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Efecto de los tratamientos pregerminativos en la emergencia y en el desarrollo inicial del cotoperiz [Talisia oliviformis (Kunth) Radlk]

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Scientific Paper

Effect of pre-germination treatments on the emergence and initial growth of *Talisia oliviformis* (Kunth) Radlk

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Abstract

The objective of the trial was to evaluate the effect of pre-germination treatments on the emergence and initial growth of *Talisia oliviformis* (Kunth) Radlk, through six pre-germination treatments: seeds without seed coat (SC) without soaking (T1), T1 plus soaking during 24 h (T2) and 48 h in drinking water (T3), seeds with SC without soaking (T4), T4 plus soaking during 24 h (T5) and 48 h (T6). A completely randomized design was used, with six repetitions and fifty seeds as experimental unit. The measurements performed were: emergence percentage (EP), plant height (PH) and number of leaves (NL). Interaction of the factors seed coat (SC) and soaking time (ST) was found for the EP. The pre-germination treatments were different among themselves; T5 reached the highest percentage (85 %), although T4 achieved 72 % of EP, value which is also considered adequate. The interaction between SC and ST significantly influenced the PH; T1, T2, T4, T5 and T6 did not differ among themselves, although T6 had equal performance as T1 and T3. Regarding the NL, no differences were found among the treatments for any of the studied factors. It is concluded that the interaction of the seed coat and the soaking time influenced the EP and PH. The treatments with seeds with SC soaked during 24 h and without soaking reached the highest emergence percentages (85 and 72 %, respectively). Both techniques are environmentalist, simple, practical and economical.

Keywords: height, leaves, seedlings, seed treatment

Introduction

In recent years, in Venezuela large extensions of forests have been lost, as well as many native and introduced trees in several livestock production areas of the country, due to deforestation, the changes in rainfall (quantity and distribution) and the increase of the ambient temperature, these two last factors associated to the El Niño climate phenomenon, oceanic event that causes alterations in the global climate system (INAMEH, 2015); it has also been remarkably influenced by the demographic and anarchical growth of cities (Ramírez *et al*., 2014a), of industry and plant and animal production systems, among others.

On the other hand, in several localities of the country the benefits and importance of trees as shade element of the livestock –cattle, sheep, goat– production ecosystem and for animal welfare are unknown, for which generally when tree death or loss occurs, they are not replaced, which has generated enclosed pastures with little or scarce shade for the animal.

In Venezuela, just like in Colombia (Navas, 2010), grass monocrop is privileged, the tree cover has been eliminated from grazing areas, and the climate conditions of the different tropical ecosystems, in which such variables as temperature and relative humidity can limit the productive and reproductive efficiency of the animals, are not taken into consideration. The incorporation, replacement and growth of the trees that are present or which grow naturally in enclosed pastures and fields (Ramírez *et al*., 2013, 2014a, 2014b), without affecting forage production and quality, lead to improve the animal welfare due to decrease of the heat stress, because below the canopy temperature is reduced between 2 and 9 ºC with regards to the enclosed pasture areas without trees.

In Latin America, several studies (Delgado and Ramírez, 2008; Lamela *et al*., 2010; Petit *et al*., 2010; Ramírez *et al*., 2012, 2013, 2014b, 2014c) have mentioned agroforestry, agrosilvopastoral and silvopastoral systems as one of the alternatives for livestock production which include the incorporation

of trees with multipurpose qualities. *Talisia oliviformis* is a multipurpose tree that maintains leaves in its crown throughout the year, even in long drought periods (up to nine months), in livestock production systems of the Zulia state; its fruits and leaves are browsed by cattle.

T. oliviformis (Kunth) Radlk, synonyms *Talisia oliviformis* (HBK) Radlk, *Talisia olivaeformis* (HBK) Radlk and *Melicoccus olivaeformis* HBK belongs to the *Sapindaceae* family and is distributed from Mexico to the northern part of South America, even in the West Indies. This tree is 6-18 m high; shows long life, slow growth, densely thick semi-spherical crown, and is very appropriate to provide shade in enclosed pastures; it is little demanding in soil and climate, although it prefers abundant light and heat, and good-drainage soils (Hoyos, 1994).

This fruit tree is generally reproduced by seeds; however, the information is scarce and refers to botanical descriptions (Avilan *et al*., 1992; Geilfus, 1994; Hoyos, 1994), for which several aspects should be studied, such as the propagation techniques by seed. For such reason, the objective of this research was to evaluate the effect of the presence or not of seed coat and the soaking time in water on the emergence and initial growth of *T. oliviformis*.

Materials and Methods

Location. The trial was conducted in the university nursery of the School of Agronomy, University of Zulia, Maracaibo municipality, Zulia state, Venezuela (10º 41' 12" L.N., 71º 38' 05" L.W.); framed in a zone classified as intervened very dry tropical forest, at 25 m.a.s.l. and with an average annual rainfall of 500 mm, temperature of 29 ºC, relative humidity of 79 % and evapotranspiration of 2 500 mm (Huber and Oliveira, 2010).

Plant material and seed preparation. Mature fruits, of green-yellowish shell with some light brown spots, were collected from trees planted in El Taparo farm, El Laberinto sector, Yépez parish, Lossada municipality, Zulia state, Venezuela. For the seed preparation the fruits were shelled; then, they were soaked in drinking water with liquid soap (Brisol®) at 2 % (active compound: dodecilbenzene, sodium sulfonate) and chlorine Nevex® at 5 % (active compound: sodium hypochlorite at 0,26 %) during an hour to separate the aril or pulp that surrounds the seeds, repeatedly rubbing with a metallic mesh until removing it. Afterwards, they were rinsed several times with drinking water with the aid of the metallic mesh until the smell of chlorine disappeared.

Then, the seeds were placed on newspaper, and those that were very small, misshapen or with mechanical damage were discarded. They were preventively sprayed with the fungicide Vitavax® (17 % Carboxin + 17 % Thiram) at 1 % until impregnating all the seeds (Ramírez *et al*., 2012, 2013); they were dried under shade (28 ºC) during four days and later stored at 10 ºC for 10 days.

Experimental design and treatments. A completely randomized design was used with three repetitions and a 2 x 3 factorial arrangement, corresponding to two levels of seed coat (with and without seed coat) and three soaking times in water (0, 24 and 48 h), for a total of six treatments. The experimental unit was formed by 50 seeds.

The six evaluated pre-germination treatments were: seeds without seed coat and without soaking (T1), seeds without seed coat (SC) soaked during 24 h (T2) and 48 h in drinking water (T3), seeds with seed coat without soaking (T4), seeds with seed coat soaked during 24 h (T5) and 48 h (T6). In T1, T2 and T3 the seed coat was removed a day before seeding, and immediately the seeds –without seed coat– were sprayed with the above-mentioned fungicide. The soaking in water in T2, T3, T5 and T6 was performed at ambient temperature (28 ºC) with changes every 12 h.

Seed sowing. The seeds were sown in plastic polyethylene trays with 50 holes (5 cm wide x 8,5 cm wide), which had a substratum in 2:1 ratio of sand (plant layer) and washed cattle manure previously disinfected three times with hot water. A seed was placed per hole at a depth of 1 cm and six trays were used per treatment. During this stage, irrigation and weed control were manually performed every day and once a week, respectively. The trial was placed in the nursery area that was covered by a saran-type mesh, with 40 % shade.

Measured variables. Every seven days the number of emerged seedlings or plumules was counted to determine the emergence percentage (EP), according to the recommendation made by Flores *et al*. (2009). Twenty eight days after seeding the plant height (PH) and number of leaves (NL) were evaluated. The PH was measured in centimeters, from the shoot apex to the plant basis.

Statistical analysis. To explain the effect of the factors under study a variance analysis was made through the GLM procedure of the Statistical Analysis System program. Before comparing the means through Tukey's test, it was verified that the

data fulfilled the assumptions of variance homogeneity and normal distribution. In the case of the variable EP the data were transformed through the equation arcsin $(x + 1)^{1/2}$ to adjust them to normality.

Results and Discussion

The variance analysis showed significant differences ($p < 0,05$) for the effect of the interaction of the factors seed coat and soaking time in water (SC x ST) on the variable EP, 21 days after sowing (table 1). The treatment which consisted in sowing seeds with SC soaked in water during 24 h (T5) showed the highest EP and differed significantly ($p < 0,05$) from the others, with a mean of 85 % at 21 days. For treatment T4 –seeds with SC and without soaking– 72 % was obtained, value which is also considered adequate.

It is important to emphasize that the six pre-germination treatments were different among themselves 21 days after planting (table 1); however, when T1, T2 and T3 were applied with ST of 24 h and 48 h for the seeds without SC emergence was inhibited. Similar performance was found for T4, T5 and T6 with ST of 0 and 48 h. The inhibitory effect of soaking was associated to the fact that at higher ST the embryo will reduce more the oxygen supply due to the anaerobic condition created by a water excess trapped between the cotyledons, which could have suffocated the embryo because oxygen is essential in the respiration process of seeds that occurs during germination (Hartmann and Kester, 2001; Ramírez *et al*., 2013). The emergence of *T. oliviformis* started eight days after sowing, with increases in the EP at 14 and 21 days, and since then it became constant (table 1).

When comparing the seeds without SC and with SC without soaking (T1 and T4) at 21 days, it was found that in T1 several seeds did not emerge or lost their viability (table1), which proves that it is not necessary to take off the coat that protects the embryo. The high emergence in T5 and T4 –seeds with SC soaked during 24 h and without soaking– indicates that such structure allowed to preserve the moisture content and viability.

Several reports point at the presence in the seed coats of substances that promote the germination process ((Hartmann and Kester, 2001; Azcón-Bieto and Talón, 2008; Taiz and Zeiger, 2013), among which are gibberellins, growth and germination stimulators (through the loss of dormancy). These phytohormones favor the mobilization of reserves and regulate the synthesis of hydrolytic enzymes, mainly α-amylases and proteases that degrade starch and proteins, respectively. The accumulation of

Table 1. Effect of the interaction of the factors seed coat and soaking time in water (SC x ST) on emergence, during the initial growth of *T. oliviformis*.

Treatment	SC	ST (h)	EP (%)			
			7 days	14 days	21 days	28 days
1	Without SC	0	6,3	26,3	52^{d}	52
2		24	6,3	20	48^{e}	48
3		48	5,4	21,5	34^{f}	34
4	With SC	0	3,8	40	72^{b}	72
5		24	5	45	85^{a}	85
6		48	5	22,5	61^{c}	61
SE ±			0,05	0,59	1,01	1,01
	Without SC				44,7^{b}	
	With SC				72,7^{a}	
SE ±					0,78	
		0			62^{ab}	
		24			66,5^{a}	
		48			47,5^{b}	
SE ±					0,45	

Means with different letters differ significantly ($p < 0,05$). SE: standard error.

gibberellins occurs during the development of the seed and embryo; they are required in some stages of the germination process, and are found in low concentration in seeds with dormancy and in high concentration in developing seeds (Azcón-Bieto and Talón, 2008; Taiz and Zeiger, 2013).

The high EP in the seeds with SC soaked in water during 24 h (T5) could be ascribed to the softening this technique exerts on them, which facilitated the hydration phase and the onset of enzymatic processes which accelerated the emergence of the seedlings (Ramírez *et al*., 2012). It could also be related to the washing or removal of growth-inhibitor substances which are produced and accumulated in the fruit pulp and the seed coats (Hartmann and Kester, 2001), usually identified as abscisic acid, and which in high concentrations suppress germination (Azcón-Bieto and Talón, 2008; Taiz and Zeiger, 2013).

Abscisic acid tends to increase with the fruit maturation and can be involved in the prevention of viviparity and the induction of dormancy in the seeds. Such compound tends to disappear with stratification, is antagonistic or counteracts the effects of gibberellins, appears in high concentrations in the seed coats, and can be sometimes lixiviated with water; nevertheless, the disappearance does not necessarily coincide with the onset of germination (Hartmann and Kester, 2001). The content of abscisic acid in the seeds is very low during early embryogenesis, increases to a maximum towards the middle and late stage of embryogenesis, and then decreases with their maturation. The normal increase of the abscisic acid content at the beginning and during the middle stage of seed development controls the accumulation of reserve proteins (Azcón-Bieto and Talón, 2008).

The effect of the SC x ST interaction on PH, 28 days after seeding, is shown in table 2. The PH values in T1, T2, T4, T5 and T6 did not differ among themselves, although T6 had equal performance as T1 and T3, which showed the lowest height, ascribed to the delay the 48-h ST caused in the emergence of seeds without SC.

The PH was similar in T1, T2, T4, T5 and T6, which could have been related to the fact that in such treatments emergence started at the same time and then weekly there were increases in the EP, situation that produced many plants with equal quantity of days in growth. However, T1 and T6, in spite of having the same performance as T2, T4 and T5, showed the lowest PH values, which were statistically equal to those of T3. This indicates that the seeds with SC exposed to 48-h ST (T6) and

Table 2. Effect of the interaction of the factors seed coat and soaking time in water (SC x ST) on plant height, during the initial growth of *T. oliviformis*.

Treatment	SC	ST (h)	PH (cm)
1	Without SC	0	6,7[ab]
2		24	7,6[a]
3		48	6,4[b]
4	With SC	0	7,5[a]
5		24	7,6[a]
6		48	6,9[ab]
SE ±			0,04
	Without SC		6,7
	With SC		7,6
SE ±			0,04
		0	6,8
		24	7,6
		48	6,7
SE ±			0,03

Means with different letters differ significantly ($p < 0,05$).
SE: standard error.

Table 3. Effect of seed coat on the number of leaves, during the initial growth of *T. oliviformis*.

Treatment	Seed coat	Number of leaves
	Without SC	3,5
	With SC	3,5
SE ±		0,10

Table 4. Effect of soaking time in water on the number of leaves, during the initial growth of *T. oliviformis*.

Treatment	Soaking time (h)	Number of leaves
	0	3,6
	24	3,5
	36	3,6
SE ±		0,11

the seeds without SC without soaking (T1) had the same response as T3, for which it would be convenient to not apply these treatments in *T. oliviformis*, also considering their effect on the EP.

The plants showed homogeneous growth during the evaluation phase, associated to the seed quality. The success in the transplant of *T. oliviformis* to bag was high (100 %). Regarding the NL, no interaction was found of the studied factors and there were no significant differences of the individual effects (SC and ST) on this variable (tables 3 and 4); this indicates that the presence of SC, ST and SC x ST interaction did not affect the quantity of leaves in the plants (table 3); three leaves for seeds without SC as well as with SC, and for 24-h ST (table 4); three leaves for ST of 0 and 48 h. This was associated to the fact that *T. oliviformis* showed slow growth during the evaluation period of the experiment.

Regarding the variables EP, PH and NL, no information was found in literature that allowed to make comparisons and there are only works related to botanical descriptions, such as the ones written by Geilfus (1994) and Hoyos (1994); the latter author mentions the most widely used propagation type (seed).

The results with *T. oliviformis* are considered pioneering and represent a high contribution for this species present in Venezuela, and in the Zulia state, because the information about the propagation and cultivation techniques are very scarce. *T. oliviformis*, because of growing naturally, shows high potential in different natural as well as farming, agroforestry, horticultural and ornamental production systems; and in semiarid zones with irrigation limitations, due to the little water availability, which in many cases is restricted only for human consumption.

The performance of EP, PH and NL during the initial growth of *T. oliviformis* is essential for decision making about the site or sowing container, in order to prevent malformations of the root system and, thus, low-quality plants. The above-mentioned variables and the success in the transplant to bag allow to suggest seeding in the multiple trays used in this research (5 cm wide x 8,5 cm deep), considering the report by Ramírez *et al*. (2013) about the transplant being made as early as possible, as plants reach a minimum of three true leaves, independently from most not having emerged. It is stated that the success of the transplant decreases as the plants are larger, because this causes higher physiological stress (Flores *et al*., 2009; Ramírez *et al*., 2012; 2013).

As *T. oliviformis* is a tree and emergence was high and occurred in a 21-day period, another option would be to perform the direct seeding in bags with a depth higher than 20 cm, considering the stem growth –because in several tree species the root length doubles or exceeds the PH– and a short time of permanence in the nursery (it is suggested to be not higher than 60 days after sowing). In the case the plants require higher size or should remain for a longer time in the sale area of the nurseries, they should be transferred every certain time to larger bags in order to prevent the damage of the root system and the decrease of its quality and,

thus, that the success of the transplant to the field is also reduced.

It is concluded that the interaction of the factors seed coat and soaking time influenced the emergence percentage and plant height. The treatment which consisted in seeds with SC soaked during 24 h, with water changes every 12 h, allowed 85 % emergence. The sowing of seeds with SC without soaking also reached high EP (72 %). Both techniques are environmentalist, simple, practical and economical.

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