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Physiological quality of forage palm seeds and seedlings associated with the level of ploidy

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ABSTRACT. The forage palm is widely cultivated in semiarid regions. It has a basic chromosome number of x = 11 and polyploids are the main variation. It is propagated via vegetative reproduction, and sexual propagation is rarely used. The objective was to associate the physiological quality of seeds of different cultivars of forage palm with the level of ploidy in the genera *Opuntia* and *Nopalea*. The cultivars used were: diploids (2n = 22) 'F8', 'F21', and 'IPA Sertânia'; tetraploids (2n = 44) 'Clone 6 African Elephant Ear', 'Mexican Elephant Ear', and 'V19', and octaploids (2n = 88) 'Round', 'Giant', and 'IPA Clone 20'. Thousand seed weight, length, diameter, number of seeds per fruit, first count and emergence percentage, shoot and root length, fresh and dry mass, electrical conductivity, and length, width, and fresh and dry weight of cladodes were measured. A nested scheme design was used, with three levels of ploidy and three cultivars within each level. The ploidy level positively influenced the number of seeds per fruit and the physiological quality of the seeds. Cultivars behave differently within the same ploidy level.

Keywords: diploids; Opuntia ficus-indica; polyploids; vigor.

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Introduction

The Cactaceae family has a basic chromosome number of x = 11, and the main variation is polyploidy. Within the *Opuntia* genus, the species can be diploids (2n), and the polyploids are triploids (3n), tetraploids (4n), and octaploids (8n). Polyploidy is also common in the genus *Nopalea*, with 2n = 4x = 22 to 2n = 4x = 88 occurring, which shows the polyploid evolution of the genera. Variations in ploidy cytotypes provide evidence of the different breeds of the same species (Majure, Judd, Soltis, & Soltis, 2012).

Among the cacti, the forage palm (*Opuntia ficus-indica*), native to Central America, and currently widespread in Spain, Italy, Greece, Portugal, Turkey, and the Mediterranean islands (Celesti-Grapow et al., 2016; Puddu et al., 2016), has drought tolerance, and manages to grow in arid and semi-arid regions. This is because Cactacea has a photosynthetic process of crassulacea acid metabolism (CAM), with stomata that open at night and are kept closed during the day to prevent water loss (Taiz, Zeiger, Moller, & Murphy, 2017).

The cultivation of cacti is usually carried out via vegetative propagation, and sexual propagation is seldom used due to the lack of information in this area. In sexual propagation, seedlings grow slowly, but seeds can be produced in large quantities. In addition, they can provide important genetic variation, maintain the genetic diversity of populations and species, and present several desirable, morphological characteristics, which are greatly appreciated in breeding genetics (Abud, Gonçalves, Reis, & Pereira, 2010). This variability allows researchers to select genotypes with desirable characteristics for breeding purposes such as tolerance to different stress factors, biomass production, superior racket size, fruit quality, and resistance to carmine cochineal, which can be used for breeding purposes (Ochoa, González-Flores, Cruz-Rubio, Portillo, & Gómez-Leyva, 2015).

The ploidy level can have a direct effect on seed characteristics. Cota-Sánchez and Bomfim-Patrício (2010) observed an increase in seed size related to the level of ploidy in *Rhipsalis*, with the polyploid populations of Africa having maximum expression. Thus, the objective was to associate the physical and physiological quality of seeds of different cultivars of forage palm with the level of ploidy in the genera *Opuntia* and *Nopalea*.

Material and methods

The experiment was carried out at the Seed Analysis Laboratory (LAS) of the Agricultural Sciences Center (CCA) at the Federal University of Paraíba (UFPB), Areia, Paraíba State, Brazil. Fruit was harvested rom forage palm belonging to the Agronomic Institute of Pernambuco (IPA) in the city of Arcoverde, Pernambuco State, Brazil (8°25' S; 37°05' W), a microregion of the Moxotó hinterland at an altitude of 680.70 m, with average annual temperature 22.90 ± 1.68°C, and average annual accumulated precipitation of 798.1 mm. The cultivars used for fruit collection and seed analysis were subjected to chromosome number counts at the Plant Cytogenetics Laboratory at the same institution (Table 1).

Table 1. Common name, species, and chromosome number of forage palm cultivars used in the trial.

Common name	Species	2 <i>n</i>	
1 – 'F8'	Nopalea cochenillifera	22	
2 – 'F21'	Nopalea cochenillifera	22	
3 – 'IPA – Sertânia'	Opuntia atropes Rose	22	
1 – 'Clone 6 Orelha de Elefante Africana'	<i>Opuntia</i> sp	44	
2 – 'Orelha de Elefante Mexicana'	Opuntialarreyi F.A.C.	44	
- 'V19'	<i>Opuntia stricta</i> (Haw)	44	
1 – 'Redonda'	Opuntia ficus-indica	88	
2 – 'Gigante'	Opuntia ficus-indica	88	
3 – 'IPA Clone 20'	Opuntia ficus-indica	88	

For every four plants cultivated, four fruits per plant were harvested, and after collection, they were placed in a thermal box and sent to LAS for processing. After being rinsed in running water with the aid of a sieve, the seeds were dried at room temperature and stored in a glass jar for two months, until physiological quality was assessed. The water content of the seeds was calculated using four sub-samples of 10 g of seeds per cultivar, using the greenhouse method at 105 ± 3 °C for 24h, and the result was expressed in g of water per kg of seeds. The weight of a thousand seeds was calculated by weighing eight repetitions of 100 seeds of each cultivar with the results expressed in grams (Brasil, 2009).

For the emergence test, 100 seeds were divided into four repetitions of 25 seeds per cultivar and were sown in plastic trays drilled at the bottom ($0.40 \times 0.25 \times 0.70$ m), containing previously washed and autoclaved sand, moistened at 60% of field capacity. The percentage of seedlings that emerged was recorded at 25 and 47 days. The first emergence count was performed in conjunction with the emergence test, where the percentage of normal seedlings was counted 25 days after the test was initiated (Guedes, Alves, Gonçalves, Viana, & Moura, 2009).

At 90 days after sowing, the length of the aerial part and the root of the seedlings was recorded with the aid of a ruler graduated in centimeters. Using a carbon fiber digital caliper, the length and the width of the cladodes were measured. The seedlings were weighed to obtain the fresh mass, packed in Kraft paper bags and dried in a forced air circulation oven at 65°C until a constant weight was observed. Subsequently, the seedlings were weighed in an analytical balance to a precision of 0.0001 g and expressed as g plant⁻¹.

The electrical conductivity was measured using four replicates of 25 seeds per cultivar that were previously weighed and placed in disposable 200 mL cups containing 75 mL of distilled water. The cups were kept at 25°C for 24h before conductivity was recorded with a conductivity meter (Vieira & Krzyzanowski, 1999). The results were expressed in μ S cm⁻¹ g⁻¹, by dividing the reading by seed weight.

The experimental design used was nested with two factors (level of ploidy and cultivars within ploidy). The data were subjected to analysis of variance and the means were compared using the Tukey test at 5% probability, using SAS[®] statistical program (Cody, 2015).

Results and discussion

The water content of forage palm seeds ranged from 45 to 69 g of water per kilogram of seeds in the tetraploid cultivars 'V19' and 'Mexican Elephant Ear, respectively, i.e., there was a variation within the same level of ploidy (Table 2). These values differ from those found by Souza, Bruno, Andrade, Dornelas, and Brito Primo (2009), in which *Opuntia ficus-indica* seeds were subjected to different fermentation periods and the humidity was practically constant throughout the period (80 g of water per kilogram of seeds). The water content of the seeds can vary depending on the drying conditions, temperature, and air humidity, *inter alia* (Marcos Filho, 2015).

Cultivars	2 <i>n</i>	Degree of humidity (g water kg ⁻¹ of seeds)
'IPA – Sertânia'	22	58
'F21'	22	65
'F8'	22	67
'Clone 6 Orelha de Elefante Africana'	44	49
'V19'	44	45
'Orelha de Elefante Mexicana'	44	69
'Redonda'	88	49
'Gigante'	88	51
'IPA Clone 20'	88	64

Table 2. Water content in seeds of different cultivars of forage palm (Opuntia and Nopalea).

The variables 'weight of 1,000 seeds', percentage of emergence, first emergence count, seedling fresh and dry mass, length, and fresh and dry mass of cladodes were different in terms of isolated factors and ploidy level (p < 0.05).

Seedling emergence also differed statistically between ploidy levels, varying from 23 to 44%, in diploid and octaploid cultivars, respectively (Figure 1A). When the emergence is compared with other species of cacti, it is considered low, since research involving facheiro seeds (*Pilosocereus catingicola*) with fruits harvested in different municipalities in Paraíba, resulted in an emergence percentage ranging from 60 to 80% (Medeiros et al., 2015). However, it is superior to that observed by Guedes et al. (2009) when evaluating the germination and vigor of forage palm seeds after treatment to overcome seed dormancy. They observed that in the control seeds, the percentage emergence was 20%, reaching a maximum of 80% in scarified seeds with mechanical sandpaper.



Figure 1. Percentage of emergence (A), first emergence count (B), weight of a thousand seeds (C), and electrical conductivity (D) of cultivars of forage palm genera, *Opuntia* and *Nopalea*, depending on the level of ploidy (88, octaploid; 44, tetraploid; and 22, diploid). Averages followed by the same letter do not differ statistically. Tukey's test was applied at the 5% probability level. The bars represent the means (n = 4) ± standard error of the mean.

The percentage of normal seedlings in the first count ranged from 10% in diploid cultivars to 20% in octaploids (Figure 1B). The vigor evaluated by the first count test is based on the principle that the cultivars with the highest percentage of normal seedlings in the first count (established by the Rules for Seed Analysis; Brasil, 2009), are the most vigorous, which correlates with emergence speed (Carvalho & Nakagawa, 2012). It is expected that the vigor of polyploid plants will increase with an increase in the level of ploidy. Polyploids have more than two complete genomes, which become an important force in the evolution of plants, as they affect the genetic and genomic constitution and the phenotype of an organism (Weiss-Schneeweiss, Emadzade, Jang, & Schneeweiss, 2013).

The reason for the low percentage emergence in forage palm may be associated with seed dormancy (due to the physical resistance of the seed coat that makes it difficult for water to enter) and may be a consequence

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of the germination and emergence process (Gonzalez-Cortés, Reyes-Valdés, Robledo-Torres, Villarreal-Quintanilla, & Ramírez-Godina, 2018). Palm seeds have a lignified integument, containing ~55% lignin and cellulose, enveloping the embryo at 90-95% of seed weight, which results in a blocking of radicle protrusion (Habibi, Heux, Mahrouz, & Ignon, 2008).

The seeds from octaploid and tetraploid cultivars have a higher weight than those harvested from diploid cultivars (1.38, 1.33, and 1.16 g, respectively, Figure 1C), i.e., the translocation of plant reserves from polyploid cultivars to seeds is superior to that of diploids. Thus, it is expected that cultivars with heaviest seeds have a higher percentage emergence, a fact observed in the present study, which was also found by Almeida, Pinheiro, Lessa, Gomes, and Medeiros Filho (2014) when assessing water stress and seed weight in the germination and seedling growth of *Amburana cearenses*.

There was no difference in electrical conductivity among the ploidy levels (Figure 1D). This vigor test is based on the principle that, when the seeds are soaked in water, it causes deterioration due to the leaching of the cellular constituents, as a result of the loss of the integrity of the cell membrane systems. In this way, low conductivity means high quality of the seed, and high conductivity suggests less vigor in the seeds due to a greater output of leachate from the seed (Vieira & Krzyzanowski, 1999). Even though they do not differ from polyploids, the seeds of diploid cultivars present greater leaching (1,662 μ S cm⁻¹ g⁻¹) and are therefore less vigorous in relation to tetraploids and octaploids (1,127 and 1,061 μ S cm⁻¹ g⁻¹, respectively).

There was no significant difference in the length of the aerial part and the roots among ploidy levels. The aerial part measured an average of 1 cm (Figure 2A), and the root size was 3 cm (Figure 2B). This response may be due to the slow growth that occurs in the forage palm. Seedling length testing is important because seedlings of greater length are more vigorous and can complete development faster (Nakagawa, 1999).



Figure 2. Length of aerial part (A) and root (B), fresh mass (C), and dry seedling weight (D) of cultivars of forage palm genera, *Opuntia* and *Nopalea*, depending on the level of ploidy (88, octaploid; 44, tetraploid; and 22, diploid). Averages followed by the same letter do not differ statistically. Tukey's test was applied at the 5% probability level. The bars represent the means (n = 4) ± standard error of the mean.

The largest fresh mass of seedlings was found in octaploid cultivars (2.214 g), which, together with the seeds of tetraploid cultivars (1.792 g), differed statistically from diploid cultivars (1.052 g) (Figure 2C). The greater accumulation of fresh mass in seedlings of polyploid cultivars may be due to the seeds of these cultivars being more vigorous, thus providing greater transfer of dry mass from their reserve tissues to the embryonic axis in the germination phase (emergence), resulting in seedlings with greater weight due to the greater accumulation of matter (Nakagawa, 1999).

For the seedling dry mass, the highest value (0.213 g) was obtained in octaploid cultivars, followed by tetraploids (0.13 g), which did not differ significantly from that in diploids (0.09 g) (Figure 2D). The value obtained in octaploid cultivars does not differ from that found by Guedes et al. (2008), who evaluated pregerminative treatments in forage palm seeds. They observed a dry matter value of seedlings from seeds without overcoming dormancy, of approximately 0.200 g. The authors did not mention which cultivar of forage palm they used; however, the dry mass indicates that it could be polyploid. The use of polyploid cultivars has become more frequent, because they have different characteristics (Farinatti et al., 2006) such as high total mass production, rapid development and initial production, and a better quality and size of seed, when compared to diploid cultivars (Shao, Chen, & Deng, 2003).

There was a significant difference in seedling cladodes in all variables, except the width of the cladode. The length varied from 6.72 to 9.45 mm in cultivars with 2n = 22 and 2n = 88, respectively (Figure 3A), i.e., octaploid cultivars promote seedlings with larger cladodes in relation to diploids because polyploid individuals tend to show an increase in the size of organs and tissues (Cohen & Tel-Zur, 2012), in addition to an increase in enzyme activity (Stanys, Weckman, Staniene, & Duchovskis, 2006).

As the animals consume the cladodes, this variable is important, as it reflects final crop production. The width of the cladode did not differ between the ploidy levels, ranging from 4.66 mm in the diploid cultivars to 5.18 mm in the octaploids (Figure 3B). The diploid cultivars had a significantly lower fresh weight of cladode (0.802 g) than the octaploids and tetraploids (1.742 and 1.581 g, respectively; Figure 3C). Octaploid cultivars had the highest dry mass of cladode (0.105 g), followed by tetraploids (0.100 g), which did not differ from diploids (0.05 g) (Figure 3D).



Figure 3. Length (A) and width (B), fresh mass (C), and dry cladode weight (D) of cultivars of forage palm genera, *Opuntia* and *Nopalea*, depending on the level of ploidy (88, octaploid; 44, tetraploid; and 22, diploid). Averages followed by the same letter do not differ statistically. Tukey's test was applied at the 5% probability level. The bars represent the means $(n = 4) \pm$ standard error of the mean.

The forage palm has a high amount of water in the cladodes, reaching 90% of the total weight, this was confirmed by the fresh mass of the cladodes, with a variation from 0.8 to 1.7 g (Figure 3C). The cultivars under study registered an average of 90% of the total water weight (unpublished data). However, this water content can change depending on growing treatments, and the cultivation environment (Lopes, Brito, & Batista, 2009).

When cultivars were evaluated within ploidy level, a significant difference was observed for all variables, showing that forage palm cultivars behave differently with the same number of chromosomes. The tetraploid cultivars differed in terms of the percentage of emergence, with the highest value in the 'Mexican Elephant Ear' (55%) (Figure 4A). Although they do not differ from each other, the seeds of octaploid cultivars also indicated a higher percentage emergence, ranging from 32 to 53%. The evaluation of the cultivar within the ploidy level is important for breeders, as it is not enough to state that polyploidy influences emergence; it is necessary to indicate which cultivars should be crossed. However, other factors must be considered when assessing the emergence of cactus seeds, such as shape, size, weight, structure, and number of seeds per fruit. These are important characteristics that, in many cases, are related to the dynamics of germination (Altare, Trione, Guevara, & Cony, 2006).



Figure 4. Percentage of emergence (A), first emergence count (B), thousand seed weight (C), and electrical conductivity (D) of forage palm cultivars of the genera, *Opuntia* and *Nopalea*, as a function of ploidy level. Diploids (2n = 22) (1 - 'F8', 2 - 'F21', and 3 - 'IPA Sertânia'); tetraploids (2n = 44) (1 - 'Clone 6 African Elephant Ear', 2 - 'Mexican Elephant Ear', and 3 - 'V19'), and octaploids (2n = 88) (1 - 'Round', 2 - 'Giant,' and 3 - 'IPA Clone 20'). Averages followed by the same letter, within the same ploidy level, do not differ by the Tukey test at the 5% probability level. The bars represent the means (n = 4) ± standard error of the mean.

For the first emergence count, there was a difference only between octaploid cultivars (Figure 4B), with a percentage of normal seedlings of 26% in 'Redonda'. Diploid cultivars, contrarily, remained constant and presented a lower percentage of normal seedlings (10%). The tetraploid cultivars did not differ, with values varying from 14 to 21%. When it comes to the edaphoclimatic conditions of the semiarid region, forage palm cultivars that have a fast initial growth, stand out in relation to slower growing ones, as they can benefit from the first rains.

Cactus seeds in general are small and light (less than 5 mm and 2 g), as observed in the present study. There was a difference between cultivars within the three ploidy levels. Among the diploid cultivars, the highest weights came from the seeds 'F8' and 'IPA Sertânia' (1.4 and 1.3 g) (Figure 4C). As for tetraploids, the cultivars 'Clone 6 African Elephant Ear' and 'Mexican Elephant Ear' had heavier seeds (1.53 and 1.35 g). The seeds of the cultivar, 'Redonda' with 2n = 88, had a weight of 1.60 g, differing from the others. The weight of the seeds can be influenced by several factors, including the presence of air (empty spaces) inside, chemical composition, maturity, and water content (Carvalho & Nakagawa, 2012). Larger mass seeds have more reserves, which will be translocated to the seedlings during germination and emergence.

The diploid cultivar F21' had a lower seed weight (0.76 g), and this may be related to the production of unviable, or even immature, seeds that have not yet completed their development, causing less vigorous seeds (as evidenced by the higher electrical conductivity of the cultivar 3,105 μ S cm⁻¹ g⁻¹) (Figure 4D). Contrarily, the cultivar 'Mexican Elephant Ear' (2n = 44) had seeds with high vigor, due to their low electrical conductivity (629 μ S cm⁻¹ g⁻¹). There is a large variation within the same ploidy level in electrical conductivity. This depends on the membrane constituents of each cultivar.

The evaluation of the seedlings by means of the length of the aerial part, shows that only the tetraploid cultivars differed, with emphasis on the 'Mexican Elephant Ear' (1.45 cm), followed by 'V19' (1.06 cm) (Figure 5A). The growth of forages is not only represented by the aerial part, but also by root development. If any factor limits the growth of the roots, it can harm the production of dry biomass of the plant and consequently the final productivity, since the root is the portal of entry of water and nutrients. Thus, to better understand a plant species, especially in conditions of water limitation, it is important to understand what occurs in the aerial part and in the root (Andrade et al., 2006).

Only the diploid cultivars showed a difference in root length, and the cultivar, 'IPA Sertânia', surpassed the others, with its roots measuring 4.1 cm (Figure 5B). The tetraploid and octaploid cultivars did not differ, with roots measuring ~3 cm. The root system of the forage palm has several thin roots close to the topsoil

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high utilization of rainwater, despite the irregular distribution of rainfall (Ramos et al., 2015).

Figure 5. Length of aerial part (A) and root (B), fresh mass (C), and dry seedling mass (D) of forage palm cultivars of the genera *Opuntia* and *Nopalea*, as a function of ploidy level. Diploids (2n = 22) (1 - 'F8', 2 - 'F21', and 3 - 'IPA Sertânia'); tetraploids (2n = 44) (1 - 'Clone 6 African Elephant Ear', 2 - 'Mexican Elephant Ear', and 3 - 'V19'), and octoploids (2n = 88) (1 - 'Round', 2- 'Giant', and 3 - 'IPA Clone 20'). Averages followed by the same letter, within the same ploidy level, do not differ by the Tukey test at the 5% probability level. The bars represent the means (n = 4) ± standard error of the mean.

The evaluation of fresh seedling mass showed that there was only a difference among the tetraploid cultivars. The 'V19' cultivar has the highest value (3.45 g) (Figure 5C). The lowest fresh mass was obtained from the cultivar 'IPA Sertânia' (0.50 g). The cultivars of the three ploidy levels differed in terms of seedling dry mass. Of these, the 'F8' diploid had the highest mass (0.14 g). The cultivar 'Orelha de Elefante Mexicana' was the one with the greatest fresh mass among the tetraploids (0.19 g), and 'IPA Clone 20' was the octaploid cultivar of highest weight (0.29 g) (Figure 5D). Freitas et al. (2021) when evaluating the growth and production of essential oils in polyploids of *Lippia alba*, found that the increase in plant biomass is directly proportional to the ploidy number.

The diploid and polyploid cultivars differed from each other when evaluated on the length of their seedling cladodes (Figure 6A). The longest octaploid (10.85 mm) was observed in the cultivar 'IPA Clone 20', and 'F8' was the longest (9.27 mm) diploids. These results differ from those observed by Vichiato, Vichiato, Pasqual, Castro, and Dutra (2007) when studying stomatal and morphometric analysis of leaves of diploid and tetraploid plants of *Dendrobium nobile*. They observed length of diploid leaves to be greater than the leaves of polyploids (tetraploids), i.e., it is not enough to be polyploid; it depends on the number of chromosomes and the cultivar. The tetraploid cladodes maintained a constant length (~9 mm).

In the same study, these authors observed that diploid plants had greater leaf widths (2.4 cm), differing from polyploids, which concurs with the results of the present study, in which the diploid cultivars 'F8' presented cladodes 6.11 mm wide (Figure 6B), differing from the cultivars within the same ploidy level. The tetraploid and octaploid cultivars did not differ, with an average diameter of 5 mm. This characteristic may be a result of lower rates of cell division, especially because polyploid species demand more time for the replication and organization of chromosomes in mitosis and meiosis (Tate, Soltis, & Soltis, 2005).

The diploid cultivars did not differ in terms of the fresh weight of the cladodes; even so, the cultivar 'F8' had a higher value among diploids (1.46 g). The fresh mass of the cultivar 'Orelha de Elefante Mexicana' was 2.73 g, which differed from the other tetraploid cultivars. The octaploids maintained a constant weight of approximately 1.5 g (Figure 6C). The cultivar 'Orelha de Elefante Mexicana' also had a larger area of the cladode, resulting in an increase in productivity, and the area index of its cladodes stood out in the semi-arid conditions (Silva et al., 2014). The ploidy level of a plant can cause the size of its tissues to increase, due to the greater cell volume.



Figure 6. Length (A), width of cladodes (B), fresh mass (C), and dry mass of cladodes (D) of cultivars of forage palm of the genera, *Opuntia* and *Nopalea*, depending on the level of ploidy. Diploids (2n = 22) (1 - 'F8', 2 - 'F21', and 3 - 'IPA Sertânia'); tetraploids (2n = 44) (1 - 'Clone 6 African Elephant Ear', 2 - 'Mexican Elephant Ear', and 3 - 'V19'), and octoploids (2n = 88) (1 - 'Round', 2- 'Giant', and 3 - 'IPA Clone 20'). Averages followed by the same letter, within the same ploidy level, do not differ by the Tukey test at the 5% probability level. The bars represent the means (n = 4) ± standard error of the mean.

The dry mass of the cladodes followed the same behavior as the fresh mass; only the tetraploid cultivars differed from each other, with a higher value for the cultivar 'Mexican Elephant Ear' (0.1793 g) (Figure 6D), concurring with the results obtained by Alves et al. (2017), who assessed the chemical and nutritional variability of forage palm. The nutrient contents are determined from the dry mass; therefore, since such cultivars present higher contents of dry matter, they contain more total digestible nutrients (Cavalcante, Santos, Silva, Fagundes, & Silva, 2014). The diploid cultivars, contrarily, maintained a dry mass of ~0.04 g and 0.10 g in octaploids.

Conclusion

The ploidy level positively influences the physiological quality of seeds and seedlings; cultivars behave differently within the same ploidy level. The vigor and emergence of polyploids stands out from the diploids. Phenotypically, the cultivar 'Mexican Elephant Ear' gave rise to heavier cladodes.

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