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Original Article

# Seed selection of *Dimorphandra gardneriana* using the tetrazolium test[1]

## Seleção de sementes de *Dimorphandra gardneriana* pelo teste de tetrazólio

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*HIGHLIGHTS:*

*The exposure time of 120 min. to the solution allowed the best action of tetrazolium.*
*The exposure time of 30 min. to the solution generated the least promising results.*
*The grouping of matrices was similar for the tetrazolium, germination, and emergence tests.*

**ABSTRACT:** 'Faveira' (*Dimorphandra gardneriana* Tul.) is a medicinal plant of great commercial value, primarily due to its ability to produce rutin on a global scale. Furthermore, it serves as a source of raw materials for the extraction of other secondary metabolites. With this, this study aimed to standardize the methodology of the tetrazolium test and evaluate its applicability in estimating the viability of seeds from different parent plants of faveira. The viability and vigor of the seeds were determined using tetrazolium (2, 3, 5-triphenyl-tetrazolium chloride) in four concentrations (0.025, 0.050, 0.075, and 0.1%) and four immersion periods: 30, 60, 90, and 120 min, with the viability percentage and vigor levels being considered according to the topological staining pattern. Germination and seedling emergence tests were also conducted. The most suitable preparation for *D. gardneriana* seeds is preconditioning for 78 hours at 25 °C, followed by cutting in the region opposite the embryo. The tetrazolium test efficiently assesses the viability and vigor of *D. gardneriana* seeds, whose ideal seed color is obtained using a 0.075% tetrazolium solution for 120 min at 40 °C. In 20 parent plants, the seeds from the parent plants 2, 3, 6, 8, 9, 12, and 13 stood out as the most vigorous.

**Key words:** Faveira, viability, germination, physiological quality

**RESUMO:** Faveira (*Dimorphandra gardneriana* Tul.) é uma planta medicinal de grande valor comercial, principalmente devido à sua capacidade de produção de rutina em escala mundial. Além disso, ela serve como fonte de matéria-prima para a extração de outros metabólitos secundários. Com isso, estudo teve como objetivo padronizar a metodologia do teste de tetrazólio e avaliar sua aplicabilidade na estimativa da viabilidade de sementes provenientes de diferentes plantas matrizes de faveira. A viabilidade e o vigor das sementes foram determinados pelo teste de tetrazólio (cloreto de 2, 3, 5-trifenil tetrazólio) em quatro concentrações (0,025; 0,050; 0,075 e 0,1%) e quatro períodos de imersão: 30, 60, 90 e 120 min, sendo a porcentagem de viabilidade e os níveis de vigor considerados de acordo com o padrão topológico de coloração. Os testes de germinação e emergência de plântulas também foram realizados. O preparo mais adequado das sementes de *D. gardneriana* é o pré-condicionamento por 78 horas, a 25 °C, seguido de corte na região oposta ao embrião. O teste de tetrazólio é eficiente na avaliação da viabilidade e do vigor de sementes de *D. gardneriana*, cuja coloração ideal das sementes é obtida utilizando-se a solução de tetrazólio a 0,075%, por 120 min, a 40 °C. Em 20 plantas matrizes, as sementes das plantas matrizes 2, 3, 6, 8, 9, 12 e 13 se destacam como as mais vigorosas.

**Palavras-chave:** Faveira, viabilidade de sementes, germinação, qualidade fisiológica

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## Introduction

'Faveira' (*Dimorphandra gardneriana* Tul.) produces fruits with high concentrations of bioflavonoids, substances with various pharmacological properties, being exploited by extractivist communities in the mosaic of protected areas in Chapada do Araripe, Ceará (Alcântara et al., 2020), so there is a lot of research as it is an important source of compounds used in pharmacology (Nunes et al., 2018; Rechenchoski et al., 2019; Fideles et al., 2020), and its application in veterinary medicine (Pinto et al., 2019).

*D. gardneriana*, previously referred to as *Dimorphandra biretusa* Tul., is a symbolic tree of the Brazilian Cerrado, widely exploited for its fruits, which are rich in rutin (flavonoid); this substance has pharmacological properties of great commercial interest, notably as a vasodilator and antioxidant (Leite et al., 2020). Among the pharmacological properties, the following stand out: anti-inflammatory and anti-carcinogenic activities, action on the immune system, anti-viral activity, reduction of the effect of cataract formation in people with diabetes, hepatoprotective and gastroprotective; in short, there are numerous applications in medicine, mainly in the treatment of circulatory and capillary problems (Silva, 2007). The presence of *D. gardneriana* Tul. has been confirmed in several regions of Brazil, covering the North, Northeast, Central-West, and Southeast, with possible occurrences in the states of Paraíba and Rio Grande do Norte. Its distribution covers different phytogeographic domains, including the Amazon, Caatinga, Cerrado, Atlantic Forest, and Pantanal, and it is found in a variety of vegetation types, such as Caatinga (*stricto sensu*), Campinarana, Campo Rupestre, Cerrado (*lato sensu*), Ciliary or Gallery Forest, Igapó Forest, Terra Firme Forest, Semideciduous Seasonal Forest, Ombrophilous Forest (Rain Forest), and Amazonian Savannah (Souza et al., 2024).

Seeds in the forests are responsible for the maintenance and perpetuation of plant communities through regeneration and are a basic input in recovery programs and ecosystem conservation; therefore, physical health and genetic seed quality have great value in the production of healthy and viable seedlings, aiming at adaptability and persistence of populations in the long term (Bhering et al., 2005). The production of high-quality seeds of forest species has become increasingly crucial, as it ensures the proper development of seedlings, which are essential for reforestation programs, the recovery of degraded areas, urban afforestation, and the preservation of native forest species threatened with extinction (Vieira et al., 2001). Therefore, it is essential to conduct tests to assess the physiological quality of seeds, allowing them to be classified into lots with different levels of vigor.

The tetrazolium test stands out as an essential tool, frequently used to assess the seed quality of forest species, as established by the Rules for Seed Analysis (BRASIL, 2009). Its main purpose is to distinguish between viable and non-viable seeds. This test is based on the fundamental principle of the activity of dehydrogenase enzymes, which participate in the respiratory process of seeds. Thus, viable and dead seed tissues are distinguished by the presence or absence of red coloration, respectively, which is induced by the action of the 2,3,5 triphenyl tetrazolium chloride salt (França Neto, 1999).

Based on the above, this study aimed to standardize the tetrazolium test methodology and assess its applicability in estimating the viability of seeds from different *Dimorphandra gardneriana* Tul. plants.

## Material and Methods

The experiments were conducted in the Seed Analysis and Plant Ecology Laboratories of the Agricultural Sciences Center of the Universidade Federal da Paraíba, in Areia - PB, with *D. gardneriana* seeds obtained from 20 parent plants located in the Chapada do Araripe, situated at an altitude of 608 m. Chapada do Araripe has the following geographic coordinates: Latitude: 7° 12' 6" S, Longitude: 40° 1' 55" W (https://www.cidade-brasil.com.br/municipio-araripe.html), in the municipalities of Crato and Jardim - Ceará, Brazil. After harvesting, the fruit with coloration ranging from dark brown to almost black, opaque, was opened manually to extract the seeds and eliminate those poorly formed, considering the size of the seeds. After, they were subjected to the following determinations and tests, with seeds from 20 parent plants, using four replications of 25 seeds evaluating the percentage of seeds not colored, seeds weakly colored, seeds normally colored (SNC), and seeds strongly colored (Delouche et al., 1976; Bhering et al., 1996; França Neto, 1999). The experimental design was entirely randomized in a factorial scheme composed of four concentrations and four exposure times to tetrazolium solutions.

Germination test: it was conducted in a B.O.D. (*Biological Oxygen Demand*) incubator set at a constant temperature of 25 °C, with a photoperiod of eight hours of light and 16 hours of dark, using daylight-type fluorescent lamps (4 × 20 W), with 100 seeds mechanically scarified with 120 grit sandpaper on the side opposite the hilum to overcome tegument dormancy. The seeds were then divided into four replicates of 25 seeds, distributed over two sheets of germination paper (Germitest), covered with a third sheet, and arranged in a roll, where the paper was moistened with distilled water equivalent to 2.5 times its dry mass. The rolls were placed in plastic bags to avoid water loss through evaporation. Counts were taken daily from the 13$^{th}$ to the 25$^{th}$ day after the test was set up, using the criterion of normal seedlings, and the results were expressed as a percentage. The germination test was conducted following the methodology of Ursulino et al. (2019).

Emergence test in the greenhouse: it was conducted with 100 scarified seeds from each parent plant, divided into four replicates of 25 seeds. The seeds were sown in plastic trays measuring 49 × 33 × 7 cm, containing washed and autoclave sterilized sand to a depth of 2.0 cm. The substrate humidity was maintained by daily irrigation using a manual watering can, and the counts were conducted daily from the 13$^{th}$ to the 25$^{th}$ day after the test was set up when the number of emerged seedlings had stabilized. The criterion used was normal seedlings; the results obtained were expressed as a percentage. The emergence test was conducted following the methodology of Ursulino et al. (2019).

Tetrazolium test: before the reaction period in the tetrazolium solution, the seeds were manually scarified (with sandpaper nº 80 on the area opposite the hilum) and then soaked for 72 hours in distilled water at 25 °C, after which the tegument was manually removed, except for the control treatment. All the seeds were submerged in tetrazolium solution at four concentrations: 0.025, 0.050, 0.075, and 0.1% for four periods: 30, 60, 90, and 120 min. In each repetition, 25 mL of tetrazolium solution was added for the seeds to soak in the dark in a becker wrapped in aluminum foil, previously identified. The samples were kept in B.O.D. incubators at 40 °C in the dark during the staining period. Four replicates of 50 seeds were used for each combination of tetrazolium solution concentration and staining period. After these periods, the seeds were evaluated individually by sectioning them longitudinally through the center of the embryonic axis using a scalpel. The color differentiation of the tissues was observed according to the criteria established for the tetrazolium test (Delouche et al., 1976; França Neto, 1999): bright red or pink (living and vigorous tissue), strong carmine red (deteriorating tissue), and milky white or yellowish (dead tissue). After detecting the best time and concentration of the tetrazolium solution, the viability of 30 seeds from each *D. gardneriana* parent plant was assessed. A representation of viable and non-viable seeds was drawn up, observing the presence and location of damage, and the physical condition of the embryonic structures was elaborated to characterize the levels of vigor.

The results obtained in the germination, emergence, and tetrazolium tests for each parent plant were transformed into arcsine since they did not present normal distribution according to the Lilliefors test and subsequently submitted to the analysis of variance using the Sisvar 5.6 software (Ferreira, 2003). The means were grouped using the Scott- Knott test at a 0.05 probability level. Due to the significant dispersion in the data, which hindered the visualization and explanation of the results, a regression test was not conducted for the qualitative factor. Instead, a mean comparison test was chosen.

## Results and Discussion

The color of the seeds from the control treatment was not satisfactory for evaluation by the tetrazolium test, regardless of the solution concentrations and exposure time. This lack of color can be attributed to two aspects of *D. gardneriana* seeds, the first being the presence of a rigid tegument and the second being the presence of a gelatinous substance protecting the embryo, which prevents or hinders the uniform imbibition of the tetrazolium solution, making it necessary to remove the tegument to conduct this type of test.

When the *D. gardneriana* seeds were scarified and subjected to tetrazolium solution for 30 min (Table 1), the coloration was uneven in all concentrations, with only 38% of the seeds showing adequate coloration in the 0.1% concentration. During this same period, many seeds showed no coloration in the central region of the reserve tissue or the embryonic axis because 30 minutes was not enough for the dehydrogenase enzymes present in the living tissue of the embryo to reduce the 2,3,5 triphenyl tetrazolium chloride salt into triphenylformazan. In the reduced form, the 2,3,5-triphenyl tetrazolium chloride is a red-colored, stable, nondiffusible substance called triphenylformazan or formazan.

**Table 1.** Percentage of seeds not colored (NC); seeds weakly colored (SWC); seeds normally colored (SNC), and seeds strongly colored (SSC) by the tetrazolium according to the concentration (%) and exposure time (min) to tetrazolium solutions*

| Evaluation criteria | Time (min) | Concentrations (%) | | | |
|---|---|---|---|---|---|
| | | 0.025 | 0.050 | 0.075 | 0.1 |
| NC | 30 | 32 aB | 32 aB | 14 bB | 0 cC |
| SWC | | 68 aA | 68 aA | 86 aA | 62 aA |
| SNC | | 0 bC | 0 bC | 0 bB | 38 aA |
| SSC | | 0 aC | 0 aC | 0 aB | 0 aC |
| NC | 60 | 12 aB | 0 bB | 8 aB | 0 bB |
| SWC | | 88 aA | 54 bA | 38 bA | 8 cB |
| SNC | | 0 cB | 46 bA | 54 bA | 80 aA |
| SSC | | 0 aB | 0 aB | 0 aB | 12 aB |
| NC | 90 | 50 aA | 2 bB | 0 bB | 0 bB |
| SWC | | 40 aA | 66 aA | 56 aA | 12 bB |
| SNC | | 10 cB | 32 bA | 44 bA | 76 aA |
| SSC | | 0 aB | 0 aB | 6 aB | 12 aB |
| NC | 120 | 8 aB | 0 bB | 0 bB | 0 bB |
| SWC | | 12 aB | 10 aB | 4 aB | 2 aB |
| SNC | | 78 aA | 90 aA | 94 aA | 80 aA |
| SSC | | 2 bB | 0 bB | 2 bB | 20 aB |

*Means followed by the same lowercase letter in the row and uppercase letter in the columns belong to the same group by the Scott Knott test at 0.05 probability level.

Most of the seeds that spent 60 min in the 0.025% tetrazolium solution were weakly colored, while the 0.1% concentration showed the highest percentage of seeds that were appropriately colored (Table 1), a total of 88%, which is important because it allows observation of the inner part of the seed, which according to Fogaça et al. (2006), is the main structure to be analyzed when determining the viability and vigor of seeds.

Table 1 also shows the 90 min exposure time, under the concentration of 0.025% did not cause the seeds to color; however, in the concentrations of 0.050 and 0.075%, most of the seeds colored weakly, while the concentration of 0.1% was the one in which the greatest number of seeds with ideal coloring for assessing vigor were observed. The tetrazolium solution in low concentrations does not allow adequate coloring of the seeds, with less precision in the visualization of recent mechanical damage caused by abrasion, which would not normally be detected using more concentrated solutions.

The 120 min exposure time to the solution (Table 1) was the one that allowed the best tetrazolium action on *D. gardneriana* seeds in all concentrations, with the majority of seeds showing an adequate and uniform coloration, which is a fundamental factor that allows for safety and efficiency in the use of this test, which is in agreement with Bhering et al. (2005). However, it is important to note that the 0.1% concentration caused excessive coloration in 20% of the seeds, and it is not recommended to use this combination when conducting the tetrazolium test. The gradual increase in the concentration of the tetrazolium solution resulted in darker colors in the seeds, and the darker the color of the seed, the greater the difficulty in visualizing the tissues and identifying injuries, which could lead to confusing living tissues with those in deterioration.

For the coloring of *D. gardneriana* seeds, a concentration of 0.075% and an exposure time of 120 min at 40 °C is recommended because the concentration, soaking time, and temperature together provide a uniform coloration. The concentration of the 0.075% tetrazolium solution was also effective for assessing the viability of *Aspidosperma pyrifolium* Mart. (Cunha et al., 2021), *Colubrina glandulosa* Perkins (Moraes et al., 2019) and *Himatanthus sucuuba* Spruce (Ramírez et al., 2021), and the concentration of the 0.01% tetrazolium solution was also effective for assessing the viability of *Handroanthus spongiosus* (Rizzini) S. Grose seeds (Silva et al., 2023a).

However, when assessing the viability of seeds of *Eugenia stipitata* McVaugh ssp. Sororia McVaugh., the combination indicated was the 1.0% concentration, with a staining period of 26 hours (Maia et al., 2023). The 1.0% solution of tetrazolium salt for 4 hours at 30 °C allowed uniform coloration and efficiently assessed the viability of *Joannesia princeps* seeds (Alves et al., 2022).

It can be seen that the preparation of the seeds, the concentration of the tetrazolium solution, and the coloring time are specific to each species, so it is necessary to conduct previous tests to determine the best methodology for the tetrazolium test (Fogaça et al., 2006).

Table 2 and Figure 1 show the classification of viability levels established in the tetrazolium test for *D. gardneriana* seeds considering the characteristics proposed by Delouche (2002) and Fogaça et al. (2006) as criteria for classifying seeds: 1. tissues with a uniform light red or pink color are typical of healthy tissue; 2. tissues with white or yellowish color are dead tissues; 3. tissues with intense red color are tissues in which the patterns and progressive nature of the deterioration are evident.

**Table 2.** Description of the seed embryo coloration and characteristics for each category and class used to assess the viability of *Dimorphandra gardneriana* Tul. seeds

| Categories | Classes | Characterization |
|---|---|---|
| Viable and vigorous | Class I | Seed with uniform light pink color and all tissues looking normal and firm. |
| Viable and not vigorous | Class II | Seed showing less than 50% of the cotyledons with an intense red color, typical of deteriorating tissue. |
| | Class III | The end of the radicle with a milky white color without reaching the central cylinder, as well as the cotyledonary region with a light pink color. |
| | Class IV | Seed showing less than 50% of the cotyledonary region with yellowish-white coloration, characterizing dead tissue. |
| Non-viable | Class V | The embryonic axis and more than 50% of the cotyledonary region show an intense red color typical of deteriorating tissues. |
| | Class VI | The seed completely colored deep red, indicating a marked deterioration process. |
| | Class VII | Seed with the embryonic axis and central cylinder colored intense red. Cotyledon region showing more than 50% milky white color, with scattered intense red spots. |
| | Class VIII | Seed with severe lesions on the embryonic axis. |

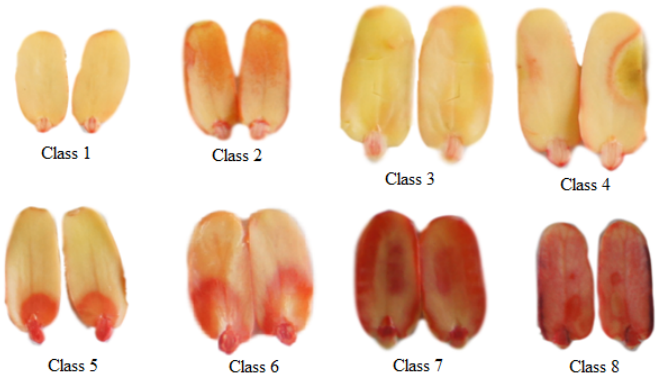


**Figure 1.** Representation of the seed classes established in the tetrazolium test for *Dimorphandra gardneriana* Tul

The results for the moisture content indicated a difference of 2.9% between the seeds from the parent plants, although there was no statistical difference (Table 3). According to Marcos Filho (2015), variations in the water content of seeds from different lots can interfere with the results of vigor tests since wetter seeds are more sensitive and subject to more intense deterioration.

The initial quality of the seeds from different parent plants was assessed to identify those with the highest and lowest physiological potential since to make the tetrazolium test truly reliable, it is necessary to compare its physiological quality tests that show the presence of seeds with different vigor levels. Concerning germination percentage, the seeds from the parent plants were distributed into three quality levels. Parent plant 10 had the seeds with the lowest quality; parent plants 1, 4, 5, and 17 had intermediate-quality seeds, and the

**Table 3.** Seed moisture content, seed germination, seedling emergence, and viability by the tetrazolium (Tz) test in seeds from different parent plants of *Dimorphandra gardneriana* Tul.*

| Parent plant | Moisture content | Germination | Emergence | Viability (Tz) |
|---|---|---|---|---|
| | (%) | | | |
| 1 | 5.62 a | 91 b | 80 a | 93 a |
| 2 | 5.52 a | 100 a | 88 a | 86 a |
| 3 | 6.12 a | 100 a | 85 a | 96 a |
| 4 | 6.65 a | 90 b | 85 a | 76 b |
| 5 | 5.12 a | 91 b | 85 a | 80 b |
| 6 | 6.92 a | 100 a | 94 a | 90 a |
| 7 | 6.11 a | 100 a | 89 a | 80 b |
| 8 | 6.32 a | 100 a | 88 a | 90 a |
| 9 | 8.02 a | 100 a | 90 a | 96 a |
| 10 | 7.12 a | 78 c | 93 a | 76 b |
| 11 | 7.23 a | 94 b | 90 a | 93 a |
| 12 | 5.92 a | 100 a | 88 a | 100 a |
| 13 | 5.52 a | 100 a | 86 a | 93 a |
| 14 | 7.02 a | 100 a | 68 b | 86 a |
| 15 | 6.01 a | 100 a | 70 b | 93 a |
| 16 | 7.14 a | 100 a | 70 b | 86 a |
| 17 | 5.70 a | 94 b | 72 b | 93 a |
| 18 | 5.59 a | 100 a | 62 b | 73 b |
| 19 | 6.17 a | 100 a | 70 b | 93 a |
| 20 | 5.82 a | 100 a | 64 b | 80 b |

*Means followed by the same lowercase letter in the column belong to the same group by the Scott Knott test at a 0.05 probability level

other parent plants produced high-quality seeds (Table 3). The germination test also made it possible to distinguish vigor between seed lots of *Panicum maximum* Jacq. (Machado et al., 2019), *Aspidosperma discolor* A. DC (Lima et al., 2024) and made it not possible to distinguish vigor between seed lots of *Myracrodruon urundeuva* Allemão (Silva et al., 2024).

Seedling emergence is another test used to help separate seed lots regarding vigor. The performance of *D. gardneriana* seeds from parent plants 14, 15, 16, 17, 18, 19, and 20 was inferior, and they were therefore considered to be of low vigor when compared to the other parent plants, whose seeds had a high standard of physiological quality.

Concerning the number of viable seeds using the tetrazolium test, the parent plants were separated into two groups, the first consisting of the most vigorous seeds from the parent plants 1, 2, 3, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, and 19 (Table 3). The second comprises seeds of lower physiological quality from the parent plants 4, 5, 7, 18, and 20. This test has been applied to the seeds of many forest species, as it helps quickly and reliably to differentiate seed lots, as was observed in *D. gardneriana*.

Table 3 shows some similarities among the three vigor tests conducted, with the seeds from *D. gardneriana* parent plants 2, 3, 6, 8, and 13 having the same quality estimates. In addition, the similarities between the tetrazolium and germination tests were also analyzed, with parent plants 4, 5, 14, 15, and 16 standing out, while concerning the emergence and tetrazolium tests, only parent plants 1, 11, and 20 showed similarities concerning the vigor of their seeds.

Therefore, these results confirm the possibility of using the tetrazolium test to assess the viability of *D. gardneriana* seeds, providing results correlated with germination as seen in several species, such as *Bertholletia excelsa* Humb. & Bonpl. (Borella et al., 2020), *Glycine max* (L.) Merrill (Tavanti et al., 2020), *Tamarindus indica* L. (Cordeiro et al., 2022), *J. princeps* Vell. (Alves et al., 2022), *Handroanthus spongiosus* (Rizzini) S. Grose. (Silva et al., 2023a), *Pterogyne nitens* Tul. (Silva et al., 2023b), *Switenia macrophylla* King, *Cedrelinga cateniformis* (Ducke) Duckey and *Ochroma pyramidale* (Cav. ex Lam) Urb. (Quintana et al., 2023), and *Bertholletia excelsa* Humb. & Bonpl. (Souza et al., 2023).

## Conclusions

1. The tetrazolium test efficiently assesses the viability and vigor of *Dimorphandra gardneriana* Tul. seeds.

2. The most suitable preparation for *D. gardneriana* seeds is preconditioning for 78 hours at 25 °C, followed by mechanical scarification in the opposite region to the embryo.

3. The ideal coloration of *D. gardneriana* seeds is obtained using a 0.075% tetrazolium solution for 120 minutes at 40 °C.

4. The seeds from the parent plants 2, 3, 6, 8, 9, 12, and 13 are the most vigorous.

**Contribution of authors:** Marina M. Ursulino: collected the seeds, conducted the experiment, and wrote the article; Edna U. Alves: acted as research advisor, participated in conceptualizing the problem, improving and correcting the article, and data analysis and interpretation.; Paulo A. F. R. de Melo, Sueli da S. Santos-Moura, and Rosemere dos S. Silva: helped with the analysis of the experiment and article writing; Flávio R. da S. Cruz: helped with the processing of seeds and analysis of the experiment; Jean P. C. Ramos: participated in data analysis and article writing.

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