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Pre-germinative treatments in seeds of *Leucaena leucocephala* (Lam.) de Wit. and *Prosopis juliflora* (Sw.) DC.

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Abstract

Leucaena and cuji are forage species that have great importance in the animal food. The effect of pre-germinative treatments on germination and the morphological characteristic were evaluated in both species. In Leucaena, seeds were treated for 10 min in hot water (80ºC), two hours for being soaked (25ºC), and scarification with sandpaper # 80 for 20 and 40 min, and a witness. In Cují, seeds with and without the knuckle were sowed with 21 days of being stored or fresh. Each treatment had four replications of 100 seeds each. The germination percentage (PG) and the germination rate (TG) were evaluated every four days, and passed 32 days were evaluated the number of leaves (NH), height of the plant (AP), longitude of the root (LR), diameter of the root (DR) and diameter of the stalk (DT). The germination started on day fourth and remained constant through 20 days in Leucaena and through 16 days in Cují. In Leucaena, the treatment with hot water (80ºC) for 10 min was the best with 91.5% of germination. TG varied from 12.82 to 14.88 days. A positive correlation was found ($P<0.01$) in AP, LR, DR, DT and NH, excepting DR with AP and LR. In Cují, the sow of fresh seeds with the knuckle showed the highest PG (29%). TG fluctuated from 7.43 and 10.01 days. A positive relation was found in AP with LR and NH, LR with NH and DR with DT. Pre-germinative treatments increased the germination in leucaena and cují seeds.

Key words: germination, morphological characteristics, seedlings, *Leucaena leucocephala, Prosopis juliflora.*

Introduction

Leguminous are widely distributed throughout the world and have an important role in the agriculture and on the fertilization of soils (4). Forages plants *Albizia lebbeck, Albizia samar, Cassia grandis, Erythrina indica, Leucaena leucocephala, Prosopis juliflora,* have

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had a great importance on the production systems, mainly on the bushy due to their huge diversity, erosion control, reforest, wood production and their derivates such as shadow tree, organic fertilization as food for birds and cover crop. Leguminous have a great ability of fixing nitrogen in the air and are a highly productive source of excellent alimentary quality for the cattle (25). Also, they represent an alternative inside genus, species, and ecotypes that guarantee successful biological and ecological improvements of the soils belonging Tropical America (19, 31).

The scarcity and high prices of the concentrates, especially of those with high protein contents have generated the necessity of looking other alternatives to supply the food in the Tropic (6). Trees and bushes have provided food to animals raised by the man probably from their domestication (29), therefore these may be considered as an alternative.

L. leucocephala (Lam) of Wit belongs to the Mimosaceae family (21). It is native from Mexico and have distributed to all tropics of the new and old world especially in most areas of Tropical countries. It is a big tree, that might reach even 15 m of height, though generally it is not higher than 3 m or less (32, 36). Its adaptation capacity allows it to develop on the sea level (21), in areas where precipitations of 760 mm occurred; it prefers neutral soils with an adequate Ca and pH content higher of 5.5, though it also adapts to clayey, heavy and saline soils (25).

Leucaena has a high nutritive value, conformed by 25.9% of raw protein, 22.4% of true protein, 75.9% of digestibility of organic manner, 2.4% of calcium, and 0.23% of phosphorus, among others (28). Additionally, leucaena has a good yield of dry matter and its bromatologic composition is acceptable. This leguminous with 10 and 12 $kg.ha^{-1}$ of seeds has achieved a production that fluctuates from 7 to 14 $ton.ha^{-1}.year^{-1}$ without irrigation, and from 12 to 19 $ton.ha^{-1}.year^{-1}$ of dry matter with irrigation (24). Arid and semiarid regions cover approximately a third of the land's surface where approximately 15% of the worldwide population live. However, in these regions, the production is restricted due to the limited humidity and/or lack of natural resources. Species of the *Prosopis* genus have an important role, since these stabilize the environment; also, they provide a huge variety of useful products that may improve the life's quality of rural populations (8). *P. juliflora* (Sw) DC., Mimosaceae, is native from the Caribbean Coast of Colombia and Venezuela and adjacent islands (27) and it is worldwide distributed in several regions of Africa, Asia and America. It is a perennial and arboreal plant, with a varied habit growth, with a height from 2 to 12 m; the plant represents an important source of food for the cattle as well as for the human being. The nutritional value is of 12.4% of raw protein, 12.2% of humidity, 48.9% of carbohydrates, 22% of fiber and 1.3% of lipids. Its yield varies from 300 kg of dry matter per hectare to 800 kg when cultural labors are done (9).

Cuji develops in very scarce

precipitation areas from 150 to 250 mm and in some places from 500 to 1000 mm.year^{-1}; high temperatures, scarce atmospheric humidity and intense sunshine. It grows in a huge variety of soils, even in very poor soils as dry and vetch dunes. On clayey sandy soils, saline of even 3.2% of NaCl (similar salinity to the one of sea water), easily eroded soils, rocky, sandy, cast lithology, limestone and clay stone. Cuji develops easily in soils with pH from 6.5 to 8.3 and it is able to grow in sodium soils with a pH until 10.4 (12).

However, cuji as well as leucaena, have some disadvantages as the slow establishment due to the aliveness of seeds, caused by the highest quantity of growth inhibitors respect to the promoters substances, or due to the presence of a waterproof or oxygen cuticle, which causes a variation on the germination of these species (35).

The most common method to propagate these species is through asexual seeds, taken directly to the field. This is a relatively fast and cheap method (10). Also, this method allows obtaining new cultivars (genetic variability); the obtained plants have a good catch, are vigorous and live longer among other characteristics (20).

According to Machado *et al*. (26), leucaena produces huge quantity of seeds in almost all kinds of weather where it is sowed, with the problem that they have a low germination percentage (15) due to the hardening of the superficial surface of the testa, not allowing the entrance of oxygen, light and water for the growth of the embryo (30)

Ruiz and Febles (33) suggested that the aliveness on the seeds of these leguminous was progressive. The problem for the establishment of the specie have become in one of the main obstacles causing that these species will not adapt well to the animal production system (9). This characteristic have conducted to use other scarification methods that would soften of would break the external surface of seeds, with the aim of obtaining a higher germination percentage on the establishment phase (26).

Prior research recommend diverse treatment for softening the seeds, among these are the use of water at 80ºC for 2 min (18), 3 min (30); to leave seeds to soak for 24 hours at an environment temperature (7) and water at 60ºC for 10 min (15), among others.

The inhibition treatment of seeds for 30 min is a simple, practical and cheap method that might be used to increase the germination percentage in different species (28). Generally, treatments with hot water have been the most favorable, being effective, safe and easy to apply (10).

Scarification done at hand or with sandpaper is a process that allows the water to enter into the seed, as well as, the gas interchange necessary so germination would start (38). Additionally, leucaena seeds treated with sulfuric acid have increased germination from 22 to 83%, at the time that increased the concentration of acid (14), being the scarification methods the most efficient, however, it is a process with practical limitations (28).

The objective of this research was to evaluate the germination and some morphologic characteristics of *Leucaena leucocephala* and *Prosospis juliflora* seedlings under pre-germinative treatments.

Materials and methods

The experiment was carried out at the greenhouse of the Agronomy Faculty of the University of Zulia (Universidad del Zulia), which is located on a very tropical forest area, with annual precipitations from 400 to 500 mm, temperature of 28°C, relative humidity of 75% and evapotranspiration of 2500 $mm.year^{-1}$. The material used of leucaena and cují, was recollected from trees located near the Agronomy Faculty of the University of Zulia. Leucaena and cují seeds were extracted from ripped sheaths. In the specific case of leucaena, seeds were collected before falling from the plant, because fruits or sheaths are dehiscent, and were only considered those sheaths with a brown color (36). Seeds were divided from sheaths manually, and were selected those with higher size and with the darkest coffee color, discharging seeds from the extremes of the sheath and with mechanic damages or caused by insects.

In cují, ripped sheaths were collected with yellow colors (12), which were previously left to soak for 24 hours in water, eliminating the extremes and cutting them in pieces in order to facilitate the removal of the sheath. The next day, seeds with the natural cover or the knuckle (squared and hardened structure that encloses the seed) were divided from the sheaths and left to soak for 14 days, changing the water daily to eliminate the residues of fruits. Subsequently, leucaena seeds were stored on a refrigerator at a temperature of 10+1°C for 21 days and cují seeds for 7 days.

The seed-bed substrate consisted on a mix of sand (vegetal surface) and organic matter (river manure) in a 2:1 proportion, which was previously disinfected with quaternary formol at 37% at doses of 150 $ml.L^{-1}.m^{-2}$.

In leucaena five treatments were evaluated: a witness, seeds submerged in hot water at 80°C for 10 min and left to soak on it for 2 hours at environment temperature (26°C); seeds left to soak for 2 hours at environment temperature, scarification of seeds with sandpaper # 80 from 20 to 40 min. The scarification was done in cycles of 5 min each. In cují, four treatments were done: seeds with 21 days of being collected with the knuckle and without it, and fresh seeds collected the same day of the crop with and without the knuckle.

For the sow of each treatment, four rows of a meter of length each with 7 cm of division between them were used, putting 100 seeds at once per row at a depth of approximately 1.5 cm. Irrigation was done every four days. The propagator was covered with a net that offered 40% of shadow.

A completely randomized design was used with four replications. Evaluations were done every four days for 32 days; the number of germinated seeds was registered in order to calculate the germination percentage (PG) and the germination rate (TG) through the followings equations: PG = (number of germinated seeds x number of total seeds^{-1}) x 100 and TG = (N1*T1 + N2*T2 +. . . . Nn*Tn x number of germinated seeds^{-1}). Where, N: number of non-accumulated germinated seeds, and T: time expressed in days.

Within 32 days, were measured height of the plant (AP), longitude of the root (LR), diameter of the stalk (DT), diameter of the root (DR) and number of leafs (NH). For AP, the measurement was done from the base of the stalk to the apex of the plant, and for LR from the base of the stalk to the apex of the root. Respect to NH, were considered those that were completely extended and in DR and DT, was measured from a centimeter of the base.

The information was processed using SAS (37). For evaluating the treatments effect on the species, the variance analysis technique was employed (ANOVA), and Tukey means test was used when significant effects were found. PG was transformed with the arch sine $(X + 1)^{1/2}$ to adjust it to the normality. The descriptive statistic was applied; mean, standard deviation, minimum and maximum values for variables AP, LR, DT, DR and NH. Pearson's correlation coefficient was used to evaluate the correlation between the growth variables.

Results and discussion

There were significant differences within 32 days of the sow ($P<0.05$) among treatments for the germination of percentage, excepting for the germination rate (table 1). The treatment that consisted on putting seeds in water at 80ºC for 10 min was the best registering 91.5% of germination, maybe because the high temperature favored the impermeability elimination of the seminal cover (11) occasioning a higher rupture of the testa, entrance of water and gas interchange, which are necessary for the germination (38) and therefore, it allowed a higher emergency of the embryo. These results were similar to those mentioned by Lulanda (23), who incremented the germination on leucaena seeds treating them with hot water at 90ºC.

Gonzáles and Mendoza (16, 17) indicated that hot water allowed a higher germination velocity and eliminated completely the aliveness of leucaena seeds, employing water at 80ºC for 5 min. On the other hand, the results that were obtained were higher than those reported by Razz and Clavero (28), who obtained 54.48% of germination when they used immersion in hot water for 30 min in *L. leucocephala* y

Table 1. Pre-germinative treatments effect in the germination percentage of leucaena seeds.

Treatment	Germination(%)	Germination rate (days)
Witness	4.75[d]	14.00[a]
10 min in water at 80ºC	91.50[a]	13.80[a]
2 hours in environment water (26ºC)	6.00[d]	12.82[a]
20 min scarification, sandpaper # 80	24.75[c]	14.88[a]
40 min scarification, sandpaper # 80	43.75[b]	14.12[a]

Means with different letters of columns differ statistically (P<0.05)

Humboldtiella ferruginea; however, they indicated that boiled water for 5, 10 and 30 min caused an inhibitory effect on the seeds germination. Amador *et al*. (2) obtained values from 30.55 to 52.80% of germination 30 days after the sow, submerging the seeds in hot water (100ºC) for three seconds three times.

The treatments with sandpaper mechanical scarification showed significant differences (P<0.05) between them, which proves that it is necessary a higher scarification time of the seed's cover in order to improve the germination. Scarification through the use of sandpaper had 43.75% of germination for 40 min and 24.75% for 20 min. These percentages were higher to the one of 16.43% germination of Razz and Clavero (28).

The witness presented 4.75% of germination, value that was statistically the same compare to leaving the seeds to soak in water at an environment temperature for 24 hours. These percentages might be due to the high impermeability of the seeds, and also the temperature used (26ºC) was not enough to favor the inhibition of seeds, to break the hardness of the cover and to allow the fast exit of the embryo.

The germination rate or averaged days to the germination in leucaena varied from 12.82 to 14.88 days (table 1). The germination was epigeal and started 4 days after the sow. It was observed that through 8 days, the exposure of the seeds for 10 min in hot water at 80ºC surpassed the rest of the treatments until 32 days (91.5%), tending to be constant 20 days and on after the sow (figure 1).

The variance analysis indicated that there were significant differences (P<0.05) between pre-germinative treatments for the height of the plant (AP), longitude of the root (LR) and number of leafs (NH); though differences were not detected (P>0.05) for the diameter of the root (DR), and diameter of the stalk (DT) (table 2). The results showed that leaving seeds to soak at 80ºC for 10 min allowed a high germination percentage (table 1) and a good development of seedlings. This treatment presented AP values of 11.16 cm, LR of 10.18 cm and NH of 4.17, though the rest of the

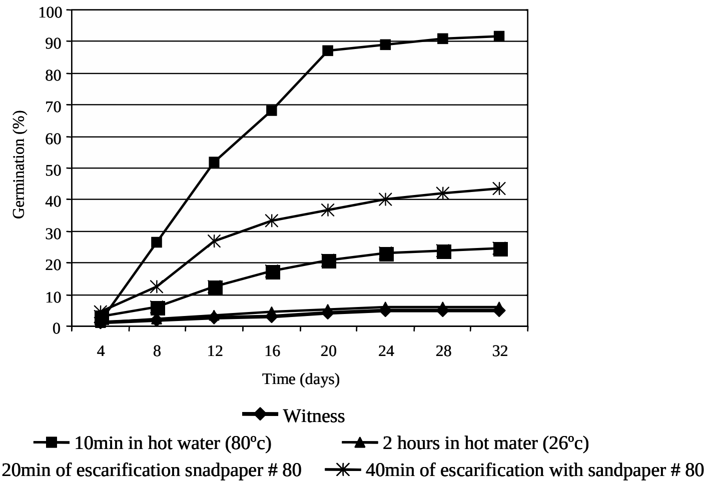

Figure 1. Pre-germinative treatments effect in the germination of leucaena seeds during 32 days of evaluation.

treatments were the same of those with the water at 80ºC in AP and LR, excluding the witness. NH in water at 80ºC registered similar values to the rest of the treatments.

Observing the standard deviation values of each variable was found that the morphological characteristics (AP, LR and NH) revealed that seedlings have a low variability, due to the variation coefficients (CV) were lower or equal to 20%. This low variability has also been reported in medlar seedlings (5). This behavior is associated to the high quality of seeds employed, that were seedlings with normal and homogeneous development, very important aspects in the quality measure of seeds (3). The variability observed in seedlings was related to the heterozygote aspect and the cross-pollination of these species.

Correlations between AP-LR (r= 0.23421), AP-DT (r= 0.12668), AP-NH (r= 0.38095), LR–DT (r= 0.16063), LR–NH (r= 0.37715), DR–NH (r= 0.19406) and DT-NH (r= 0.21532) were positive and highly significant (P<0,01), DR-DT (r= 0,08756) positive and significant (P<0.05) and the other correlations were positive between them (table 3). Even though there was a positive and significant correlation, correlation values were low. In AP, was observed that at the time that this increases, LR also increases significantly and vice versa, maybe due to the root has to reach new absorption areas to provide the necessary nutrients for the growth of all the plant (22). On the root/shoot relation, the root constitutes an important organ for the outbreak, besides its catching function, since it supplies water to all mineral elements required for this process. On the other hand, the shoot provides the photosynthetic products to the roots and apexes in growth (1).

Table 2. Ofect of pre-germinative treatments on morphological characteristics of leucaena, 32 days of hab been sowed.

Treatment	Height of the plant (cm)			Longitude of the root (cm)			Diameter of the stalk (mm)			Diameter of the root (mm)			Number of leaves		
	M	DE	Vm-VM	M	DE	Vm-VM	M	DE	(Vm-VM)	M	DE	(Vm-VM)	M	DE	(Vm-VM)
Witness	6.71^{b}	2.23	1.0-10.1	7.00^{c}	1.82	4.0-10.0	9^{a}	2	6-10	7^{a}	3	2-10	3.57^{b}	1.83	2-6
10 min in water at 80ºC	11.16^{a}	2.66	4.4-16.4	10.18^{ab}	5.51	2.9-21.7	9^{a}	1	1-10	7^{a}	2	1-10	4.17^{ab}	1.18	1-8
2 hours in environment (26ºC)	9.91^{a}	2.01	6.4-14.5	10.90^{a}	2.80	6.5-17.9	8^{a}	2	4-10	6^{a}	2	1-8	4.62^{a}	0.96	3-6
20 min escarification, sandpaper # 80	9.03^{ab}	2.03	2.0-14.0	8.45^{bc}	3.02	3.0-16.2	8^{a}	2	1-12	6^{a}	2	2-10	3.89^{ab}	1.49	1-8
40 min escarification, sandpaper # 80	9.46^{ab}	2.89	1.5-15.7	9.27^{ab}	3.45	1.0-18.6	8^{a}	6	4-11	7^{a}	2	1-12	3.80^{ab}	1.66	1-7
CV (%)	20.86			15.23			12.46			10.59			6.24		

Means with different letters inside columns differ statistically ($P<0.05$). M: mean. DE: standard deviation. VM: minimum value. VM: maximum value. CV: variation coefficient.

Table 3. Correlation of the height of the plant, longitude of the root, diameter of the stalk, diameter of the root, and number of leucaena in 32 days of had been sowed.

	Height of the plant	Longitude of the root	Diameter of the root	Diameter of the stalk	Number of leaves
Height of the plant (cm)		0.23421**	0.00512^{NS}	0.12668**	0.38095**
Longitude of the root (cm)			-0.07431^{NS}	0.16063**	0.37715**
Diameter of the root (mm)				0.08756*	0.19406**
Diameter of the stalk (mm)					0.21532**

** Highly significant (P<0.01). * Significant (P<0.05). NS: no significant.

At the same time, there was a significant relation between the number of leafs with LR and AP, since at a higher NH a higher production area of photo assimilated, which are responsible of the growth in AP, LR or any other organ of the plant. The significant relation between DR and DT is a prove of the growth of the radical system and its absorption capacity, due to at the time that it has a higher growth of the root there will be at the same time an increment of the shoot, in order to obtain a morphologic equilibrium since the growth of the plant depends in both the aerial and the underground components (1, 22, 34).

In cují, the highest germination percentage (29%) was obtained when fresh seeds with their knuckle were used, followed by seeds with 21 days of being collected without the knuckle with 19.2% of germination. Seeds with 21 days of being collected with the knuckle had a lower germination (11.2%) (table 4). These values were different to those obtained by Catalán and Macchiavelli (8), who obtained lower germination percentages when the seed was covered by the knuckle, and high percentages in pealed seeds or without the knuckle. However, none of these scarification treatments employed could increase the germination more than 10%.

Likewise, the obtained results were higher to those reported by D'Aubeterre *et al.* (13), 19% of germination, when seeds were scarified with sulfuric acid for 5 min, 5% of germination treating then with sodium hydroxide and chlorhydric acid for 5 and 10 min, and leaving them soaking in water at environment temperature during 24 and 48 h.

The low germination rate obtained in cují might be due to a possible allelopathic effect of seedlings in development on seeds, since it has

Table 4. Pre-germinative treatments effect in the germination percentage of cují seeds, 32 days of had been sowed.

Treatment	Germination (%)	Germination rate (days)
Fresh with knuckle	29.00[a]	9.34[a]
Fresh without knuckle	15.66[b]	7.43[a]
21 days of been collected without knuckle	19.50[b]	10.01[a]
21 days of been collected with knuckle	11.50[c]	8.60[a]

Means with different letters inside columns differ significantly (P<0.05).

been reported that *P. julifora* plants have allelochemicals that have inhibited the germination and the dispersion of other species such as *P. cineraria* (1).

Regarding the germination rate, it oscillated from 7.43 to 10.01 days. Germination was hypogeal and started 4 days after the sow, Fresh seeds with the knuckle showed higher germination percentage 8 days after the sow up to 32 days (29%), tending to be constant 24 days and on after the sow (figure 2).

Pre-germinative treatments in cují, showed significant differences in DT, DR and NH but not on AP and LR (table 5). DT and NH were higher in the seed's treatment with 21 days of being collected and without the knuckle, 8 mm and 4 respectively. Though, DT was statistically similar to the one of seeds with knuckle and with the same collection time. The highest DR was obtained in seeds with 21 days of collected with the knuckle. The values with standard deviation indicated that there was a morphological variability in seedlings, which was considered low (CVd»20%) (5) compare to the high quality of seeds used (3).

It was found that AP in the variables correlations studied in cují had a positive and significant relation (P<0.05) with LR (r= 0.21975) and highly significant (P<0.01) with NH (r= 0.48561) (table 6). This is explained if it is considered that the canopy as well as the root is involved in the growth and development of the plant. At the same time, LR resulted non significant with DT and DR, but was significant (P<0.05) with NH (r=0.23316). Comparing DR with DT a significant difference was presented between them (r=0.24696). Even though there was a positive and significant correlation, values were low.

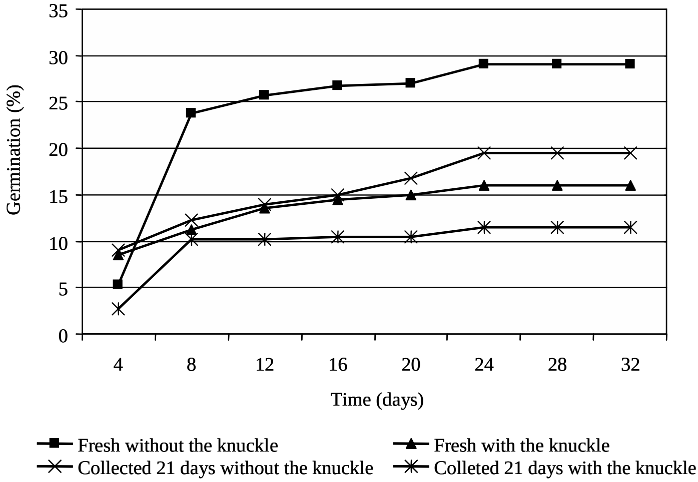

Figure 2. Pre-germinative treatments effect in the germination of cují seeds during 32 days of evaluation.

Conclusions

Pre-germinative treatments incremented the germination of leucaena and cují seeds when leucaena seeds were left for 10 min in hot water at 80ºC (91.5%) and sowing cuji seeds fresh with the knuckle (29%).

The germination started on day fourth and stabilized 20 days after in leucaena and 16 days in cují. The germination rate was from 12.82 to 14.88 days for leucaena and from 8.6 to 10.01 days for cují.

The exposure of leucaena seeds for 10 min in hot water at 80ºC improved considerable the germination and development of the plants. Pre-germinative treatments in cují did not affect the height of the seedling and the longitude of the root.

Recommendations

It is recommended to continue investigating in order to increase the germination percentage in cují and the fast development of leucaena and cují seedlings, on their greenhouse phase

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Table 5. Knuckle presence effect and storage time of the seed in the germination and some morphological characteristics of cují seedlings. Mean standard, deviation, minimum and maximum values 32 days after the sow.

	Height of the plant (cm)			Longitude of the root (cm)			Diameter of the stalk (mm)			Diameter of the root (mm)			Number of leaves		
Treatment	M	DE	Vm-VM	M	DE	Vm-VM	M	DE	(Vm-VM)	M	DE	(Vm-VM)	M	DE	(Vm-VM)
Fresh with knuckle	5.46^{a}	2.56	1.6-14.5	5.53^{a}	2.73	1.2-11.5	5^{b}	3	1-9	2^{b}	2	1-9	2.07^{c}	1.65	1-5
Fresh without knuckle	5.88^{a}	1.59	2.7-10.3	5.30^{a}	2.46	1.7-15.7	5^{b}	2	1-9	2^{b}	1	1-8	3.21^{b}	1.71	1-7
21 days of been collected without knuckle	6.90^{a}	1.27	3.7-8.0	5.38^{a}	3.21	1.6-15.0	8^{a}	2	3-10	2^{b}	2	1-8	4.00^{a}	1.03	2-5
21 days of been collected with knuckle	5.48^{a}	1.61	2.0-8.7	5.53^{a}	2.90	1.6-12.2	6^{ab}	2	2-10	3^{a}	2	1-9	2.83^{bc}	1.60	1-6
CV (%)	19.02			18.98			15.85			16.37			9.63		

Means with different letters inside columns differ statistically ($P<0.05$). M: means. DE: standard deviation. VM: minimum value. VM: maximum value. CV: variation coefficient.

Table 6. Correlation of the height of the plant, longitude of the root, diameter of the stalk, diameter of the root, number of leaves in cují 32 days of had been sowed.

	Height of the root	Longitude of the root	Diameter of the root	Diameter of the stalk	Number of leaves
Height of the root (cm)		0.21975*	-0.05433^{NS}	-0.08951^{NS}	0.48561**
Longitude of the root (cm)			-0.10478^{NS}	-0.03379^{NS}	0.23316**
Diameter of the root (mm)				0.24696*	-0.00880^{NS}
Diameter of the stalk (mm)					-0.05951^{NS}

** Highly significant (P<0.01). * Significant (P<0.05). NS: non significant.

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