



Physicochemical characterization of fruits, biometry and dormancy of marula seeds

João Rafael Prudêncio dos Santos^{1*} ^{id} Victor Martins Maia¹ ^{id}
Andréia Marcia Santos de Souza David¹ ^{id} Fernanda Soares Oliveira² ^{id}
Mirna Ariane Taveira de Sousa e Souza¹ ^{id} Bruno Soares da Silva¹ ^{id}

¹Universidade Estadual de Montes Claros (Unimontes), Campus Janaúba, 39440-000, Janaúba, MG, Brasil. E-mail: joaorafaelantos2@yahoo.com.br.
^{*}Corresponding author.

²Universidade Federal do Oeste da Bahia (UFOP), Barra, BA, Brasil.

ABSTRACT: This study recorded the physicochemical properties of the marula fruits, the biometry and breaking the dormancy in the marula seeds. Using 200 ripe fruits, the physicochemical characterization was done. From a random sample of 400 seeds, an assessment of the biometrics was performed, and the weight, length, width and thickness of the fruits and seeds were evaluated. Two experiments were conducted to study dormancy breaking using pre-germination treatments, which included immersing the marula seeds five times (0, 15, 30, 45 and 60 minutes) in concentrated sulfuric acid (96%) and water, at 65 °C temperature. The control was time zero, using plain distilled water, with 25 seeds per treatment and four replications. The marula fruits revealed average values of 21.65 ± 3.44 g, 35.5 ± 2.38 and 34.8 ± 2.73, respectively, for weight, diameter and length. The soluble solids (SS), Hydrogenonic Potential (pH), titratable acidity (AT) and ratio (AT/SS) were 14.74 ± 1.0° Brix, 3.93 ± 0.49 pH, 0.63 ± 0.22 and 27.07 ± 12.10, respectively. For the weight of one thousand seeds, the average value was 3.576 ± 188.4 g. The mean values for the dimensions of the width, length and diameter of the seeds were 22.9 ± 2.11; 23.7 ± 2.04; and 14.9 ± 2.09 mm, respectively. The physiological quality of these seeds was enhanced by immersing the seeds from freshly picked fruits in sulfuric acid for a maximum of 30 minutes and storing them for six months.

Key words: *Sclerocarya birrea*, *Anacardiaceae*, sulfuric acid, storage and scarification.

Caracterização físico-química de frutos, biometria e dormência de sementes de marula

RESUMO: Objetivou-se com este trabalho estudar as características físico-químicas de frutos, biometria e superação da dormência de sementes de marula. Para a caracterização físico-química foram utilizados 200 frutos maduros. Realizou-se a biometria utilizando uma amostra aleatória de 400 sementes e foram avaliados: peso, comprimento, largura e espessura de frutos e sementes. Para o estudo da superação de dormência foram realizados dois experimentos com os tratamentos pré-germinativos constituídos pela imersão das sementes em ácido sulfúrico concentrado (96%) e água a temperatura de 65 °C por cinco tempos (0, 15, 30, 45 e 60 minutos), sendo o tempo zero a testemunha, onde utilizou-se apenas água destilada, com quatro repetições de 25 sementes por tratamento. Os frutos de marula apresentaram valores médios de 21,65 ± 3,44 g, 35,5±2,38 e 34,8±2,73 mm de peso, diâmetro e comprimento, respectivamente e sólidos solúveis (SS), *Potencial Hidrogeniônico* (pH), acidez titulável (AT) e ratio (AT/SS) foram de 14,74 ± 1,0 °Brix, 3,93 ± 0,49 pH, 0,63 ± 0,22 e 27,07 ± 12,10 respectivamente. As sementes apresentaram valores médios de 3.576 ± 188,4 g para o peso de mil sementes. Para as dimensões, largura, comprimento e diâmetro de sementes, apresentaram valores médios de 22,9 ± 2,11; 23,7 ± 2,04; e 14,9 ± 2,09 mm respectivamente. A imersão das sementes procedentes de frutos recém colhidos em ácido sulfúrico por até 30 minutos e o armazenamento por seis meses promove incrementos na sua qualidade fisiológica.

Palavras-chave: *Sclerocarya birrea*, *Anacardiaceae*, ácido sulfúrico, armazenamento e escarificação.

INTRODUCTION

Belonging to family *Anacardiaceae*, the marula (*Sclerocarya birrea* subsp. *Caffra*) is a fruit tree, which originated in the regions of South and eastern Africa, in the savannah biome (TAPIWA, 2019 and MOUK et al., 2007). It ranks high among the most valued trees in southern Africa, renowned for its high medicinal and nutritional properties (ELOFF, 2001). The fruit pulp is used commercially to manufacture an alcoholic drink (liqueur) under the Amarula® brand, made by fermenting its juice. As this beverage is popular, most of the marula fruits are used in drink.

Marula, normally cultivated in Africa, possesses high potential for production if it is raised in a dry tropical climate (semi-arid) because this environment resembles the edaphoclimatic conditions of its place of origin. The plants raised in the Brazilian semiarid environment are perennial and dioecious, with small red flowers, which bloom in early spring.

Sexual and asexual propagation takes place, and the seedlings arise normally from the seeds. The propagation methods in marula bear similarity to those of the umbu tree (*Spondias tuberosa* Arr Câmara); which although, is a member of the same family as the marula, and shows mainly seminiferous

propagation, can also be grown through grafting (REIS, 2010).

Although, this is a crop showing high potential for cultivation in the Brazilian semi-arid region, the characteristic dormancy of its seeds is a hindrance to its production, which must be overcome (PRITCHARD et al., 2004). As its emergence is slow and irregular and the seedlings display dissimilar degrees of growth, pre-germination treatments become a necessity to break the seed dormancy (MARUZANE et al., 2002).

To make valuable and scientific contributions to the studies that deal with overcoming seed dormancy in marula, generating seedlings, and obtaining new viable techniques, plus to complement the data available in the literature, works like the present study are significant. These studies widen the knowledge base regarding the propagation process and support the cultivation of this fruit species in semiarid regions.

This research studied the physicochemical characteristics of the fruits, the biometrics, as well as break the dormancy in the *Sclerocarya birrea* seeds.

MATERIALS AND METHODS

The experiments were done in the Plant Physiology Laboratory of the Department of Agricultural Sciences (DCA), in the State University of Montes Claros – UNIMONTES, Campus Janaúba-MG. When the fruits used in the experiment appeared to be at the point of maturation (yellowish-colored skin), they were picked from the plants that had been raised in the Experimental Farm of UNIMONTES, Janaúba, the geographic coordinates, which are as given — 15° 47' 50" S and 43° 18' 31" W, at an altitude of 516 m. Based on the Koppen classification, the climate of the region is type 'Aw 22' (tropical with dry winter) according to ALVARES et al., (2013).

The fruits were analyzed and the physicochemical characterization was done in terms of the length, width, diameter, weight, soluble solids (SS), titratable acidity (TA), SS/TA ratio and pulp pH.

Using digital calipers, the fruit length, width and diameter were measured and expressed in mm. Using a precision scale with two decimal places, the fruit weight was noted, and expressed in g. Thereafter, using a digital refractometer the fruit pulp was extracted and evaluated for soluble solids (SS), and expressed in Brix degrees (° Brix). Employing the titration method with NaOH 0.1 mol L⁻¹ the titratable acidity (TA) was determined and expressed in terms of % malic acid. Based on the ratio between the two variables (SS/AT) the soluble solids/titratable acidity

ratio (ratio) was determined. With the help of a digital pH meter the pH was determined.

Once the physicochemical assessments were completed, the seeds were extracted from the fruits (this involved removing the epicarp, mesocarp and endocarp) by placing them in hydrated lime and then passing the pulp through a sieve with a 5 mm mesh. The seeds were then thoroughly washed for 5 minutes, under running water. Next, the seeds were shade-dried for 10 days, under the environmental conditions prevailing in the laboratory (26 ± 3 °C and 65 ± 5% RH). Once this was done, the seeds were subjected to the oven method, set at 105 ± 3 °C, for 24 h (BRASIL, 2009), and the water content of 25 seeds was assessed, with four replications.

The seed biometrics (length, width and diameter) of 100 seeds were recorded, with four repetitions, using digital calipers (0.01 mm), and expressed in cm. The weight of one thousand seeds was established by counting eight repetitions of 100 seeds from the pure seed lot. Next, using a precision scale (0.0001g), the seeds from each repetition were weighed.

To overcome or break seed dormancy, two experiments were performed, adopting the completely randomized design (DIC), which included five treatments using 25 seeds per treatment, and four replications.

Once the fruits were harvested, the first experiment was performed. Treatments included immersing the seeds in concentrated sulfuric acid (96%) for 0, 15, 30, 45 and 60 minutes. Then the following parameters were assessed: seedling emergence, emergence speed index, hard seeds and abnormal seedlings.

Seedling emergence was performed under the environmental conditions in the laboratory (26 ± 2.3 °C). Sowing was done at 5.0 cm depth, in plastic trays which contained washed and sterilized sand, watered to a saturation equivalent of up to 60% retention capacity (BRASIL, 2009). Then, 70 days post sowing, the assessments were done. The normal seedlings were counted, as this was a mandatory criterion of assessment. In fact, the seedlings which revealed the cotyledons above the soil surface were counted, as well as the abnormal seedlings and hard seeds, and the findings were expressed in percentage.

The emergence speed index (IVE) was evaluated from the daily counts of the number of seedlings which emerged from the time of sowing up to 70 days. On completion of the experiment, the EVI was determined, using the formula proposed by MAGUIRE (1962).

In the second experiment, the seeds stored under laboratory conditions ($\pm 26\text{ }^{\circ}\text{C}$ and 65% RH) for six months were used. These were immersed in water, kept at $65\text{ }^{\circ}\text{C}$ temperature for the identical time durations of 0, 15, 30, 45 and 60 minutes. Apart from the evaluations described earlier, the lengths of the shoot and main root, as well as total dry mass of the seedlings were estimated.

On day 70 day, the lengths of the shoot and root of the normal seedlings of each replication were measured, using a ruler graduated in centimeters, and the findings were given in cm seedlings. Next, the seedlings were transferred to paper bags and oven-dried with forced air circulation, at $65\text{ }^{\circ}\text{C}$ for 72 hours. The well-dried seedlings were next cooled in a desiccator. Using an analytical scale with a precision of 0.001 g, the seedlings were weighed and recorded in g seedlings.

Applying descriptive analysis and Pearson's correlation, the data on the physicochemical characterization of the fruits and seed biometrics were analyzed. Adopting the analysis of variance at 5% probability level and subsequent regression analysis, the findings were tested for dormancy breaking. Based on the significance of the regression coefficients the models were selected, using the student's t test to a maximum of 10% probability, on the coefficient of determination and the potential to best describe the biological phenomenon. The R software was used to perform the statistical analysis.

RESULTS AND DISCUSSION

The marula fruits revealed the mean values of diameter and length as 35.5 ± 2.38 and 34.8 ± 2.73 mm, respectively. These findings were very similar to those noted by GOUWAKINNOU et al. (2011), who reported values of 35.43 ± 0.39 mm and 32.11 ± 0.36 mm in diameter and length, respectively, in the marula fruits.

The fruit weight on average was 21.65 ± 3.44 g. For the fresh fruit market, consumers were observed to prefer the heavier and, therefore, bigger fruits, according to CHITARRA & CHITARRA (2005). Hence, the marula fruit possesses great edible potential for intake *in natura*, as well as in the processing industries. In fact, GOUWAKINNOU et al. (2011) reported values of 19.90 ± 0.36 g. Earlier, HILMAN et al. (2008) had noted in different genotypes, the weight of the marula fruits, on average, having values in the range from 34.41 ± 0.91 to 56.74 ± 1.65 g.

As fruit size and mass are physical features innate to a species or cultivar, these are selected as

quality traits, based on which they are chosen and classified in terms of catering to the interest generated by the consumer trade. These variables are crucial in attracting the consumer to purchase the product (CHITARRA & CHITARRA, 2005).

The length/diameter (L/D) ratio was found to show a value of 1 ± 0.1 , signifying that the marula fruits are round in shape, concurring with the results reported by GOUWAKINNOU et al., (2011) whose findings were cited as the C/D ratio of 1.1 ± 1 ; they classified the fruit as oval in shape, as the length was mostly greater than the diameter, although some of the fruits showed the width was greater than the length.

As the length to diameter ratio of the fruits has a value of around to 1, it signifies that they are morphologically rounded in shape. This feature makes them more ideal for use in the pulp industry because the present-day machines are capable of comfortably handling fruits of this shape (LIRA JÚNIOR et al., 2005).

In this study, the parameters of soluble solids (SS), Hydrogen Potential (pH), titratable acidity (AT) and ratio (AT/SS) showed average values of 14.74 ± 1.0 °Brix, 3.93 ± 0.49 pH, 0.63 ± 0.22 and 27.07 ± 12.10 , respectively. The percentage of SS is particularly indicative of fruit flavor, which includes sugars and acids. In fact, CHITARRA & CHITARRA (2005) stated that the SS and pH 9 are the parameters that usually signify fruit acidity and are a good index (1-10) of maturity and quality for certain fruits; for the marula fruits, it can also find use as a reliable indicator of maturation 11 and consumption.

Post harvest, the seeds revealed water content of 8.2%. The water content of the seeds appears to be relatively low, a significant factor to consider during analyses. This is so because, based on the work of TAIZ et al. (2017), the water content in the dry and mature seeds is in the 5 to 15% range, similar to the findings in the marula seeds, which indicates greater reliability of the findings observed from the physiological quality tests.

After a six-month storage period, the seeds showed the water content a retained was almost identical to the post harvest value, (showing confirmed values of 7.8% moisture). In the present research, the environmental conditions, which included $26 \pm 3\text{ }^{\circ}\text{C}$ and $65 \pm 5\%$ RH observed during seed storage, were found to be the contributing factors to the water content estimated. Seed longevity is strictly linked to the water content values found, as it directly interrupts the physiological processes, thus lowering the seed quality and, therefore, directly

influencing the plant vigor, as well as germination power (MARCOS-FILHO, 2015).

Water content in the seeds is mostly influenced by the intensification of cellular respiration. When the water content of the seed reaches high levels, its respiratory activity can also dramatically increase and, correspondingly, there is quite a high utilization of reserve material and a reduction in the energy, which in turn, results in greater deterioration and decreased vigor (CARVALHO & NAKAGAWA, 2012).

The findings for the weight of a thousand marula seeds revealed mean values of $3,576 \pm 188.4$ g, with each seed having a mean weight of 3.576 ± 0.018 g. Pearson's correlation study for the dimensions of width, length and diameter of the seed showed no significance, with mean values of 22.9 ± 2.11 ; 23.7 ± 2.04 ; and 14.9 ± 2.09 mm, respectively. In the multiplication of different plant species, the popular practice of classifying seeds has been done by size, to determine the physiological quality (ALVES et al., 2000). In light of this, CARVALHO & NAKAGAWA (2012) reported that bigger seeds have a larger quantity of reserves and; therefore, greater vigor.

Seed biometry is also linked to the properties of dispersal and establishment of the seedlings (FENNER, 2006); it is also used to distinguish between pioneer and non-pioneer species in the tropical forests (BASKIN & BASKIN, 1998); and these studies are of great significance for the establishment of the marula culture.

The analysis of variance with reference to the physiological characterization of the seeds assessed post-harvest (first test) demonstrated a vital effect of the duration of immersion in concentrated sulfuric acid on all the variables analyzed, barring the abnormal seedlings.

Data related to seedling emergence (Figure 1A) displayed quadratic behavior. It was observed that for the control (without immersion), 9.03% percentage of emergence was evident. However, after the seeds were immersed in H₂SO₄, the values increased, achieving the maximum percentage of seedling emergence (52.59%) in 28.7 minutes. This resulted in a 482.39% increase, when compared with the control.

The immersion period in sulfuric acid for 28.7 minutes was sufficiently effective in reducing

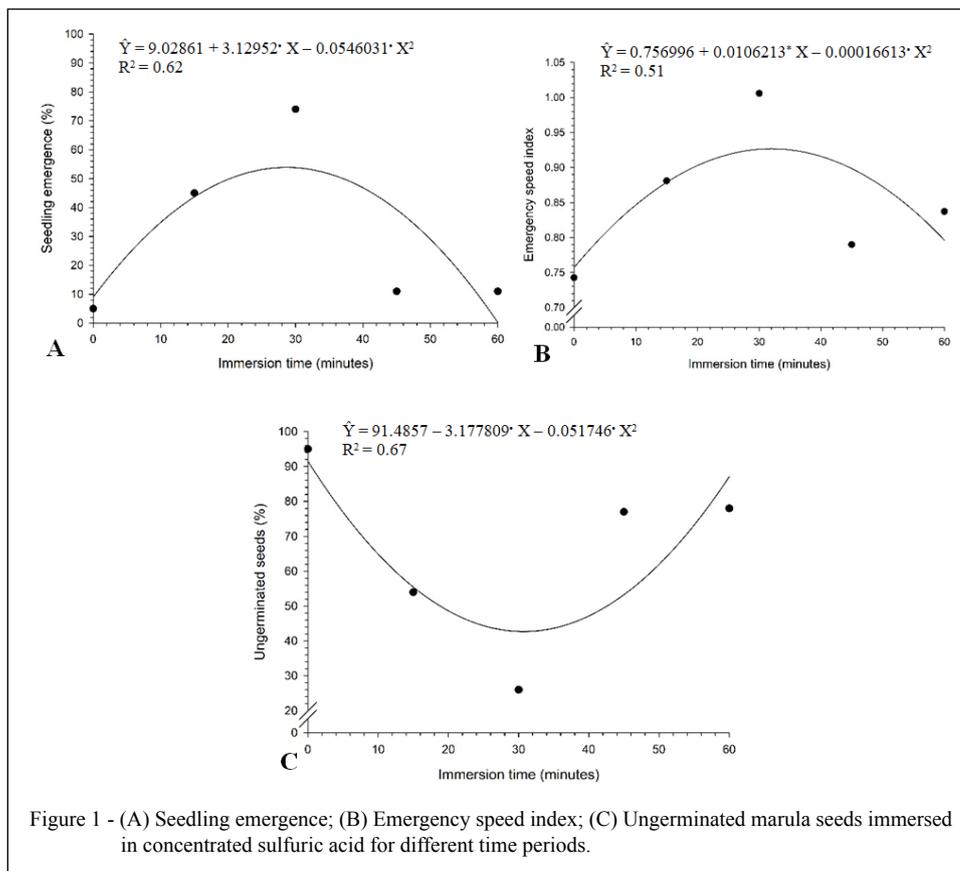


Figure 1 - (A) Seedling emergence; (B) Emergency speed index; (C) Ungerminated marula seeds immersed in concentrated sulfuric acid for different time periods.

the resistance of the tegument, raising its permeability for the entry of water and oxygen, encouraging the absorption process and, thereby, initiating the process of germination. However, from that time onwards, a decline was noted, culminating in 0.22% of emergence, after immersing the seeds in H₂SO₄ for 60 minutes. These results signify that immersing the seeds in H₂SO₄ from 28.7 minutes and above induced a negative effect on the initial physiological processes of germination and caused embryo damage, thereby compromising the seed viability.

The findings from the current study differ from those reported by MOYO et al., (2009); they reported that the immersion of the marula seeds in sulfuric acid for different time durations did not encourage increased germination, when compared with the control. Besides, VON TEICHMAN & ROBERTSE (1986) recorded the inefficiency of acid scarification in breaking the dormancy in the marula seeds.

Just like seedling emergence, the IVE results (Figure 1B) fit into a quadratic polynomial behavior regression equation. In the beginning, in the treatment of the seeds without immersion in H₂SO₄, the IVE, which was 0.75, increased to 0.92 within 31.96 minutes, registering a 16.45% increase, higher than the control, thus highlighting the efficacy of the treatment in breaking the dormancy in the marula seeds post-harvest, through tegument scarification. Higher IVE values are indicative of a more rapid and uniform emergence process, signifying heightened seed vigor. However, from the maximum point (0.92), a decline in the IVE was observed, culminating in a value of 0.79, after 60 minutes.

For the variable hard seeds (Figure 1C), quadratic behavior was confirmed. Prior to the sulfuric acid treatment, values exceeding 90% in the hard seeds were observed. However, immersing the seeds in H₂SO₄ for up to 29.9 minutes raised the efficiency, by lowering the percentage of the hard seeds, thus offering valid reason for the high values in the emergence noted during the period (Figure 1A). According to ROLSTON (1978), the water resistance of the tegument is a normal occurrence in the seeds of the Anacardiaceae family, such as marula. In RAS (BRASIL, 2009), many treatments are suggested to encourage germination in the hard seeds, focusing on imbibition, as well as mechanical and chemical scarification using concentrated H₂SO₄.

After the six-month storage-period (second test), immersing the seeds in water at 65 °C temperature induced a significant effect on all the variables studied. The data on seedling emergence

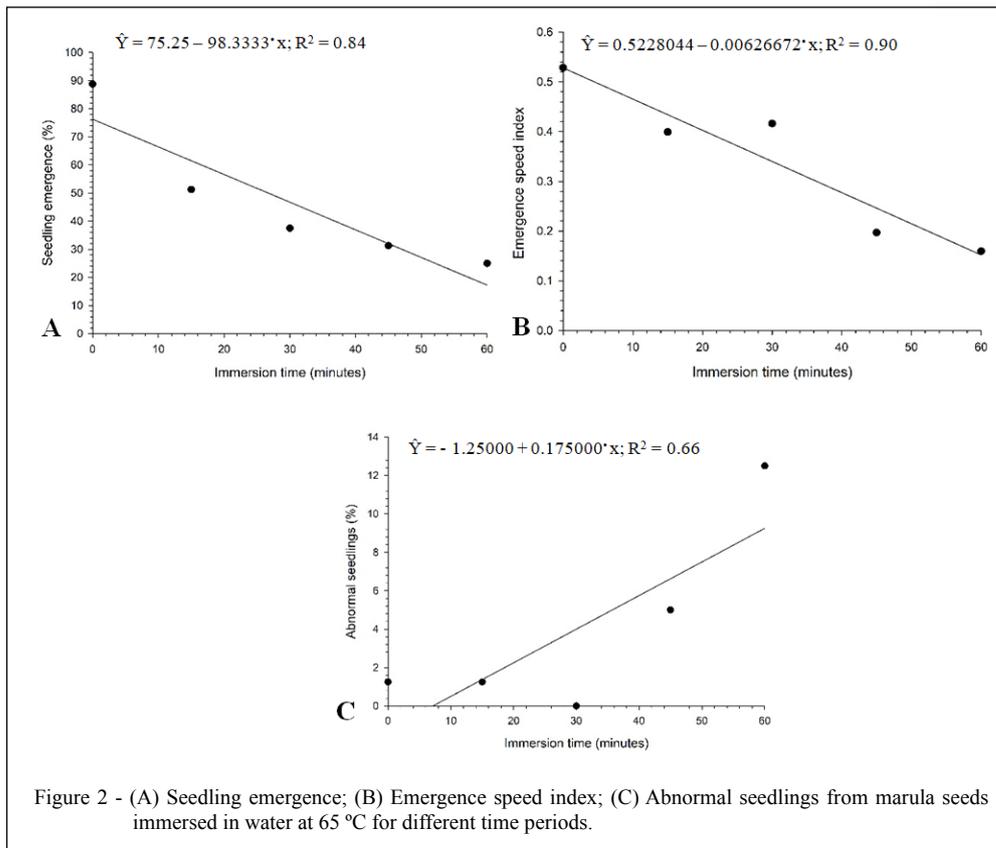
(Figure 2A) revealed linear behavior which was inversely proportional to the extended duration of the immersion time of the seeds in water, at 65 °C; this showed that as the time duration of immersion increased, the percentage of emergence decreased. In the case of the control (without immersion) 88.75% emergence was observed. However, the lowest value was achieved at 60 minutes, when the emergence of only 25% of the seedlings was seen, registering a decrease to 71.83% when compared to the control, thus suggesting the negative effect exerted by the treatment on the marula seeds.

Seeds allowed to soak in hot water can experience irreversible damage to the membrane system, resulting in leaching of the cellular contents, which in turn have a negative effect on germination. However, according to MAYER & POLJAKOFF-MAYBER (1989), the hot water can cause denaturation of the tegument proteins and raise the capacity for water absorption. In the current work though, this treatment negatively affected seedling emergence.

In their research, MOYO et al. (2009), investigated different techniques to overcome dormancy in marula, and reported that for seeds immersed in water at 100 °C temperature (boiling point), no germination occurred, revealing the possibility of treatment-induced embryo death.

For the IVE (Figure 2B), a linear behavior is confirmed bearing resemblance to seedling emergence, showing a drop in the values proportional to the increased duration of seed immersion time. The highest IVE (0.52) was observed for the control (time 0), implying that the seeds showed more rapid and uniform emergence because of heightened vigor. The immersion of the seeds in water at 65 °C for 60 minutes, however, produced the lowest index (0.16), indicating a drop in the values of 69.23%, when compared to the control. From these results it becomes evident that the dormancy present in the marula seeds can be overcome naturally; however, the time duration required may vary based on the state of the environment when the seeds were produced. This reiterates that immersion in water at 65 °C, during the time periods examined, exerts a negative effect on the initial physiological processes of germination, resulting in damage to the seed embryos and compromising their viability, as reported prior.

It was ZWIRTES (2013) who investigated the use of hot water as a low-cost technique with high efficacy for breaking dormancy in certain species. However, in the current research, this method failed to show good results with respect to breaking dormancy in the marula seeds.



Therefore, when the *Sclerocarya birrea* seeds were stored for six months, besides encouraging the germination potential, this procedure also contributed towards supporting the breaking of seed dormancy, which removes the necessity for using pre-germination treatments, like exposure to water raised to high temperatures.

Similar to the other variables, the findings regarding the abnormal seedlings (Figure 2C) were adjusted in a linear model regression equation, with a drop in the values corresponding to the duration of the time that the seeds were immersed in the water at 65 °C. This indicates the possibility that this occurrence was caused by the damage to the seed embryo induced by the duration of exposure to water at high temperature. Lower percentages were observed for the abnormal seedlings when compared with the seeds from the control (time 0).

In view of the presence of abnormal seedlings it becomes clear that the physiological quality of the seeds was weakened; however, immersion of the seeds at the temperature selected in this study may have affected the increased rapidity of the metabolic reactions in the seed, which failed to result in adequate emergence.

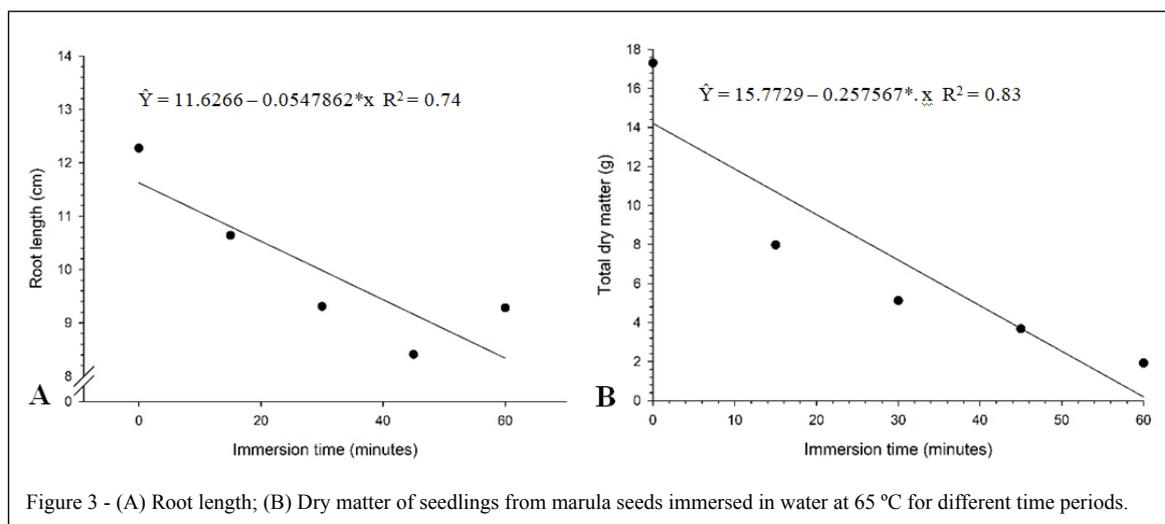
The findings for root length (Figure 3A) and seedling dry mass (Figure 3B) were observed to fit a linear behavior model. When the seeds were immersed in the in water at 65 °C the accumulated dry mass of the seedlings decreased (Figure 3B); the highest values were achieved for the seeds of the control. The lowest dry mass content was noted when the seeds remained immersed in water at 65 °C for 60 minutes. Identical behavior was seen for root length (Figure 3A), where the untreated seeds gave rise to seedlings having longer roots.

CONCLUSION

The marula fruits cultivated in semi-arid regions reveal physicochemical features that make it suitable for use both as a fresh fruit, as well as for industrial processing.

Marula fruits are almost identical in dimensions to each other, possessing an average of 21.65 ± 3.44 g, 35.5 ± 2.38 and 34.8 ± 2.73 mm in terms of weight, diameter and length, respectively.

Marula seeds too possess almost the same dimensions as each other, revealing an average weight,



width, length and diameter of 3.576 ± 0.018 g, 22.9 ± 2.11 mm, 23.7 ± 2.4 mm and 14.9 ± 2.9 mm, respectively.

When the seeds from freshly harvested fruits are immersed in sulfuric acid for a maximum of 30 minutes and placed in storage for six months, their physiological quality is enhanced.

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DECLARATION OF CONFLICT OF INTEREST

The authors have no conflict of interests to declare. With respect to the study design, collection, data analysis and interpretation, as well as to the manuscript writing and decision to publish the results. In all these aspects, the sponsors/funders had no role to play at all.

AUTHORS' CONTRIBUTIONS

All authors made equal contributions with regards to the manuscript design and writing. All the authors individually reviewed the manuscript critically, before the final version was approved.

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