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Teramnus Labialis (L.f.) Spreng seeds priming with an organic agent: Effects on germination and initial seedling growth

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Abstract

Teramnus labialis (L.f.) Spreng is a forage legume that is used for animal feed and as a cover crop in various fruit species. However, the seeds have a low germination percentage and after the emergence of the seedling establishment is difficult due to their slow growth. Seed priming treatments are an alternative to improve germination and vigor of seedlings during the initial growth phase. The objective of the present investigation was to determine the effectiveness of the use of IHPLUS™ for priming of *T. labialis* seeds. The seeds were primed with IHPLUS™ at a concentration of 5% for 3 h and its effect was evaluated during germination and on the seedlings after 14 days of cultivation under semi-controlled conditions. Priming with IHPLUS™ increased the germination percentage (from 72% to 94%) and reinvigorated the seeds. The concentration and mobilization of amino acids during germination increased after seed priming. Additionally, after 14 days of growth, the seedlings obtained from the conditioned seeds increase the fresh (from 14.43 mg to 18.69 mg) and dry mass (from 4.93 mg to 6.33 mg), the length and thickness of the stem, the number of leaves, as well as the length of the root. Therefore, the priming of *T. labialis* seeds with IHPLUS™ is effective in increasing germination and improving seedling vigor during the initial phase of growth.

Keywords: amino acids, imbibition, seedlings and seed vigor

Acondicionamiento de semillas de *Teramnus labialis* (L.f.) Spreng con un compuesto orgánico: Efectos en la germinación y el crecimiento inicial de las plántulas

Resumen

Teramnus labialis (L.f.) Spreng es una leguminosa forrajera que se utiliza para alimento animal y como cultivo de cobertura en varios frutales. Sin embargo, las semillas poseen bajo porcentaje de germinación y después de la emergencia de la plántula el establecimiento se dificulta debido a su lento crecimiento. Los tratamientos acondicionadores de semillas constituyen una alternativa para mejorar la germinación y el vigor de las plántulas durante la fase inicial de su crecimiento. El objetivo de la presente investigación fue determinar la efectividad del uso de IHPLUS® para el acondicionamiento de semillas de *T. labialis*. Se acondicionaron las semillas con IHPLUS® a una concentración del 5 % durante 3 h y se evaluó su efecto durante la germinación y en las plántulas a los 14 días de cultivo en condiciones semicontroladas. El acondicionamiento con IHPLUS® incrementó el porcentaje de germinación (de 72 % a 94 %) y revigorizó las semillas. La concentración y la movilización de los aminoácidos durante la germinación se incrementaron después del acondicionamiento de las semillas. Adicionalmente, a los 14 días de cultivo, las plántulas obtenidas de las semillas acondicionadas incrementan la masa fresca (de 14,43 mg a 18,69 mg) y seca (de 4,93 mg a 6,33 mg), el largo y el grosor del tallo, el número de hojas, así como el largo de la raíz. Por lo tanto, el acondicionamiento con IHPLUS® de las semillas de *T. labialis* es efectivo para incrementar la germinación y mejorar el vigor de las plántulas durante la fase inicial de su crecimiento.

Palabras clave: aminoácidos, imbibición, plántulas, vigor de las semillas

Preparação de sementes de *Teramnus labialis* (L.f.) Spreng com agente orgânico: Efeitos na germinação e no crescimento inicial das mudas

Resumo

Teramnus labialis (L.f.) Spreng é uma leguminosa forrageira utilizada na alimentação animal e como cultura de cobertura em diversas árvores de fruto. No entanto, as sementes apresentam uma baixa percentagem de germinação e após a emergência da plântula o estabelecimento é difícil devido ao seu crescimento lento. Os tratamentos de condicionamento das sementes são uma alternativa para melhorar a germinação e o vigor das plântulas na fase inicial do seu crescimento. O objetivo da presente investigação foi determinar a eficácia da utilização do IHPLUS® para o condicionamento de sementes de *T. labialis*. As sementes foram acondicionadas com IHPLUS® a uma concentração de 5% durante 3 h e o seu efeito foi avaliado durante a germinação e nas plântulas após 14 dias de cultivo sob condições semi-controladas. O condicionamento com IHPLUS® aumentou a percentagem de germinação (de 72% a 94%) e revigorou as sementes. A concentração e mobilização de aminoácidos durante a germinação aumentaram após o condicionamento das sementes. Adicionalmente, após 14 dias de cultivo, as plântulas obtidas a partir das sementes condicionadas aumentam a massa fresca (de 14,43 mg a 18,69 mg) e seca (de 4,93 mg a 6,33 mg), o comprimento e espessura do caule, o número de folhas, bem como o comprimento da raiz. Portanto, o condicionamento IHPLUS® das sementes de *T. labialis* é eficaz para aumentar a germinação e melhorar o vigor das plântulas durante a fase inicial do seu crescimento.

Palavras-chave: aminoácidos, embebição, plántulas y vigor de sementes

1. Introduction

The potential of *Teramnus labialis* (L.f.) Spreng to be used as animal feed[(1)] and cover crop[(2)] has been demonstrated by several researchers. However, currently it has not been possible to extend its use mainly due to the presence of physical dormancy in the seeds[(3)(4)(5)] and the slow growth of seedlings in the initial stage of field establishment[(6)].

The slow growth of seedlings is related to the small size of the seeds and the low availability of reserves necessary for emergence and initial growth[(4)], which is accentuated during seed storage[(7)(8)(9)]. During the storage period, seeds of many species experience loss of viability and vigor[(10)(11)(12)(13)]; this phenomenon being observed in *T. labialis* seeds[(8)].

Viable seeds, with high vigor, provide synchronized germination and the ability to emerge and survive under adverse environmental conditions[(14)(15)]. On the contrary, in seeds that experience certain deterioration or loss of vigor, some of the advantages explained above are suppressed. However, there are several pre-germinative treatments to reinvigorate seeds that are defined as seed priming[(16)(17)] and these are: water conditioning[(18)], solid matrix conditioning[(19)], osmotic conditioning[(20)], chemical conditioning[(21)], hormonal conditioning[(22)], thermal conditioning[(23)], nanoparticle conditioning[(24)] and organic conditioning[(25)(26)].

For organic priming, several conditioning agents are used, including: garlic extracts, algae, green tea, protein hydrolysates, food industry waste and different microorganisms[25][26][27]. In this sense, various efficient microorganisms have been used for the conditioning of seeds of *Dacus carota* L. and *Allium sativum* L.[28][29], *Brassica oleracea* L.[30] and *Triticum* spp. and *Zea mays* L.[31]. In Cuba, the formulation of efficient microorganisms IHPLUS™ has been used for seed priming in crops such as: *Sorghum bicolor* L. (Moench), *Zea mays* L. and *Cucumis sativus*[32][33], showing great effectiveness in the germination and reinvigoration of seeds. However, there is no reference in the reviewed literature of studies related to seed priming in the *T. labialis* species to enhance germination and initial growth of seedlings. Therefore, the present research aims to determine the effectiveness of IHPLUS™ for the priming of *T. labialis* seeds.

2. Materials and methods

Mature seeds were collected from 50 plants of *Teramnus labialis* (L.f.) Spreng grown in Ciego de Ávila, Cuba (21˚50`25.10"N, 78˚45`32.24"W). The seeds had humidity content of 7.96%, a viability of 98.00%, 95.00% of high vigor and 3.00% of low vigor at the time of harvest. The seeds were stored in artisanal conditions according to Acosta and others[8], during twelve months in hermetically sealed glass containers.

2.1 Seeds quality after stored

To estimate viability and vigor, the topographic test of 1% tetrazolium (2, 3, 5 triphenyl-2 H-tetrazolium chloride) was used as described by ISTA[34]. Seeds were classified as per the degree of coloration as: (1) viable with high vigor, when they were completely stained deep red; (2) viable with low vigor, when the coloration was light red or with colorless sections, and (3) non-viable without vigor, when they remained the natural coloration[35].

2.2 Seeds priming

Before seeds priming, mechanical scarification was carried out in seeds to homogenize and promote imbibition and germination according to Acosta and others[3]. Two thousand seeds were used, one thousand for control treatments (seeds without priming) and one thousand for priming with efficient microorganism. For the seeds priming, a formulation of the efficient microorganism IHPLUS™[32] was used. The seeds of this treatment were immersed directly in a Biker with 50 mL of IHPLUS™ at a concentration of 5% (v:v) and for 3 h according to Bolaño and others[36]. After 3 h, the seeds were extracted and rinsed with distilled water for 1 minute to eliminate any remains of IHPLUS™ impregnated on the outside of the seed coat and then dried for 30 minutes on felt paper at a temperature of 25 ± 1 °C. Subsequently, the seeds without priming (control) and those with IHPLUS™ priming were used in the following research experiments.

2.3 Evaluations during germination

Germination (%): For each treatment (seeds without priming (control) and seeds priming with IHPLUS™) four repetitions of 25 seeds were used (n = 4). The seeds were placed to germinate in Petri dishes (9 cm in diameter) on filter paper (FILTRAK) previously moistened with 5 mL of distilled H_2O and placed in a controlled environment pre-germination chamber (RTOP-D Series) at a temperature of 30±1 °C for 7 days. After this period of time, the germination percentage (%) was determined and some numerical variables associated with vigor were calculated: time necessary for 50% of the seeds to germinate (T_{50}), germination index (GI), mean germination time (MGT) and germination synchrony (Z) according to Ranal and others[37].

Amino acid content: Three repetitions of 60 seeds (n = 3) were used for each treatment (seeds without priming (control) and seeds priming with IHPLUS™). The seeds were placed to germinate in Petri dishes (9 cm in diameter) on filter paper (FILTRAK) previously moistened with 5 mL of distilled H_2O and placed in a controlled environment pre-germination chamber (RTOP-D Series) at a temperature of 30±1 °C for 24 h. At the beginning (0 h) and at 12 and 24 h, seed samples were taken for each treatment (20 seeds per repetition) to determine the amino acid content according to Moore and Stein[(38)].

2.4 Evaluations on seedlings after 14 days of cultivation

For the cultivation of seedlings, two samples of 300 seeds were taken for each treatment (seeds without priming (control) and seeds priming with IHPLUS™) to place them to germinate in polyurethane trays with 136 alveoli. The trays were filled with a substrate based on Typical Red Ferralitic soil (50%) and worm humus (50%) and one seed was sown for each alveolus at a depth of 2 cm, based on that recommended by Machado and Roche[(39)]. Next, the trays were placed in the growing house in semi-controlled conditions and daily watering was maintained for 14 days.

Biochemical determinations: To carry out the biochemical determinations of the seedlings 14 days after sowing, the aerial part (leaves and stems) of 30 seedlings (n = 30) for each treatment (10 per repetition) was taken. The content of chlorophyll a, b and total (µg g fresh mass^{-1}) was determined in both the leaves and stems according to the methodology described by Porra[(40)] and the nitrogen content (%) and proteins (%) by the Kjeldahl method[(41)].

Physiological determinations: To carry out the physiological determinations of the seedlings 14 days after sowing, 30 seedlings were taken for each treatment (10 per repetition) (n = 3) and the fresh and the dry mass (g) of the seedling were determined.

Morphological determinations: To carry out morphological determinations of the seedlings 14 days after sowing, 30 seedlings (n = 30) were taken for each treatment (10 per repetition) and the number of leaves per seedling, stem length (cm), stem thickness (cm), root length (cm) and root thickness (cm) were determined.

2.5 Statistical processing of results

All data were subjected to statistical analysis using SPSS (Version 8.0 for Windows, SPSS Inc., New York, NY). Data were tested for normality using a Kolmogorov–Smirnov test and means were compared using t-tests ($p \leq 0.05$).

3. Results

3.1 Seeds quality after stored

The moisture content of *T. labialis* seeds after storing them for 12 months was 7.74% and decreased slightly in relation to the time of harvest (7.96%). However, viability decreased to 93.00% (98.00% at the time of harvest) and the percentage of seeds with high vigor to 73.00% (95.00% at the time of harvest), which shows some degree of deterioration during storage (**Table 1**).

Table 1. Physiological indicators of *T. labialis* seeds after storage

Moisture content	%	7.74 ± 0.45
Viable seeds	%	93.00 ± 1.91
Seeds with high vigor	%	73.00 ± 100
Seeds with low vigor	%	20.00 ± 1.60

Mean ± Standard deviation of the mean

3.2 Evaluations during germination

Seeds priming with IHPLUSTM significantly improved the germination percentage in controlled conditions and all the variables associated with vigor (**Figure 1**, **Table 2**). Additionally, an increase in the amino acid content was observed in the seeds conditioned at 0 h (**Figure 2**). However, it was observed that the amino acid content decreased significantly in the conditioned seeds during germination at 12 h and 24 h compared to those of the control treatment.

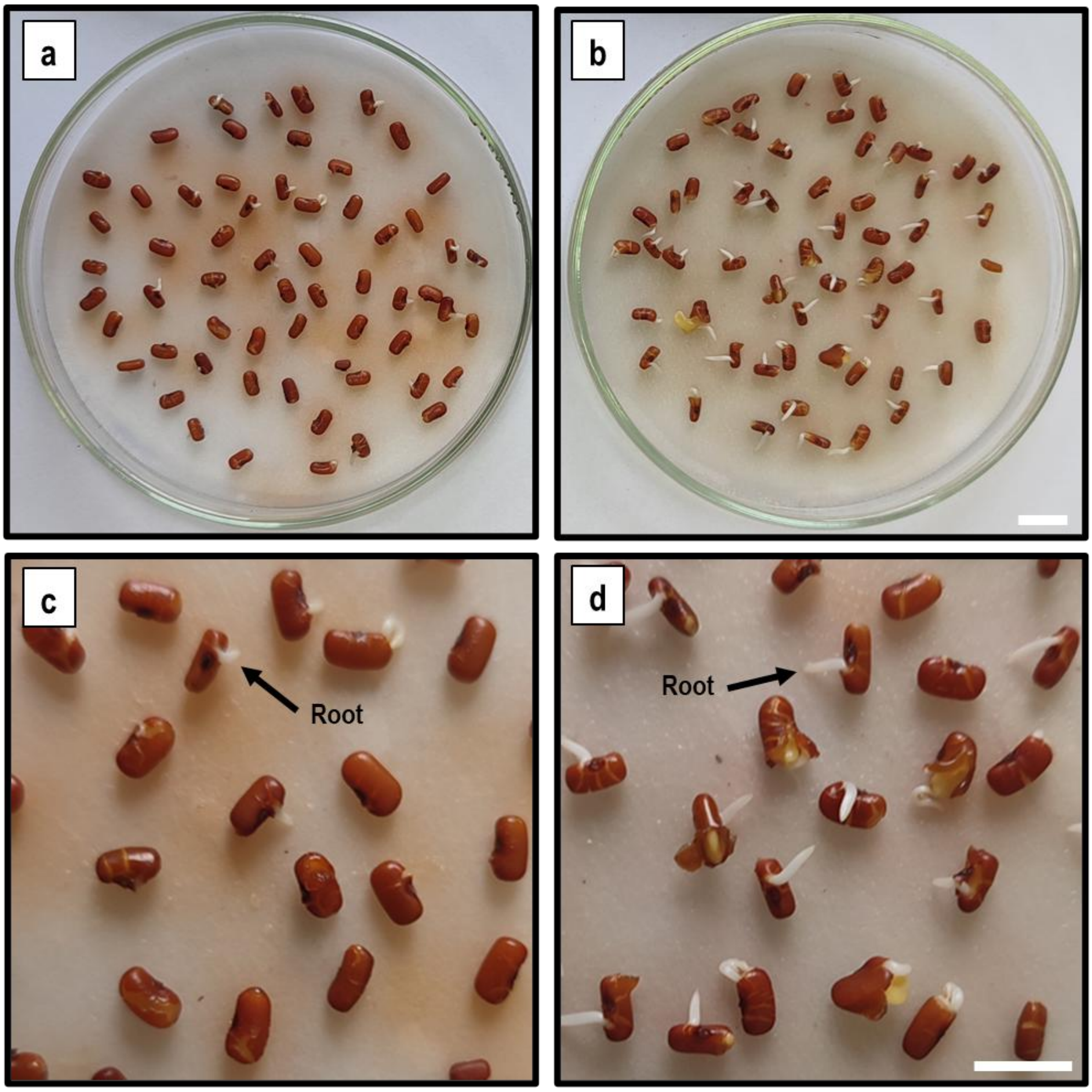

Figure 1. Germination of *T. labialis* seeds at 48 h in controlled conditions

Seeds without priming (control) (**a, c**), seeds priming with IHPLUS™ (**b, d**). The bar indicates 0.5 cm.

Table 2. Effect of IHPLUS™ on germination and some mathematical variables associated with the vigor of *T. labialis* seeds during germination in controlled conditions

	Seeds without priming (control)	Seeds priming with IHPLUS™
Germination (%)	72.00 ± 2.2 (b)	89.00 ± 2.3 (a)
Germination index (seeds day^{-1})	7.06 ± 0.48 (b)	10.75 ± 0.54 (a)
Time required for germination of 50% of the seeds (days)	3.5 ± 0.12 (a)	0.83 ± 0.04 (b)
Mean germination time (days)	3.45 ± 0.22 (a)	2.44 ± 0.16 (b)
Germination synchrony	0.23 ± 0.01 (b)	0.56 ± 0.03 (a)

Results with unequal letters, in each row, are statistically different (t-test, P ≤ 0.05). Ranges indicate the mean ± standard error of the mean.

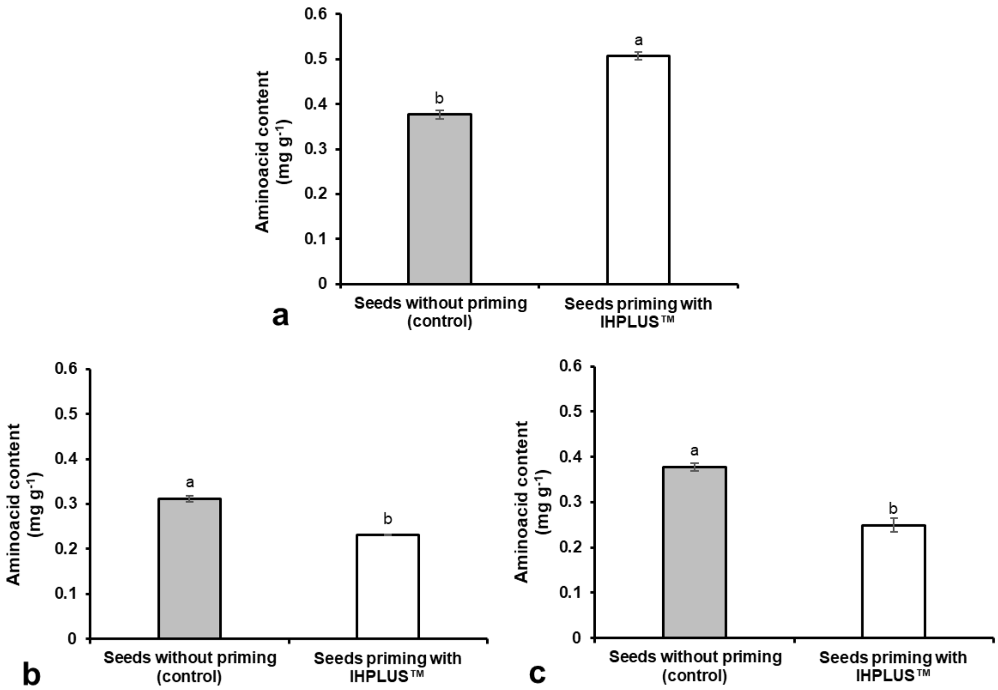

Figure 2. Amino acid content during germination of *T. labialis* seeds in controlled conditions

Amino acid content at 0 h (**a**); amino acid content at 12 h (**b**); amino acid content at 24 h (**c**). Means with unequal letters have statistically significant differences (t-test, p ≤0.05, n = 3). Vertical bars indicate mean ± standard error of the mean.

3.3 Evaluations on seedlings after 14 days of cultivation

The content of chlorophyll a, b and total 14 days after sowing the seeds did not show significant differences between the treatments evaluated (**Table 3**). However, the content of total nitrogen and total protein, in the same period of time, increased significantly in the aerial part (leaves and stems) of the seedlings obtained from seeds priming with IHPLUS™. Likewise, fresh and dry mass, the number of leaves per plant, the length and thickness of the stem, and the length and thickness of the root increased significantly in the seedlings obtained from seeds priming with IHPLUS™ after 14 days of cultivation (**Table 3**).

The increase in fresh and dry mass indicators is closely related to the growth of seedlings 14 days after sowing. As can be seen in **Figure 3**, when the seeds are primed with IHPLUS™, more vigorous and larger plants are obtained. This result is closely related and is a consequence of the increase in some morphological characters such as stem length and the number of leaves per seedling.

Table 3. Determinations on *T. labialis* seedlings after 14 days of growth in semi-controlled conditions

	Seedlings obtained from seeds without priming (control)	Seedlings obtained from seeds priming with IHPLUS™
Biochemical determinations		
Chlorophyll a (µg g^{-1} fresh weight)	76.58 ± 1.63 (a)	75.15 ± 0.52 (a)
Chlorophyll b (µg g^{-1} fresh weight)	23.41 ± 2.92 (a)	23.04 ± 3.21 (a)
Total chlorophylls (µg g^{-1} fresh weight)	100.01 ± 3.83 (a)	98.19 ± 3.63 (a)
Nitrogen contents (%)	3.86 ± 0.16 (b)	4.78 ± 0.01 (a)
Proteins (%)	24.13 ± 1.03 (b)	29.92 ± 0.09 (a)
Physiological determinations		
Fresh weight per seedling (mg)	14.43 ± 1.70 (b)	18.69 ± 1.55 (a)
Dry weight per seedling (mg)	4.93 ± 0.30 (b)	6.33 ± 0.18 (a)
Morphological determinations		
Number of leaves per seedling	1.50 ± 0.13 (b)	1.80 ± 0.13 (a)
Stem length (cm)	1.91 ± 0.12 (b)	2.44 ± 0.13 (a)
Stem thickness (cm)	0.39 ± 0.04 (b)	0.49 ± 0.03 (a)
Root length (cm)	3.16 ± 0.42 (b)	5.46 ± 0.29 (a)
Root thickness (cm)	0.36 ± 0.03 (a)	0.37 ± 0.02 (a)

Results with unequal letters, in each row, are statistically different (t-test, P ≤ 0.05). Ranges indicate the mean ± standard error of the mean.

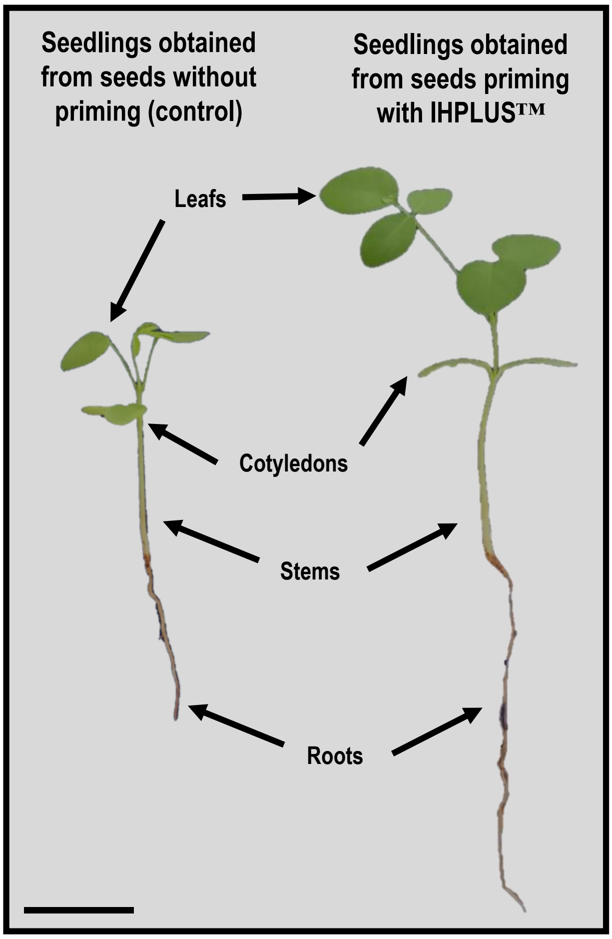

Figure 3. *T. labialis* seedlings obtained from seeds without priming and seeds with IHPLUS™ priming at a concentration of 5% for 3 h at 14 days after sowing in semi-controlled conditions

The bar indicates 1 cm.

4. Discussion

T. labialis seeds showed deterioration during storage under artisanal conditions, which coincides with what was described for this species by Acosta and others[8]. Varghese and Naithani[42] reported that deterioration during storage is due to a gradual increase in reactive substances of thiobarbituric acid, resulting from the progressive accumulation of reactive oxygen species. An aspect also described by Walters[10], who suggests that this is due to the temperature and humidity conditions prevailing during storage, which lead to a loss of viability and vigor in the seeds.

The priming of *T. labialis* seeds caused an increase in the germination percentage and the reinvigoration of the seeds. This is because seed priming causes an increase in the activity of enzymes involved in the respiration and catabolism of starch, proteins and lipids, resulting in more rapid mobilization of reserves to the growing points[43][44]. Specifically, the results of this research suggest the entry into the interior of the seeds of substances that stimulate enzymatic activity. This influences greater efficiency in metabolic processes, increasing the speed of germination and reinvigoration of the seeds[45][46]. As a result, an increase in the amino acid content was observed at 0 h in the primed seeds, but with a decrease at 12 and 24 h after they were put to germinate. Torres and others[47] indicated that the results obtained during the germination of *Cajanus Cajan* seeds showed a reduction in amino acids at a time close to the emergence of the radicle since they were used in the synthesis of new proteins. These proteins are formed with the aim of providing energy through the oxidation of their structures to promote the growth and elongation of the embryo[48].

It is known that the amino acid content varies during germination in legume seeds[49]. Some amino acids also act as precursors for the biosynthesis of spermidine and gibberellin, growth regulators, and many secondary metabolites[50]. The mobilization and transformation of reserves accumulated in seeds are crucial for the efficiency of germination and seedling establishment[51]. In conditioned *T. labialis* seeds, during germination, the amino acid levels do not present differences in the first 24 hours. This implies that germination occurred in a shorter period and in a higher percentage in seeds priming with IHPLUS™. Presumably, these levels of amino acids were provided to the seeds by the bioproduct used for priming. Amino acids act as a regulator of cytokinins and auxins, improving the elongation and growth of roots, which contributes to the absorption of nutrients by the new seedling[52]. Previously, it was observed that seeds primed increase shoot and root length, and fresh and dry mass of seedlings in rice crops[53] and wheat crops[54][55]. Furthermore, it was observed that the germination rate, the final germination and the seedling size improved in wheat cultivation[56]. Likewise, increases in the vigor index were also demonstrated in response to seed primed in rice and carrot crops[57][58].

Different studies related to primed seeds showed greater efficiency in nitrogen use, which can be reflected in resistance to phytopathogens and in vegetative development of seedlings[59][60][61]. The length and diameter of the stem are indicators of the vigor of the seedling, which is considered important since they show the strength and resistance it may have when subjected to field conditions[62]. The differences observed between seedlings obtained from seeds primed with IHPLUS™ and the control may be associated with the hormonal balance between the different endogenous and exogenous growth regulators and the nitrogen contents shown. Díaz and others[63] attribute this effect to biostimulant action mechanisms, such as auxinic amino acids, which coincides with Castillo-Portela and others[64], who also refer to this effect of the hormones that intervene in the growth of the different parts of the seedlings. In this sense, Batista-Sánchez and others[65] reported that biostimulants contain amino acid and carbohydrate chelates that are rapidly absorbed, have greater mobility once absorbed, and possess root growth-stimulating properties.

Various researchers observed an improvement in the number of leaves in corn crops[66], in leaf area in banana crops[67], in fresh and dry mass of leaves in wheat[68], and in fresh and dry mass in chickpea and wheat seedlings[69], in response to seed conditioning. The use of IHPLUS™ for the priming of *Sorghum bicolor* seeds stimulated the growth of the roots and the aerial part of the seedlings[33]. Meena and others[59], when investigating *Trichoderma harzianum* as a conditioning agent for wheat seeds, reported increases in seedling height, root length, field yield and chlorophyll content.

5. Conclusions

The use of IHPLUS™ for priming of *T. labialis* seeds stored for 12 months increased the germination percentage under controlled laboratory conditions. A reinvigorating effect was observed in the conditioned seeds, which was expressed in a better mobilization of amino acids during germination. In seedlings grown up to 14 days under semi-controlled conditions, nitrogen and protein content, fresh and dry mass, and vegetative development were significantly higher when the seeds were conditioned with IHPLUS™.

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Transparency of data

Available data: The entire data set that supports the results of this study was published in the article itself.

Author contribution statement

BL: Conceptualization; Investigation and Writing – original draft

AY: Conceptualization; Investigation; Supervision; Project administration and Writing – review & editing

LGY: Investigation

P-GL: Investigation

FP: Conceptualization; Funding acquisition and Supervision

LJC: Conceptualization; Supervision and Writing – review & editing

MC: Conceptualization; Project administration and Writing – review & editing

FD: Conceptualization; Investigation; Project administration; Supervision and Writing – review & editing

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