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Breaking the dormancy of *Momordica balsamina* L. seeds by resorting to pre-treatments

Quebra de dormência de sementes de *Momordica balsamina* L. por recurso a pré-tratamentos

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ABSTRACT

The Cucurbitaceae family has some species with dormant seeds, including *Momordica balsamina*. Therefore, several methods have been used to overcome dormancy, as these help to accelerate the germination process. In this way, the objective was to evaluate seed germination at different temperatures after seed pre-treatments. The seed pre-treatments were: removal of the integument; wetting the substrate with 0.1% KNO_3 solution; pre-cooling to 10 ºC for 3 days and control. Thereafter seeds germination temperatures were 25, 30 and 35 ºC in a BOD. Four replications of 25 seeds for each pre-treatment and temperature were used. After 21 days of the test assembly first germination count, germination, seedling emergence speed and seedling emergence were evaluated. The results showed physical seed dormancy. The removal of the tegument and the temperatures of 25 or 30 ºC, with 97 and 96% of germinated seeds respectively, provided ideal conditions for germination.

Keyword: common balsam apple, cacana, tegument dormancy, germination

RESUMO

A família Cucurbitaceae, possui algumas espécies cujas sementes apresentam dormência, inclusive *Momordica balsamina* L. Esta característica obriga à utilização de métodos de quebra de dormência para aceleração do processo de germinação. Assim, objectivou-se avaliar a germinação das sementes a diferentes temperaturas após o pré-tratamento das mesmas. Utilizou-se um delineamento inteiramente casualizado, em esquema factorial 4x3 com quatro repetições de 25 sementes por modalidade, sendo quatro pré-tratamentos: remoção do tegumento; humedecimento do substrato com solução de KNO_3 a 0,1%; pré-esfriamento a 10 ºC durante 3 dias e a testemunha e três temperaturas de germinação 25, 30 e 35ºC em câmaras do tipo BOD. Posteriormente procedeu-se à avaliação da primeira contagem de germinação, germinação, velocidade de emergência de plântulas e emergência de plântulas. Os resultados evidenciaram dormência tegumentar (física) das sementes. A remoção do tegumento e as temperaturas de 25 ou 30 ºC, com 97 e 96% de sementes germinadas respectivamente, proporcionaram condições ideais para germinação.

Palavras-chave: balsâmina-de-purga, cacana, dormência tegumentar, germinação

INTRODUCTION

Momordica balsamina L. (common balsam apple) is a plant species belonging to the family Cucurbitaceae (Nagarani *et al*., 2014). It is a native plant of South Africa and it can also be found with greater predominance in regions of tropical climate in Africa, Asia, Arabia, India and Australia (Duenas, 2019).

The species *M. balsamina* nevertheless being an invasive plant in several parts of the world (GBIF, 2021), is also a plant which is considered medicinal due to its phytotherapeutic properties that gives it the aptitude to treat various diseases such as ulcers, internal and external control of parasites, fever, malaria, wound healing and measles (Ramalhete *et al*., 2010).

The seeds of *M. balsamina,* although they are viable even with all the appropriate conditions, they do not germinate (Carvalho and Nakagawa, 2012). Therefore, the research involving seed pre-treatments in order to overcome dormancy has shown promising results, especially with regard to the impermeability of the integument. Some studies with seeds of other species of the family Cucurbitaceae report satisfactory results. For example, Oliveira *et al*. (2018) overcome dormancy of *Luffa operculata* (L.) Cogniaux by exposing the seeds to mechanical scarification with sandpaper number 80 and topping of the integument in the distal region of the embryo and Schmidt *et al*. (2017) got good germination results by submitting the seeds of *Cucumis anguria* L. to soaking for four days in distilled water, immersion in 10% NaClO solution for 10 minutes, and mechanical scarification with sandpaper for 10 seconds. In the case of the related species *Momordica charantia* L. seeds, the best results were obtained when the seeds were immersed in concentrated sulfuric acid (H_2SO_4) for 3 minutes (Parreira *et al*., 2012).

However, research using methods to overcome dormancy in cucurbit seeds, with an emphasis on pumpkins, melons and watermelons, has been underway for decades (Haim, 2007), and the efficiency of applying these methods is limited by the variability of the degree of dormancy between different species and between seeds of the same fruit (Carvalho and Nakagawa, 2012).

In this sense, among the different methods used to overcome integumentary dormancy, pre-cooling, treatment with strong acids and bases (Brasil, 2009), immersion of seeds in hot water, and chemical and mechanical scarifications have been showing satisfactory results (Oliveira *et al*., 2003).

Furthermore, the rules for seed analysis (Brasil, 2009) standardize the germination test and the first count of germination (vigor) in seeds of *M. charantia* and *Momordica* spp., but do not make any observations regarding the use of methods to overcome dormancy.

Thus, despite the great medicinal importance of *M. balsamina* there are few studies on the germination of seeds of this species. With this stated, the objective was to evaluate and compare the efficiency of different pre-germinative treatments for overcoming dormancy of seeds of *M. balsamina* L. (common balsam apple) under the effect of different temperatures.

MATERIAL AND METHODS

The fruits of *M. balsamina* L. used to carry out the experiment, were collected in April of 2019, in the city of Maputo, Mozambique. The fruits were selected at random, and fruits were collected at a similar stage of maturation, with a yellow-orange color (Figures 1, 2 and 3).

The seeds were extracted from the fruits with the aid of a knife, making a vertical cut dividing the fruit in halves with the subsequent removal of the seeds manually, then proceeding the separation of the seeds from the aryl washing them through a sieve in running water until they were completely clean. Finally, the seeds were placed to dry in shade at room temperature, revolving them until they were dry, which took 72 hours. Then the seeds were packed in kraft paper bags and sent to the Central Seed Analysis Laboratory of the Agriculture Department of the Federal University of Lavras, Brazil, for analysis.

Due to the scarcity of information on the germination of *M. balsamina* seeds, the degree of seed moisture was first determined by the greenhouse method at 105ºC ± 3ºC for 24 hours,

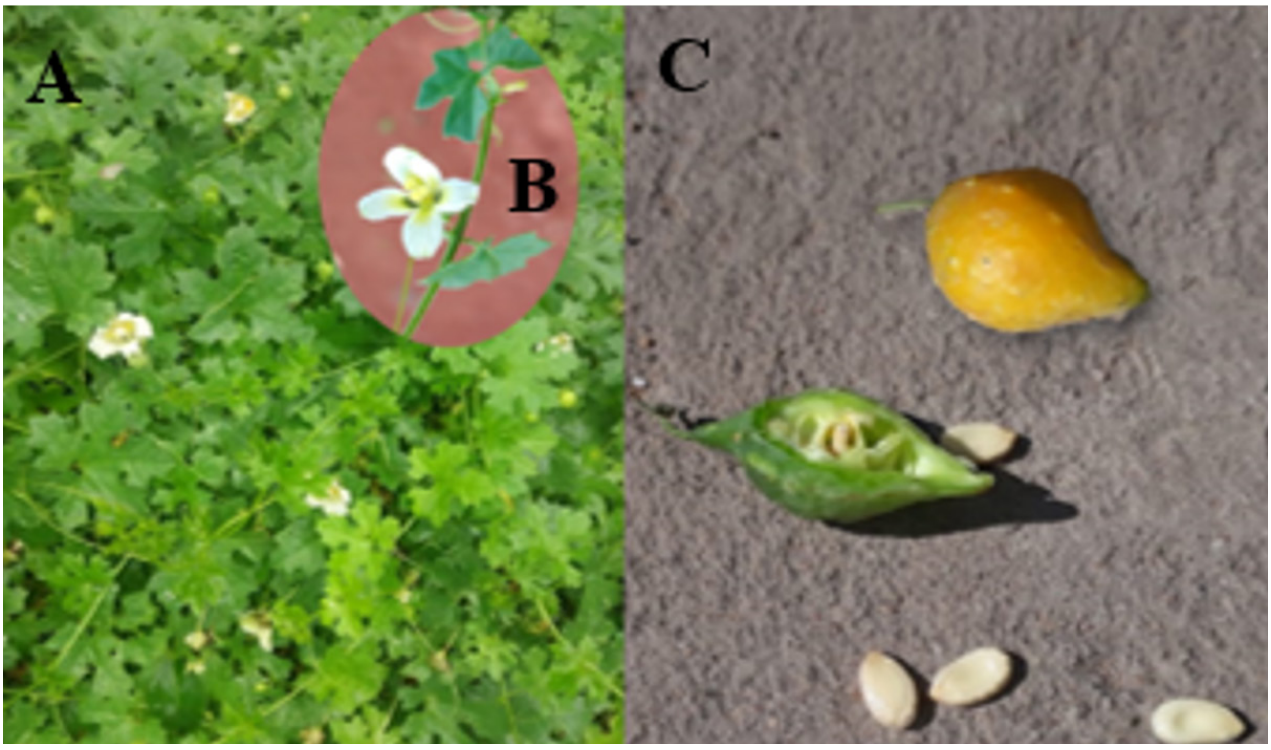

Figure 1 - A - Plant of *Momordica balsamina* L.; B – Flower; C - Ripe fruit and open green fruit with immature seeds.

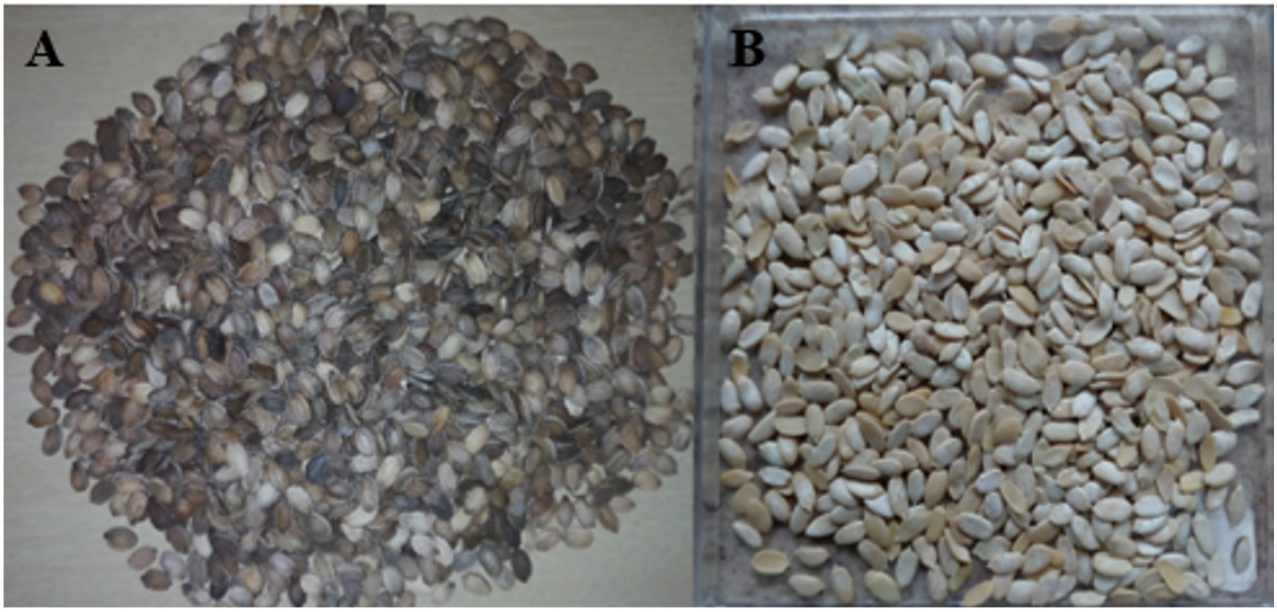

Figure 2 - A - Seeds with tegument; B - Seeds without tegument.

according to Brasil (2009). Subsequently, a pre germination test was performed, to find out what would be the germination potential of the seeds.

A germination pre-test was performed using four repetitions of 25 seeds, which were distributed on a roll of germitest paper moistened with distilled water, in an amount equivalent to 2.5 times the weight of the dry paper. The paper rolls were

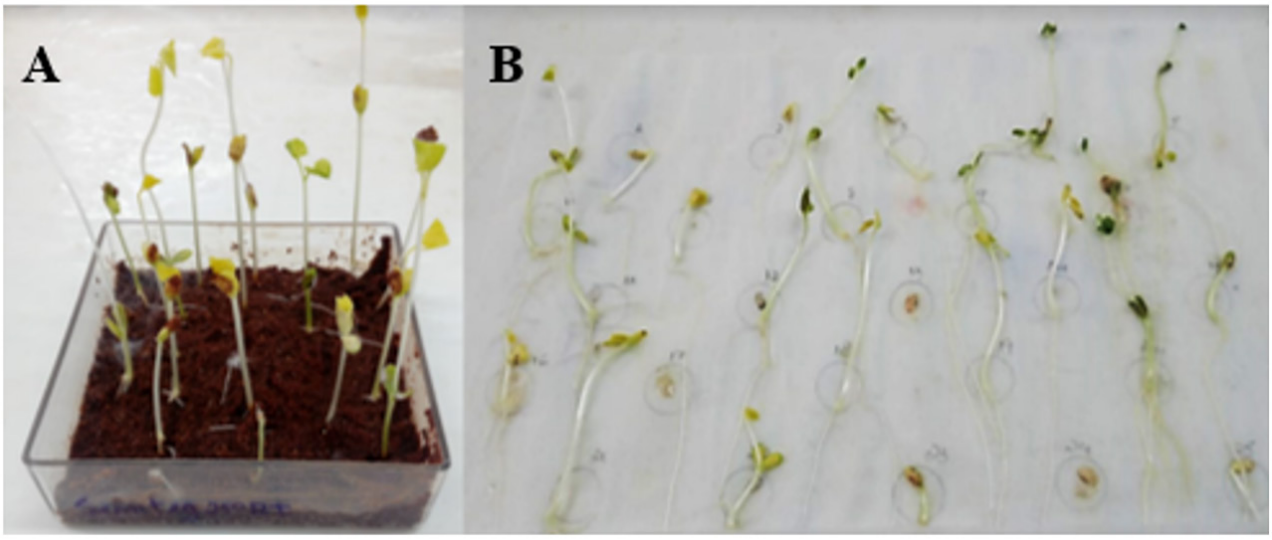

Figure 3 - A - Seedlings on a sand substrate; B - Seedlings on paper substrate.

stored in germination chambers, type BOD, at a constant temperature of 25 ºC, with 12/12 hours of light and dark. After 21 days of the test set-up, a germination of 10% was observed, considering the seeds with a radicle of 2 mm or more in length, according to Brasil (2009).

Due to the low percentage of germination observed in the pre-test and based on the reports of some studies that report physical dormancy in cucurbit seeds, it was decided to submit the seeds to X-ray image analysis to verify if the seeds were full, empty and or deformed.

Based on the result of the X-ray analysis, which showed that the seeds were completely full, the second germination pre-test was carried out, removing the entire seed coat with the aid of a scalpel, making a small cut at the opposite end of the embryo and the removal of the remaining integument was completed manually. After the total removal of the tegument, the germination test was assembled using two repetitions of 25 seeds, after 21 days of the test assembly, 100% of germinated seeds were obtained.

Based on the results obtained by the two pre-tests, an experiment was carried out using a completely randomized design in a 4x3 factorial scheme, in which four seed pre-treatments and to three temperature regimes were tested to determine the best method of germination and seedling emergence.

The pre-treatments carried out were: tegument removal - before sowing, the seed tegument was removed with the aid of a scalpel, making a small cut at the opposite end of the embryo and the removal of the remaining tegument was done manually; moistening the substrate with 0.1% KNO_3 solution - instead of distilled water, the paper was moistened with KNO_3 solution at a concentration of 0.1%; pre-cooling at 10 ºC for 3 days before the germination was assembled, the paper rolls were stored in a cold chamber and dried at 10 ºC for 3 days; and control - consisted of intact seeds, no treatment was performed on the seeds. After the treatments, the rolls were placed in BOD chambers at temperatures of 25, 30 and 35 ºC.

The effects of seed pre-treatments and germination temperatures were assessed using the following tests:

First count of germination - It was conducted along the germination test, consisting of recording the number of normal seedlings verified on the 14th day after sowing, according to the rules for seed analysis Brasil (2009), and the results were expressed as a percentage of normal seedlings.

Germination - Performed by moistening sheets of germitest paper 2.5 times the weight of dry paper with distilled water followed by sowing in accordance with Brazilian seed rules (Brasil, 2009).

The germination of the seeds corresponded to the number of seeds that produced seedlings classified as normal, presenting their complete essential structures such as primary root, hypocotyl, and cotyledons. The count of normal seedlings was performed on the 21 day after the sowing of the test and the results were expressed as a percentage (Brasil, 2009).

Seedling emergence - The test was carried out on a soil and sand substrate in the proportion of 1:2 respectively, they mistured was sieved and subsequently sterilized at 200 ºC for two hours, then the substrate was placed in plastic boxes (gerbox) and sowing proceeded, followed by moistening with water at 60% of the holding capacity, and for the treatment using KNO_3, instead of distilled water, the substrate was moistened to 60% of the retention capacity with the KNO_3 solution at a concentration of 0.1%. For pre-cooling, after the test set-up, the gerboxes were stored in a cold chamber and dried at 10 ºC for 3 days.

The moisture content of the substrate was maintained with irrigation whenever necessary. The results obtained were expressed as a percentage of the number of normal seedlings that emerged up to the twenty-first day after sowing, and seedlings above the substrate were considered normal seedlings.

Seedling emergence speed - The seedling emergence speed was performed along the seedling emergence test where the number of seedlings emerged up to the emergence stabilization at 21 days was recorded daily at the same time. The seedling emergence speed was calculated using the formula proposed by Maguire (1962), the result being expressed in index.

Statistical analysis

For the statistical analysis of the data, the results were subjected to analysis of variance by the F test and comparison of means by the Tukey test, at 5% probability with the aid of the Sisvar software (Ferreira, 2014).

RESULTS AND DISCUSSION

The moisture content of the seeds used in the experiment was 12.1%. Due to the low percentage of germination observed in the control, the results showed that the seeds of *M. balsamina* have dormancy, a fact that was confirmed with the increase in the percentage of germination verified when the seeds were subjected to pre-treatments.

From the results of the vigor measured by the first count of germination it was found that this was different for each pre-treatment used in the seeds (Table 1).

Table 1 - First count of germination (%) of *Momordica balsamina* L. seeds submitted to four pre-treatments under different germination temperatures

Seed pre-treatment	Seed germination temperature (ºC)		
	25	30	35
Removal of the integument	87 aA*	89 aA	90 aA
Moistening the substrate with 0.1% KNO_3	43 bA	18 bB	23 bB
Pre-cooling to 10ºC for 3 days	28 cA	23 bBC	14 bcC
Control	28 cA	15 bB	8 cB
CV** = 15.31			
Average = 38.83			

* The averages followed by the same lowercase letter in the column and uppercase in the row do not differ by the Tukey test, at the level of 5% probability. **Coefficient of variation

It was observed that the treatment that showed greater efficiency in overcoming dormancy was the removal of the integument, having been superior and significantly different from the other treatments, with 87, 89 and 90% of normal seedlings on the fourteenth day of the evaluation of the first count of germination at temperatures of 25, 30 and 35 ºC, respectively.

The three assayed temperatures were favorable, for the vigor expressed in the first germination count. However, in the control (integument not removed) seed germination was lower than the other treatments with 28, 15 and 8% of germinated seeds up to fourteen days at temperatures of 25, 30 and 35 ºC, respectively.

According to Vasconcellos *et al*. (2010), integumentary dormancy is characterized by the difficulty in absorbing water by the seed, making hydration impossible, limiting the metabolic processes of germination.

Therefor the superiority verified in the seeds submitted to the removal of the integument was already an expected result, since the entire seed coat was removed which at some point constituted a physical barrier preventing the entry of water. Thus, with the total removal of the tegument it was possible to promote rapid germination of the seeds, a fact that was not verified in the control.

The removal of the integument is a time-consuming procedure and requires special attention in its handling so as not to compromise the integrity of the embryo, and due to this delay, it becomes an inefficient procedure to be used on a large scale. However, this method of keeping the seeds without tegument was very advantageous in relation to the other treatments studied, not only for the vigor expressed in the first germination count but also for the significant increase in the germination percentage in naked seeds, and for the speed of the germination process, since the seedling emergence speed index was also higher in seeds without tegument.

Therefore, further studies are needed to facilitate the removal of the tegument in seeds of *M. balsamina*. It was observed that this method significantly reduced the percentage of dormant seeds, because the images captured by X-rays, the other treatments had full seeds and even so the vigor was low, due to the permanence of the seed coat impermeability.

According to the results of germination (Table 2), similarly to the first count of germination, it was found that the seeds submitted to the removal of the integument, were statistically superior in relation to the other treatments, with 97, 96 and 90% of germination at temperatures of 25, 30 and 35 ºC, respectively.

The efficacy of the removal of the integument to overcome physical dormancy was also observed by Silva *et al*. (2014). They observed a better germination result when the seeds were totally free of physical obstructions, without endocarp and integument.

Table 2 - Germination (%) of *Momordica balsamina* L. seeds submitted to four seed pre-treatments at different germination temperatures

Seed pre-treatment	Seed germination temperature (ºC)		
	25	30	35
Removal of the integument	97 aA*	96 aA	90 aA
Moistening the substrate with 0.1% KNO_3	51 bA	58 bA	23 bB
Pre-cooling to 10ºC for 3 days	28 cA	23 cAB	14 bcB
Control	28 cA	15 cB	8 cB
CV** = 12.31			
Average = 44.25			

* The averages followed by the same lowercase letter in the column and uppercase in the row do not differ by the Tukey test, at the level of 5% probability.
**Coefficient of variation

Once the seeds of this experiment were subjected to the removal of the integument in its entirety, having no coating, the seed was exposed, being in direct contact with the substrate, and in this way, it was sensitive to the action of the moisture of the substrate, light, and temperature that interacting with each other promoted the rapid resumption of the metabolic activity of the seeds, thus providing greater germination when compared to the seeds of the other treatments with tegument.

Temperatures of 25 and 30 ºC had a positive influence on germination, with a higher percentage of seed germination. Similar results were observed by Parreira *et al*. (2011) in their study with seeds of *Momordica charantia*, verified the reduction of germination (less than 5%) when using a temperature of 15 ºC and an increase when the seeds were submitted to 25 ºC. These observations corroborated Kraemer *et al*. (2000) who consider the ideal temperature range that favors seed germination of *M. charantia* between 20 and 30ºC, highlighting that temperatures below and above this range compromise the speed of the reactions involved in the germination process, reducing the germination capacity and in some cases, germination does not occur.

For seeds subjected to wetting with 0.1% KNO_3 solution, the germination was 51, 58 and 23% at temperatures of 25, 30 and 35 ºC, respectively.

Seed germination of at least 50% was observed at temperatures 25 and 30 ºC, what means that possibly the concentration used was not enough to break the seed coat, requiring some other way proceed for example by immersing the seeds so that they can be in direct contact with the solution, which would favor the rapid degradation of seed integument.

On the other hand, the seeds that were submitted on pre – cooling, the germination did not even reach 50%, it was below 30% in all temperatures studied meaning that the low temperature condition did not favor germination.

For seedling emergence (Table 3), the results were similar to what was observed in germination and seedling emergence speed, the treatment that showed high seedling emergence values was the seed removal of the integument, having been superior to the other treatments with 71, 58 and 47% of seedlings emerged at temperatures of 25, 30 and 35 ºC, respectively; the germination effect of the studied temperatures differed from each other, with the temperature of 25 ºC being statistically higher than the other temperatures. These results corroborate the ones reported by Rocha *et al*. (2018) also with seeds without tegument but with immersion in gibberellic acid (GA_3). Also in this test, seed moistening treatments with 0.1% KNO_3 solution and pre-cooling responded in a similar way, not reaching 20% of emerged seedlings by the end of the test. Considering that the emergency test simulates field conditions, specifically for seed wetting treatment with KNO_3 when mixing the solution with the soil and sand mixture, this condition reduced the contact of the seed with the solution, limiting the solution absorption consequently it also reduced the emergency percentage.

The highest values for variable seedling emergence speed (Table 4) were achieved in the seeds removal of the integument treatment, with 2.85, 1.45 and 1.28 seedling emergence speed index at temperatures of 25, 30 and 35 ºC, respectively. The temperature of 25 ºC was statistically higher than the others.

It was possible to prove once again the efficiency of seed tegument removal in overcoming dormancy in *M. balsamina* seeds, since the seeds submitted to this pre-treatment expressed their maximum vigor potential measured by the speed of emergence speed and seedling stabilization in comparison to the other treatments studied that obtained lower results (less than 1) of seedling emergence speed. Similar results were found by Souza and Gentil (2012) with other plant species. They observed higher seedling emergence speed values in the treatment with seeds without tegument.

The results observed in this work evidenced the occurrence of physical dormancy in seeds of *M. balsamina*, caused by the impermeability of the integument, restricting the entry of water and gas exchange of the seed with the environment. Fact proven in the experiment because the seeds that were subjected to tegument removal obtained the

Table 3 - Seedling emergence (%) of *Momordica balsamina* L. seeds submitted to four pre-treatments under the effect of different germination temperatures

Seed pre-treatment	Seed germination temperature (ºC)		
	25	30	35
Removal of the integument	71 aA*	58 aB	47 aC
Moistening the substrate with 0.1% KNO_3	15 bA	8 bA	7 bA
Pre-cooling to 10ºC for 3 days	4 cA	6 bA	3 bA
Control	0 cA	0 bA	0 bA
CV** = 28.24			
Average = 18.25			

* The averages followed by the same lowercase letter in the column and uppercase in the row do not differ by the Tukey test, at the level of 5% probability.
**Coefficient of variation

Table 4 - Seedling emergence speed (index) of *Momordica balsamina* L. seeds submitted to four pre-treatments under different germination temperatures

Seed pre-treatment	Seed germination temperature (ºC)		
	25	30	35
Removal of the integument	2.85 Aa*	1.45 aB	1.28 aB
Moistening the substrate with 0.1% KNO_3	0.38 bA	0.18 bA	0.33 bA
Pre-cooling to 10ºC for 3 days	0.16 bA	0.24 bA	0.073 bA
Control	0 bA	0 bA	0 bA
CV** = 43.95			
Average = 0.57			

* The averages followed by the same lowercase letter in the column and uppercase in the row do not differ by the Tukey test, at the level of 5% probability.
**Coefficient of variation

best results in all tests evaluated when compared to the other treatments.

The tegument removal, despite being efficient in overcoming seed dormancy, it is a very slow and inadequate process on a large scale, so other pre-germinative treatments need to be studied, treatment involving immersion of seeds in different concentrations of the potassium nitrate solution (KNO_3), since in this experiment seeds submitted to this treatment showed promising results. For seeds submitted to pre – cooling, the low performance verified in seeds of this treatment can be explained by the fact that the *M. balsamina* seeds have a physical tegumentary dormancy and pre – cooling was not enough to break the hard integument of seeds.

CONCLUSIONS

The seeds of *Momordica balsamina L.* have physical dormancy.

The removal of the integument from the seeds allowed the complete overcoming of physical dormancy.

The temperatures of 25 and 30 ºC were favorable for the germination of seeds.

The wetting of the substrate with the KNO_3 solution at a concentration of 0.1% and the pre-cooling to 10 for 3 days, did not favor the overcoming of dormancy of the *M. balsamina L.* seeds.

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