms for the next crop. White lupin can take up more P from soil than alfalfa, clover, peas, vetch and wheat grown in P- deficient soil [5, 16]. With respect to the capacity of mobilization and recycling of nutrients between legumes and grasses used as green manure, pea and sunhemp showed better results compared to grass, due to higher biomass and higher nutrient concentrations in biomass [25].

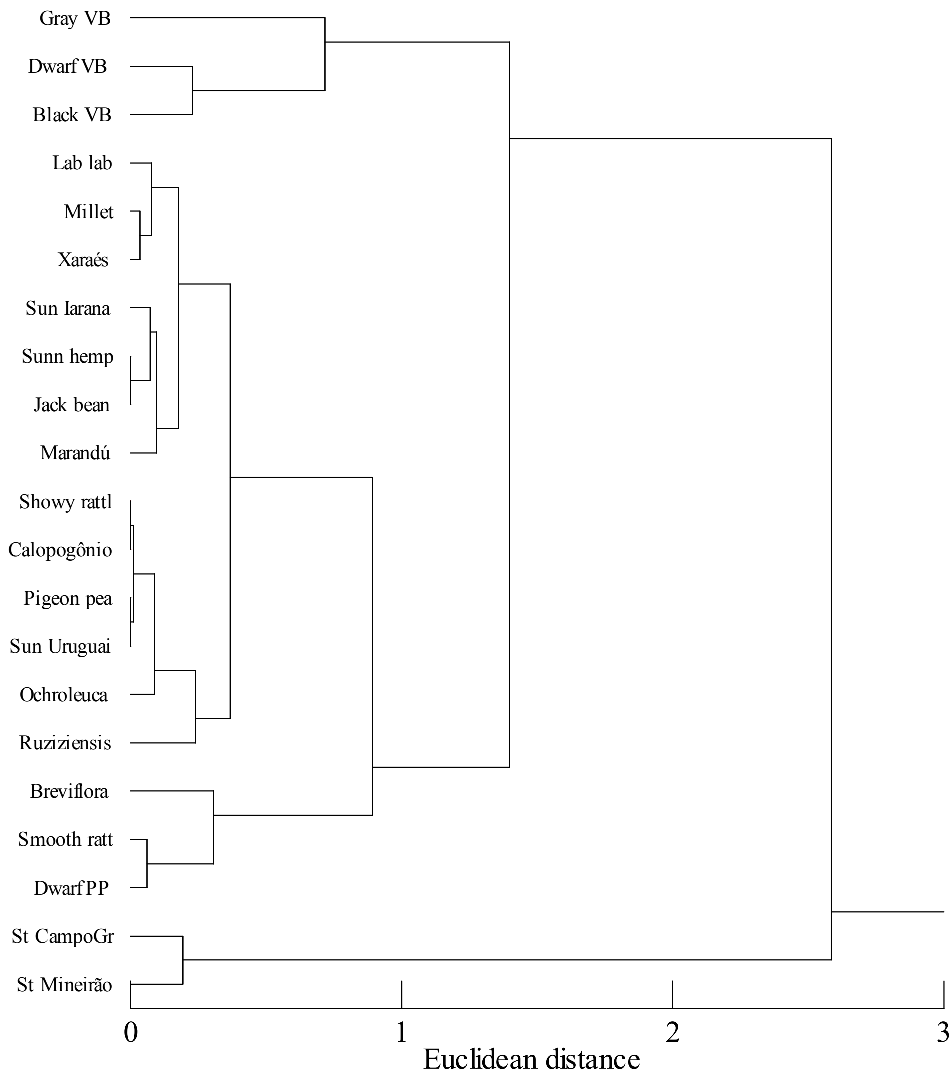

FIG. 3. Dendrogram obtained by hierarchical method of nearest neighbor based on distance of 0.5 for the green manure species. Hierarchical Cluster Analysis (HCA) - Average Method - for L-value discounting the P in the plant derived from the seed (L-s).

White lupin, pigeon pea and *Stylosanthes* are well adapted to acid soils deficient in P [3, 5, 26]. The white lupin roots secrete large amounts of citric acid, which solubilizes the fixed P [27–30], pigeon pea releases P from iron and aluminum phosphates through the secretion of piscidic, malonic and oxalic acids by roots [12, 31, 32]. *Stylosanthes* roots exude citrate and release P from Fe and Al phosphates. These organic acids solubilize the P, thus increasing P uptake by the plant subsequently [3, 33–35], making these species resistant to P deficiency in soil.

Legumes and grasses have been used as cover crops. However, they must have some desirable features, for their beneficial effects to succeed in the main crops, and facilitate the cultivation system [36]. The species to be used as green manures should provide enough biomass to cover the surface area and improve the yield of the main crops, such as providing nutrients as well as being compatible with the management of the main crop [37].

The L-values, considering or not the seed derived P, are illustrated in the Table 2. There was increasing variation in the differences between the L and L-s values of the green manure species with higher values of seed derived P, varying form 1% for *Brachiaria ruziziensis* (lower seed P content, Table 1) up to 80% for grey velvet bean (Table 2), suggesting that the higher the seed P, the greater the influence on the correction of the seed P effect on the L-value (Tables 1 and 2). Reducing the L-value to more realistic levels by correcting the seed derived P were also shown in an experiment with ryegrass grown in three soils of very low, low and medium P status (soil I, II and III), containing 4, 14 and 37 mg initial $NaHCO_3$-soluble P kg^{-1} soil, and supplied with increasing amounts of added P. The L-values after correcting the seed-derived P were reduced by 69% in soil I, 18% in soil II and 10% in soil III. This effect was still much higher, when L-values were reduced by increasing levels of fertilizer P [22]. In a study on the dynamics of P uptake by wheat using radioisotopes ^{32}P and ^{33}P, the specific activities of the plants in the early development stage for both radioisotopes were much lower, presumably due to the high amount of seed- derived P in the plant [38]. Chickpea, faba bean, white lupin, canola and wheat were evaluated in the greenhouse for P uptake and growth in three soil types with low, medium and high P content, by labeling with ^{32}P. It was concluded that the L-value for faba bean grown in soils with low P was compromised by the large proportion of the seed P in relation to P taken up in shoots, making the calculation of L-value very sensitive to the estimated value of P derived from the seeds accumulated in the shoot [39].

Stylosanthes cv. Minerão and *Stylosanthes* cv. Campo Grande showed no difference between L-value and L-s value, probably because they have lower seed P contents (Table 1). Jack bean was classified in this study as the species that absorbed more P (Fig. 1), with higher SDM, P content and P uptake (Table 1), and highly efficient in P absorbing capacity (Fig. 2). However, when discounting the P from the seed (Table 1), it was the least efficient in P uptake (Fig. 3), despite its high SDM and P uptake in SDM (Table 1). The L-s value was the most trustworthy, because a fraction of the amount of P translocated from the seed is subtracted from the total P uptake by the plant [40, 41].

The specific activity which is inverse to the L-value showed no correlation between the L-s value, seed SA and L-value (-0.095^{ns}) and L-s value and L-value (-0.0012^{ns}), due to differences in the seed P contents of the green manures studied. Therefore, the results could be erroneous when the L-values are calculated without considering the P in the plant derived from the seed.

4. CONCLUSIONS

- Jack bean was more efficient in P utilization, i.e., the most productive under low soil available P conditions.
- *Stylosanthes*, regardless of cultivar Mineirão or Campo Grande, was the most efficient green manure species for P uptake.
- The seed-derived P in the green manures, when L-value is used, affects the identification and classification of P uptake efficiency

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