Morphology and germination of *Acmella oleracea* L. R. Jansen under different temperatures and photoperiods

Erivanessa Costa Sousa Sarmento*, Kleane Targino Oliveira Pereira, Fernando Sarmento de Oliveira, Caio César Pereira Leal, Salvador Barros Torres and Alek Sandro Dutra

**ABSTRACT** - The objective was to evaluate the physical characteristics and physiological behavior of *A. oleracea* seeds during imbibition and germination, under different temperatures and photoperiods, as well as to determine the duration of the germination test. For this, biometric parameters (length, width and thickness) were measured in 100 seeds and thousand-seed weight was determined. In addition, the external structures of seeds and seedlings were described and the imbibition curve was monitored. Subsequently, the germination test was conducted at temperatures of 20, 25, 30, 35, 20-30 and 25-35 °C, under constant and alternating light and dark photoperiods (12 h), arranged in a 6 x 3 factorial scheme, with four replicates of 50 seeds. Seeds were sown in transparent plastic boxes with blotting paper as substrate, germinated in Biochemical Oxygen Demand (B.O.D) incubator, and evaluated for six days. The following variables were analyzed: germination, germination speed index, seedling length and seedling dry mass. The imbibition pattern test was conducted at 25 °C with 4 replicates of 100 seeds, evaluated until 50% root protrusion occurred. *A. oleracea* seeds exhibit uniform physical characteristics and the three-phase model as a water absorption pattern. Constant temperatures of 25 and 30 °C and alternating temperatures of 20-30 °C, regardless of the photoperiod, led to higher germination values. For *A. oleracea*, the germination test evaluations can be performed on the fourth and sixth day after sowing, as first and last counts, respectively.

**Key words:** Asteraceae. Jambu. Imbibition. Seed morphology. Seed analysis.

**RESUMO** - Objetivou-se avaliar as características físicas e o comportamento fisiológico de sementes de *A. oleracea* durante a embebição e germinação, sob diferentes temperaturas e fotoperíodos, bem como determinar a duração para a execução do teste de germinação. Para isso, realizaram-se a biometria das sementes (comprimento, largura e espessura) de 100 sementes e peso de mil sementes. Assim como, as descrições das estruturas externas das sementes e plântulas e o monitoramento da curva de embebição. Em seguida, realizou-se o teste de germinação sob temperaturas de 20, 25, 30, 35, 20-30, e 25-35 °C, submetidas aos fotoperíodos de luz e escuro constantes e alternado (12 h), arranjados em fatorial 6 x 3, com quatro repetições de 50 sementes. As sementes foram semeadas em caixas de plástico transparente, tendo como substrato o papel mata-borrão e colocadas para germinar em Biochemical Oxygen Demand (B.O.D), sendo avaliadas durante seis dias. As variáveis analisadas foram: germinação, índice de velocidade de germinação, comprimento e massa seca de plântulas. O padrão de embebição, foi realizado com 4 repetições de 100 sementes, a 25 °C, e avaliado até o surgimento de 50% de protrusão radicular. As sementes de *A. oleracea* apresentam características físicas uniformes e o modelo trifásico como padrão de absorção de água. As temperaturas de 25 e 30 °C e alternada de 20-30 °C, independente do fotoperíodo, proporcionaram maiores valores de germinação. As avaliações do teste de germinação de *A. oleracea* podem ser realizadas no quarto e sexto dia após a semeadura, como primeira e ultima contagem, respectivamente.

INTRODUCTION

_Acmella oleracea_ (L.) R. K. Jansen - Asteraceae, known as jambu, is a widely cultivated herbaceous vegetable in the northern region of Brazil (HOMMA et al., 2011). In addition to its extensive use in gastronomy, it has several pharmacological properties, such as anesthetic, aphrodisiac, insecticide, anti-inflammatory, analgesic and antioxidant (GOUVÊA et al., 2019).

_A. oleracea_ is mainly multiplied by seeds, but there is little information regarding seed technology involving this species, especially regarding the physical and physiological characteristics during its germination, photoperiod and temperature, which influence this process.

Biometric characterization of seeds is an important tool for morpho-anatomical studies (PEREIRA; FERREIRA, 2017). It can assist in the identification and detection of genetic variability within populations of the same species, especially in those cultivated under different environmental conditions (BEZERRA et al., 2014). Moreover, it contributes to understanding the domestication processes of the species (ORRÚ et al., 2012).

Imbibition of water by the seeds varies with species, number of pores distributed over the coat surface, water availability, temperature, hydrostatic pressure, contact area between seed and water, intermolecular forces, chemical composition and physiological quality (BEWLEY; BRADFORD; HILHORST, 2013). These variables together initially result in capillarity and subsequently in diffusion, which are the processes responsible for water movement into the seed (MARCOS-FILHO, 2015). Germination can be influenced by several factors, such as water, light, temperature, salinity and oxygen, which can act alone or in interaction (NOGUEIRA et al., 2014; PAIVA et al., 2016). Usually, each species responds in a particular way to these variables (BELMEHDI et al., 2018).

The presence of light is fundamental to regulate seed germination in agroecosystems. This variable is essential for the survival of several species, because it controls where and when their germination occurs, preventing the reserves stored in the seeds from being exhausted (MOTSA et al., 2015). Some seeds germinate equally well in the presence or absence of light (STEFANELLO; VIANA NEVES, 2017), whereas others germinate better only under light or darkness (MAHMOOD et al., 2016; ZUCARELI; HENRIQUE; ONO, 2015).

Temperature is one of the environmental factors that most influence germination, given its important role in water absorption by seeds, biochemical alterations and reactivation of metabolism (BEWLEY; BRADFORD; HILHORST, 2013; MARCOS-FILHO, 2015). Seeds germinate at specific temperatures, which can be optimal, minimum and maximum. Understanding the relationship between germination and cardinal temperatures is important to identify tolerance to low and high temperatures and simulate environmental conditions under which crops can successfully germinate and establish (MOTSA et al., 2015).

Recent studies have demonstrated that light and temperature influence the germination of herbaceous species (DIAS et al., 2015; MOTSA et al., 2015; PAIVA et al., 2016). Given all the information above, the hypothesis of this study is that the interaction between light and temperature may have significant implications for successful germination and establishment of _A. oleracea_ in agroecosystems. To test this hypothesis, the biometric parameters and water absorption curve of the seeds and the effects of different light regimes and temperatures on _A. oleracea_ germination were investigated.

MATERIAL AND METHODS

The experiment was conducted in the Seed Analysis Laboratory (LAS) of the Federal Rural University of the Semi-Arid Region (UFERSA), Mossoró, RN, Brazil. _A. oleracea_ inflorescences were collected from spontaneous and random plants in the city of Belém, PA (01°27'21" S, 48°30'16" W and 10 m of altitude). These inflorescences were dried in a ventilated and shaded environment for five days (HOMMA et al., 2011). The achenes were manually removed from the inflorescences and the paleae were eliminated with a blower (General®). Subsequently, the seeds were placed in polyethylene bags and stored in a controlled environment (18°C and 50% relative humidity) until the experiment was set up.

To evaluate seed biometric parameters (length, width and thickness), 100 seeds were randomly separated and measured using a digital caliper, graduated in millimeters, with precision of 0.05 mm. Length was measured in the central region of the seeds, longitudinal direction, whereas width and thickness were evaluated in their middle portion. The means, standard deviations and coefficients of variation were obtained for each characteristic. Thousand-seed weight was determined based on the recommendations of the Rules for Seed Analysis (BRASIL, 2009), using eight replicates of 100 seeds. These seeds were weighed on an analytical scale (0.0001 g) and the results were
expressed in grams. Water content was determined before the germination test, by the oven method at 105 ± 3 ºC for 24 hours, using the weight of two seed subsamples, and the results were expressed as mean percentage based on the wet weight (BRASIL, 2009).

The imbibition curve was constructed using four replicates of 100 seeds, which were arranged on two sheets of blotting paper, according to the procedure described for the germination test. Then, the boxes (Gerbox®) were closed and placed in transparent plastic bags (0.05-mm thick) to prevent the substrate from drying and kept in a Biochemical Oxygen Demand (BOD) incubator at 25 ºC. Initially, the seeds were weighed every hour during the first 11 hours of imbibition on an analytical scale with precision of 0.0001 g. After this period, the seeds were weighed at 2-h intervals until 26 h from the beginning of the imbibition, when the primary root protrusion was observed in 50% of the seeds. Before each weighing, the seeds were lightly dried with paper towels and placed again to absorb water. The percentage of water absorbed was calculated relative to the initial and final weights of the seeds (OLIVE; BOSCO, 2013).

In the evaluation of the adequate temperature and photoperiod for the germination test, the experimental design was completely randomized, in a 6 x 3 factorial arrangement, with four replicates of 50 seeds per treatment. The temperatures used were 20, 25, 30 and 35 ºC (constant) and 20-30 and 25-35 ºC (alternating). The lighting conditions were constant light and dark and alternating light (12 h light and 12 h dark). Seeds were arranged in Gerbox® acrylic boxes (11 x 11 x 3.5 cm) on two sheets of blotting paper as substrate, moistened with distilled water in a quantity equivalent to 2.5 times the weight of the dry paper. Daylight-type white fluorescent lamps (20 W) were used for the constant light treatment. In the constant dark treatment, the acrylic boxes were wrapped with two sheets of aluminum foil and the evaluation was carried out under safety light, covered with three sheets of green cellophane paper (SOUZA; PEREIRA, 1992). Photoperiod alternation under the alternating temperatures was 8 h light at the highest temperature and 16 h dark at the lowest temperature (BRASIL, 2009).

The germination test was conducted for six days until stabilization occurred. Seeds that produced normal seedlings were considered as germinated and the results were expressed in percentage.

The germination speed index was evaluated along with the germination test. Normal seedlings were daily counted for six days from sowing, following the same time as the first evaluation (MAGUIRE, 1962).

At the end of the germination test, normal seedlings were measured from the tip of the primary root to the leaf apex, using a ruler graduated in millimeters, and the results were expressed in cm seedling⁻¹.

To obtain the dry mass, normal seedlings were placed in identified Kraft paper bags and then dried in a forced air circulation oven at 65 ± 2 ºC until constant weight. After drying, the seedlings were weighed on an analytical scale and the results were expressed in mg seedling⁻¹.

The data were subjected to analysis of variance by F test (p ≤ 0.05). In cases of significance, the means were compared by Scott-Knott test at 5% probability level. Seed biometric data (length, width and thickness) were analyzed by means of descriptive analysis and represented by graphs constructed using the program SigmaPlot 12.0®. For the water absorption curve, a third-order polynomial regression model was fitted. Statistical analyses were performed using the statistical program SISVAR (FERREIRA, 2011).

RESULTS AND DISCUSSION

A. oleracea seeds have a dark-gray, almost black coat, partially involved in membranous parts, with two marginal ribs, longitudinally elongated, ciliated, complete, with bristly faces, pairs of decentralized bristles, not divided in apices and with small burned yellow spots on the coat (HIND; BIGGS, 2003) (Figure 1).

The average water content of the seeds was 7.4% and the thousand-seed weight was 0.22 g, corresponding to 4545 seeds g⁻¹.

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Figure 1 - External morphology of Acmella oleracea L. R. K. Jansen seeds (Magnification is 3x)
In relation to seed length, width and thickness, there was a low variation between the intervals, and the classes with highest frequency were 2.16-2.28 mm (23%), 0.89-0.99 mm (36%) and 0.32-0.36 mm (35%), respectively (Figure 2). These seeds came from mother plants that grew under the same environmental conditions. This may have contributed to the low variation in the biometric characteristics of the seeds.

Mean values of 2.33, 0.96 and 0.33 mm were recorded for length, width and thickness, respectively (Table 1).

The water absorption curve fitted to the three-phase model (Figure 3), in which the phase I was characterized by a rapid increase in mass due to water absorption, from 7 to 55% after 4 hours from the start of imbibition. This rapid water absorption occurred due to the difference in the water potential between the interior of the seed and the external medium (Bewley; Bradford; Hilhorst, 2013). The imbibition phase is essential for the rupture of seed coat, hydration of the embryo and beginning of degradation of reserve substances (carbohydrates, proteins and lipids) into substances of lower molecular mass, which are used to nourish the embryo axis until the seedling develops the root system (Carvalho; Nakagawa, 2012; Marcos-Filho, 2015).

Phase II lasted up to 18 hours and showed characteristics of mass increase stabilization compared to phase I. The mass stabilization phase is characterized by a small increase in mass due to swelling of the embryo and hydration of the seed coat. This phase is essential for the hydration of the embryo and for the beginning of the degradation of reserve substances into substances of lower molecular mass, which are used to nourish the embryo axis until the seedling develops the root system.

### Table 1 - Minimum, maximum and mean values for length, width and thickness of *Acmella oleracea* L. R. K. Jansen seeds

<table>
<thead>
<tr>
<th>Variables</th>
<th>Minimum (mm)</th>
<th>Maximum (mm)</th>
<th>Mean ± deviation</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>1.86</td>
<td>2.94</td>
<td>2.33±0.2</td>
<td>8.91</td>
</tr>
<tr>
<td>Width</td>
<td>0.58</td>
<td>1.40</td>
<td>0.96±0.13</td>
<td>13.73</td>
</tr>
<tr>
<td>Thickness</td>
<td>0.18</td>
<td>0.55</td>
<td>0.33±0.05</td>
<td>16.59</td>
</tr>
</tbody>
</table>

CV = coefficient of variation

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**Figure 2** - Frequency distribution of length (A), width (B) and thickness (C) of *Acmella oleracea* L. R. K. Jansen seeds
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Figure 3 - Water absorption curve of *Acmella oleracea* L. R. K. Jansen seeds. RP = root protrusion

\[ y = -3.269 + 20.013x - 2.0827x^2 + 0.0685x^3 \]

\[ R^2 = 0.9427 \]

Phase I

Phase II

Phase III

Figure 4 - Germinative development of *Acmella oleracea* L. R. K. Jansen seeds along the first six days after sowing. rd = radicle; pr = primary root; hp = hypocotyl; h = hairs; sc = seed coat; ct = cotyledon; Magnification is 2×

Rupture of seed coat and radicle protrusion, with 5% germination, were observed on the first day after sowing (Figure 4).

Presence of hypocotyl and primary root was observed from the second day (Figure 4) and hairs appeared on the third day. On the fourth day, the cotyledons emerged from the seed coat, expanding in the opposite direction, and normal seedlings were produced (59%). On the fifth day, the hypocotyl showed a slight curvature with tapered root up to the cap. On the sixth day, the seedling was 1.92 cm long.

For the variables germination speed index, seedling length and seedling dry mass, there was interaction between the factors (light regimes and temperature), while for germination there was single effect of the factors (Table 2).

The temperatures and light regimes, separately, influenced the germination of *Acmella oleracea* seeds. Higher germination percentages (95%) were observed under the constant light condition (Figure 5A) and temperature of 25 and 30 °C (Figure 5B), but germination occurred under all light and temperature conditions.

The occurrence of germination of *A. oleracea* seeds under different light conditions may be related to the presence of an active form of phytochrome, which is responsible for inducing the germination process in the seed (MARCOS-FILHO, 2015). This characteristic suggests that the species is able to adapt to the fluctuations in the light regimes naturally found at the site of cultivation.
Researchers have already evidenced this capacity of seeds to germinate in the presence and absence of light in species such as *Piptadenia stipulacea* (NOGUEIRA et al., 2014), *Salvia hispanica* (PAIVA et al., 2016) and *Linum usitatissimum* (STEFANELLO; VIANA NEVES, 2017).

In relation to the effect of temperature, higher germination values at 25 and 30 °C can be explained possibly by a higher metabolic activity in the seeds under these conditions, which prove to be optimal for the germination of this species. On the other hand, at temperatures above 30 °C, the respiratory activity probably increased in the seeds, which caused reduction of enzymatic activity and germination due to thermal stress (PAIVA et al., 2016). However, it is worth mentioning the capacity of *A. oleracea* to germinate under a wide range of temperatures, between 20 and 35 °C. This may represent a potential of adaptation of this species to thermal variations and result in greater success in the establishment of seedlings in areas with adverse environmental conditions.

For germination speed index (GSI), the highest values were found under constant light, at constant temperatures of 20 and 35 °C and alternating temperatures of 20-30 and 25-35 °C (Table 3). On the other hand, seeds subjected to alternating light/dark conditions, in general, showed the worst indices.

The requirement of temperature in the germination speed of the seeds seems to be more important under constant conditions of light or dark. Seeds that germinate fast guarantee seedling establishment more rapidly and make better use of the growth conditions. Fast germination under constant light conditions at similar temperatures has also been reported in *Myracrodruon urundeuva* (SILVA; RODRIGUES AGUIAR, 2002) and *Caesalpinia echinata* (MELLO; BARBEDO, 2007). In general, at higher temperatures, the speed of water absorption and chemical reactions is higher and seeds germinate faster (CARVALHO; NAKAGAWA, 2012). According to these authors, the optimum temperature for germination speed is different from the optimum temperature for total seed germination.

In relation to seedling length, the values varied according to the temperatures and light regimes (Table 4). The conditions of constant light with constant temperatures (20 and 25 °C) and alternating temperature
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### Table 3 - Germination speed index of *Acmella oleracea* L. R. K. Jansen seeds under different light regimes and temperatures

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Light regimes</th>
<th>Light/dark alternation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Constant light</td>
<td>Constant dark</td>
</tr>
<tr>
<td>20</td>
<td>38.50 ± 2.39 aA</td>
<td>11.50 ± 3.06 bC</td>
</tr>
<tr>
<td>25</td>
<td>31.75 ± 8.33 aB</td>
<td>21.75 ± 5.93 bB</td>
</tr>
<tr>
<td>30</td>
<td>20.00 ± 7.23 aC</td>
<td>22.50 ± 6.62 aB</td>
</tr>
<tr>
<td>35</td>
<td>43.50 ± 4.25 aA</td>
<td>7.00 ± 2.40 bC</td>
</tr>
<tr>
<td>20-30</td>
<td>42.50 ± 4.21 aA</td>
<td>32.50 ± 3.75 bA</td>
</tr>
<tr>
<td>25-35</td>
<td>40.00 ± 6.97 aA</td>
<td>25.75 ± 5.93 bB</td>
</tr>
</tbody>
</table>

CV (%) 20.53

* ± standard deviation of the mean (n=4); means followed by the same lowercase letter in the row and uppercase letter in the column do not differ by Scott-Knott test at 5% probability level. CV = Coefficient of variation

### Table 4 - Length (cm seedling⁻¹) of *Acmella oleracea* L. R. K. Jansen seedlings under different light regimes and temperatures

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Light regimes</th>
<th>Light/dark alternation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Constant light</td>
<td>Constant dark</td>
</tr>
<tr>
<td>20</td>
<td>2.12 ± 0.16 aA</td>
<td>1.67 ± 0.21 bC</td>
</tr>
<tr>
<td>25</td>
<td>2.45 ± 0.51 aA</td>
<td>2.20 ± 0.27 aA</td>
</tr>
<tr>
<td>30</td>
<td>1.20 ± 0.14 bC</td>
<td>1.82 ± 0.09 aB</td>
</tr>
<tr>
<td>35</td>
<td>0.57 ± 0.07 aD</td>
<td>0.75 ± 0.07 aD</td>
</tr>
<tr>
<td>20-30</td>
<td>2.17 ± 0.21 aA</td>
<td>1.90 ± 0.24 bB</td>
</tr>
<tr>
<td>25-35</td>
<td>1.57 ± 0.10 aB</td>
<td>1.45 ± 0.22 aC</td>
</tr>
</tbody>
</table>

CV (%) 13.18

* ± standard deviation of the mean (n=4); means followed by the same lowercase letter in the row and uppercase letter in the column do not differ by Scott-Knott test at 5% probability level. CV = Coefficient of variation

(20-30 ºC) led to the highest values of length, as well as the conditions of constant dark and alternating light/dark at constant temperatures (25 ºC) and alternating temperatures (20-30 ºC), respectively; while the lowest values of length were recorded at 35 ºC, regardless of light regimes.

Different results were found in *Salvia hispanica* seeds by Paiva *et al.* (2016), who observed that seeds germinated under constant light had lower values of length, regardless of temperature. Greater growth of seedlings also in the absence of light is justified by the etiolation of the seedlings. Under dark conditions, the phytochrome present in the seedling triggers responses that result in the increase of the synthesis of gibberellin, a phytohormone known to promote excessive growth of the hypocotyl towards light (GOMMERS; MONTE, 2017).

Elevated temperatures can hamper seedling growth as it alters the speed of water absorption and modifies the speed of chemical reactions. It affects the synthesis and transport of essential elements for the initial seedling growth. In addition, high temperatures can modify the stability of cell membranes and affect different metabolic processes, including photosynthesis, cell respiration and germination (TAIZ *et al.*, 2017).

For seedling dry mass, the behavior was similar to that of seedling length (Table 5). Constant temperatures of 20 and 25 ºC and alternating temperatures of 20-30 ºC, under constant light conditions, caused the highest mean dry mass accumulations, along with the conditions of constant dark and alternating light/dark at 20 ºC, constant dark at 25 ºC, and alternating light/dark at alternating temperatures of 20-30 ºC. On the other hand, the temperature of 35 ºC was the most harmful to biomass accumulation under all light regimes evaluated.

The best results of dry mass under alternating temperature for both constant light and light/dark alternation can be explained by the fact that these conditions simulate thermal and light fluctuations naturally found under environmental conditions of cultivation of the species (OLIVEIRA; INNECCO,
It is also believed that a lower respiratory rate occurred within this temperature range, which allowed greater investment in biomass in the seedlings. Similar results were found by Oliveira and Innecco (2012), who obtained greater biomass of jambu seedlings under the conditions of constant light at 20 and 25 °C and light/dark alternation at 20 °C, whereas the constant dark treatment resulted in lower means for all temperatures.

The results suggest that *Acmella oleracea* seeds are able to germinate under elevated temperatures, particularly at 35 °C, but it should be pointed out that seedling growth is affected. Due to their indifference to light, as they germinate under both light and dark (neutral photoblastic), *A. oleracea* seeds are expected to germinate on the surface or buried in the soil, but further studies are needed to confirm the effect of depth on the germination of this species.

**CONCLUSIONS**

1. *A. oleracea* seeds have uniform physical characteristics and their water absorption pattern follows the three-phase model;

2. The germination test can be conducted at constant temperatures of 25 and 30 °C and alternating temperature of 20-30 °C, being indifferent to the light regime. Evaluations of germination test can be performed on the fourth day and sixth day for the first and last counts, respectively.

**ACKNOWLEDGMENTS**

To the Federal Rural University of the Semi-arid Region (UFERSA), to the Coordination for the Improvement of Higher Education Personnel (CAPES) for granting the scholarship to the first author, and to Professor Sérgio Antônio Lopes de Gusmão (UFRA) for providing the seeds of *Acmella oleracea*.

**REFERENCES**


DIAS, E. F. *et al.* Interactions between temperature, light and chemical promoters trigger seed germination of the rare Azorean

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**Table 5** - Dry mass (mg seedling⁻¹) of *Acmella oleracea* L. R. K. Jansen seedlings under different light regimes and temperatures

<table>
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<tbody>
<tr>
<td></td>
<td>Constant light</td>
<td>Constant dark</td>
<td>Light/dark alternation</td>
</tr>
<tr>
<td>20</td>
<td>6.00 ± 0.00 aA</td>
<td>4.75 ± 0.50 aB</td>
<td>5.75 ± 0.50 aA</td>
</tr>
<tr>
<td>25</td>
<td>5.25 ± 1.50 aA</td>
<td>5.75 ± 0.50 aA</td>
<td>3.00 ± 1.83 bB</td>
</tr>
<tr>
<td>30</td>
<td>4.50 ± 1.00 aB</td>
<td>4.25 ± 0.50 aB</td>
<td>3.50 ± 1.00 aB</td>
</tr>
<tr>
<td>35</td>
<td>2.00 ± 0.82 aC</td>
<td>1.25 ± 0.50 aD</td>
<td>2.25 ± 0.50 aB</td>
</tr>
<tr>
<td>20-30</td>
<td>5.75 ± 0.50 aA</td>
<td>3.25 ± 0.96 bC</td>
<td>6.00 ± 0.00 aA</td>
</tr>
<tr>
<td>25-35</td>
<td>4.50 ± 0.58 aB</td>
<td>2.25 ± 0.50 bC</td>
<td>3.25 ± 0.50 bB</td>
</tr>
</tbody>
</table>

CV (%) 18.85

± standard deviation of the mean (n=4); means followed by the same lowercase letter in the row and uppercase letter in the column do not differ by Scott-Knott test at 5% probability level. CV = Coefficient of variation.
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