MORPHOPHYSIOLOGICAL FEATURES OF SEEDS AND SEEDLINGS BETWEEN WILD AND CULTIVATED GENOTYPES OF *HEVEA BRASILIENSIS*

de Carvalho JC[1], Miranda MJDS[2], Kramer YV[1], Reis ARS[3] & Gonçalves JFDC[1, *]

[1]*National Institute for Amazonian Research (INPA-MCTI), Laboratory of Plant Physiology and Biochemistry / PO Box 2223 Brazil*
[2]*Federal Institute of Education, Science and Technology of Amazonas, Josiane Celerino Campus – Manaus Zona Leste, Brazil*
[3]*Faculty of Forestry of the Federal University of Pará, Campus – Altamira Brazil*

**jfc@inpa.gov.br*

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Morphophysiological changes related to germination and initial seedling growth of different *Hevea brasiliensis* genotypes (wild and cultivated) were investigated. The seeds were sterilised before their biometric analyses were performed and the imbibition curve was mapped. Parallel to these assays, the morphology of the germination/post-germination stages and seed health testing were monitored from seeds germinated in washed sand and on filter paper respectively. The wild and cultivated (PB260) genotypes showed different behaviour patterns during the events of germination and post-germination. The seeds and seedlings could be differentiated by morphological characteristics associated with the size and colour of the seeds, as well as by the rapid imbibition of water, germination, and initial growth. Germination was characterised as being of a hypogeal, cryptocotyledonous type, with root protrusion between 6 and 8 days, and complete seedling formation at 23 and 25 days for wild and cultivated seeds respectively. The morphophysiological variables in seeds and seedlings of *H. brasiliensis* are useful in breeding programmes where seed selection and seedling performance evaluation focus on wild genotypes, which can add functional characteristics in favour of clones currently used in rubber tree plantations.

Keywords: Morphophysiological markers, seed selection, seedlings performance, rubber tree breeding

INTRODUCTION

The genus *Hevea* (Euphorbiaceae), comprises 11 tree species from the Amazon rainforest. One of the species that form part of this genus, the rubber tree, presents economic importance since it belongs to the main group of latescent plants providing essential raw material for more than 50 thousand items, from pneumatic to surgical items (Nogueira et al. 2015, Liu et al. 2019). The species *H. brasiliensis*, popularly known as the rubber tree, stands out for having the greatest productive capacity of latex among the other species (Krickl et al. 2017). Its timber is utilised for making plywood and furniture (Hytönen et al. 2018). The reproduction of *H. brasiliensis* focuses mainly on the advanced breeding of cultivars and depends on its genetic diversity of wild species available in the germplasm banks (Vu et al. 2020). However, the success of the improvement of the rubber tree depends on the increase of this genetic diversity, as well as on the information about the important characteristics for the selection of elite genotypes (Sant'anna et al. 2021).

The main challenge in rubber tree breeding programmes is the development of early selection markers that can shorten the reproductive cycle of clones (which takes between 25 and 30 years) and increase productivity (Priyadarshan 2017). However, according to Sant'anna et al. (2020), it is possible to make an early selection of *Hevea* genotypes to increase important traits (latex productivity, stem circumference, bark thickness, and the number of laticiferous vessel rings) using the number of leaf storeys.

The classification of the morphological characteristics of the seed, embryo and seedlings constitutes one of the most used taxonomic criteria in the identification of plants at the species level since, for angiosperms, these structures are only slightly influenced by the environment, thus allowing divergence between species (Silva et al. 1995). On the other hand, the classification of seeds by size or mass can be a strategy adopted to standardise seedling emergence, size and vigour of seedlings (Silva et al. 2012).

Biometric characteristics of seeds are important in the study of any species, and are used for the recognition of species within a genus or family (Pereira & Ferreira 2017, Nascimento et al. 2019). However, the scarcity of this information limits the suitability of the morphophysiological method for seeds, making it difficult to establish adequate conservation techniques for seeds of forest species (Silva et al. 2020a).

Not much information is available on the seed and germination physiology of different *H. brasiliensis* genotypes, but its recalcitrant characteristics are well known (Berjak & Pammenter 2013, Bewley et al. 2013). Recalcitrance leads to loss of embryo viability and germination power, and therefore, the long-term storage of the seed is not viable (Genaina et al. 2018). The low physiological quality of recalcitrant seeds is due to the high content of water and metabolic components, which can favour the infestation of microorganisms, thus, reducing seed viability (Bonome et al. 2011, Dutra et al. 2019).

Research related to the morphophysiological processes of *H. brasiliensis* germination is still ongoing (De Carvalho et al. 2022). Physiological aspects associated with the morphology and germination of *H. brasiliensis* can provide important information for the breeding studies of this species. We hypothesised that wild and cultivated species may present different morphophysiological characteristics throughout the germination/post-germination processes and that these characteristics may be useful for the creation of morphological markers for the early selection of seedlings that will grow to be more productive. This information is useful in breeding programmes. In this study, we investigated the morphophysiological changes related to germination and the initial growth of seedlings of two *H. brasiliensis* genotypes.

MATERIALS AND METHODS

Collection of plant materials

Seeds from 10 matrices of native *H. brasiliensis* were collected in an area located 17 km along the Tatajuba trail of the BR 230 highway, at the municipality of Altamira, Pará state, Brazil (3° 18′ S, 52° 17′ W). Another sampling, aimed at cultivated seeds (PB260), was made from 10 matrices of *H. brasiliensis* plantation, located near the BR 163 highway, municipality of Belterra, Pará state, mesoregion of the lower Amazon (2° 38′ S, 54° 58′ W).

After sampling, the seeds were packed in plastic bags and transported to the Laboratory of Plant Physiology and Biochemistry, National Institute for Amazonian Research, where the seeds were processed for morphological (biometrics and classification of the external and internal characteristics), and technological (degree of humidity, seed health test, imbibition curve, and germination test) characterisation of the seeds.

Seed biometry

The length, width and thickness were measured using digital callipers (0.01 mm precision). A hundred seeds were randomly separated from the study lots (wild matrix and clone seeds). The degree of humidity of quiescent seeds was determined by the greenhouse method at 105 ± 3 °C, for 24 hours (Anonymous 2009). For this experiment, four repetitions of 2.5 g of seeds were used. These biometric identifiers are often described as physiological characteristics, which are related to the shape of the organ or organisms.

Health of seeds

The isolation and identification of pathogens from the *H. brasiliensis* seeds were performed according to the modified methodology of Anonymous (2009). The detection of fungi in the seeds was carried out using the filter paper method in germination boxes disinfected with 70% alcohol (blotter test). Sheets of filter paper, moistened with distilled water and sterilised in an autoclave (121 °C for 20 min), were placed in the boxes. The seeds were subjected to surface disinfestation by 70% ethyl alcohol for 3 min, followed by 1% sodium hypochlorite for 5 min, and two washes in distilled water to remove excess alcohol and hypochlorite. The seeds were then deposited on the filter paper. Each germination box contained 10 seeds. The boxes were kept in a BOD germinating chamber (12 hours photoperiod) at a constant temperature of 27 °C, for 7 days. The fungi were identified using a stereoscopic and optical microscope. This experiment was conducted in 10 repetitions.

Imbibition curve

The imbibition curve was determined using 10 repetitions for each imbibition period. Before starting imbibition, the seeds were weighed on a digital analytical balance (0.1 mg precision) to obtain the initial weight. Imbibition was performed in beakers containing distilled water with the temperature maintained at 30 °C. The seeds were weighed every 2 hours during the 12-hour period and, subsequently, were weighed every 12 hours until reaching the point of water saturation and the emergence of radicle. The imbibition curve was calculated according to the methodology described by Oliveira and Bosco (2013).

Seed morphology, germination and seedling emergence

Another batch of seeds, after asepsis in 2% sodium hypochlorite for 2 min, were placed in plastic trays containing washed sand as substrate. The seeds were left to germinate in a greenhouse at room temperature (approximately 30 °C), with daily monitoring and counting. A total of 50 seeds were used in each of the 10 replicates.

The criteria considered to define germination was the emergence of radicle (2 mm) and this was observed for 30 days. The illustrations of the different phases considered throughout the germination were done manually with the aid of a binocular loupe to identify as many details as possible. Germination was categorised into the following three stages: quiescent seed, which was considered as the first stage; embedded seed, which was the second stage, and emergence of root primordia, the third stage. The descriptions and terminologies used in the morphological description are in accordance with Anonymous (2009), Oliveira and Pereira (1987) and Camargo et al. (2008). Drawings were made at each germination stage, characterising each structure during growth and development.

Seedling emergence was categorised into the following five stages: adventitious root, primary root elongation, emergence of the epicotyl, emergence of the eophylls and the expansion of the eophylls as the first to fifth stages respectively. Every two days, the roots, stem and leaves of the seedling were drawn to characterise each structure during growth and development.

Seedling germination data were calculated from the following variables: germination percentage and germination speed index, both according to the methodology found in Maguire (1962), and average germination time following Labouriau and Valadares (1976). Soon after seedling formation, the following growth variables were determined: plant height and diameter and number of leaves up to 60 days with 15-day intervals, which totaled four measurements (15, 30, 45 and 60 days).

Experimental design and statistical analysis

The experimental design was completely randomised, with two genotypes and an appropriate number of repetitions, depending on the objective of each assay (health, biometry, degree of humidity, morphology, imbibition, seedling emergence). Initial analyses of the data were subjected to a test of normality (Shapiro Wilks) and homogeneity of variance (F test), and when in accordance with these premises, analyses of variance (ANOVA) and regression analyses were performed to obtain adjusted equations for the variables (percentage and germination speed, imbibition curve, and mass gain) as a function of time. The statistical programs SigmaPlot 12.0 and GraphPad Prism 9.0 were used to perform the analyses. Statistical analysis of seedling initial growth data (height, stem diameter, and the number of leaves) was carried out using the Mixed Model of Repeated Measures in two ways and Bonferroni test at an accuracy of up to 5% probability (Field 2009), using procedures for repeated measures over time according to the methodology adapted from Miranda et al. (2021), SPSS and SigmaPlot 12.0.

RESULTS AND DISCUSSION

Seed biometry

Hevea brasiliensis seeds showed differences ($p < 0.05$) for both wild and cultivated seeds, but the former presented higher means for width (2.0 cm), thickness (2.1 cm), length (2.9 cm), weight (3.2 g) and seed moisture (36.2% for seed cut in half and 39.1% for whole seed) (Table 1). Similar results were reported by Camargo et al. (2008) and Widyarani et al. (2014) whereby the wild *H. brasiliensis* seeds in their study had length

Table 1 Biometric characteristics and degree of moisture of wild and cultivated *Hevea brasiliensis* seeds

Hevea brasiliensis	Parameter (cm)			Weight (g)	Moisture content (%)	
	Width	Thickness	Length		SC	WS
Wild	2.0 ± 0.10	2.1 ± 0.11	2.9 ± 0.11	3.2 ± 0.40	36.2 ± 0.11	39.1 ± 0.10
Cultivated	1.5 ± 0.12	1.6 ± 0.13	2.1 ± 0.14	1.9 ± 0.16	32.4 ± 0.13	35.6 ± 0.13

Means ± standard deviation; SC = seed cut in half, WS = whole seed

values of 2.5–3.0 cm, width 2.0–2.5 cm, thickness 1.9–2.1 cm and total mass 2.0–4.0 g. Differences in seed size are important in order to understand the process of domestication of the species. Cultivated genotypes go through a genetic improvement process, hence, the size of the seeds can be considered a characteristic that is associated with this improvement. According to Bezerra et al. (2014), seed biometry is an important tool that can assist in the identification and detection of genetic variability within populations of the same species and between species of the same genus. However, it must be taken into account that seed size (total seed mass), seed quality (concentration of reserves), and the main function of cotyledons (photosynthetic material or reserve organs) are paramount factors in order to understand the best performance in seedling production, and these may vary between species (Alencar et al. 2012).

Wild and cultivated half seeds had 36.2 and 32.4% seed moisture respectively (Table 1). Paula et al. (1997) who worked on the same species observed 27% moisture content while Martins et al. (2020) reported water content of 28% for rubber seed of the clone genotype RRIM 600. This information is directly related to the issue of seed longevity, since *H. brasiliensis* seeds lose viability when their moisture levels drop below 15–20% (Chin et al. 1981), and are viable up to 2 months (Dutra et al. 2019).

Seeds with recalcitrant characteristics present difficulties regarding storage, since they cannot be stored at less than 30% humidity and have little tolerance to low temperatures. Thus, under these conditions, the metabolism is still active even in a state of rest (quiescent), and the seeds present relatively high-water content, ranging between 30–70% (Berjak & Pammenter 2013).

Seed health

Due the recalcitrancy of *H. brasiliensis* seeds and their high degree of moisture, there is a risk of greater proliferation of fungi when the seeds are exposed to high temperature. In this study, the most frequently found fungi in the seeds of *H. brasiliensis* were *Aspergilus* sp. and *Fusarium* sp., of which 52% were in the wild seeds and 16% in the cultivated seeds. *Cladiosporium* sp. was also isolated from the wild seeds. Rubber seeds are infested with fungi when they are dispersed by the explosion of fruits and have direct contact with the soil (Theodoro & Batista 2014). Natural forests have greater species diversity and, consequently, greater susceptibility to fungal diseases. Thus, wild seeds have higher rates of contamination compared with cultivated seeds. *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp. have been identified as spoilage agents in seeds of *H. brasiliensis* (Igeleke & Omorusi 2007). In addition to causing a reduction in seedling germination capacity and increased mortality, fungi in the seeds influence the progressive increase of seedling disease in the field and may reduce the commercial value of crops (Henning 1994).

Imbibition curve

The imbibition curve of wild and cultivated seeds followed the three-phase model, with rapid water absorption in the first 60 hours of imbibition (phase I), followed by slower absorption up to 144 hours (phase II), and emission of primordia of the roots, characterising the end of germination (phase III) (Figure 1).

The differences in water absorption in the seeds, whether of the same species or even of the same family, can be attributed to their origin, vigour, chemical composition, and even morphological aspects, as well as, seed size and external characteristics of the integument. *Hevea brasiliensis* seed did not show integument dormancy, which was characterised by the limitation of water entry into the seed and, therefore, the activation time of metabolism. Water is one of the most important factors

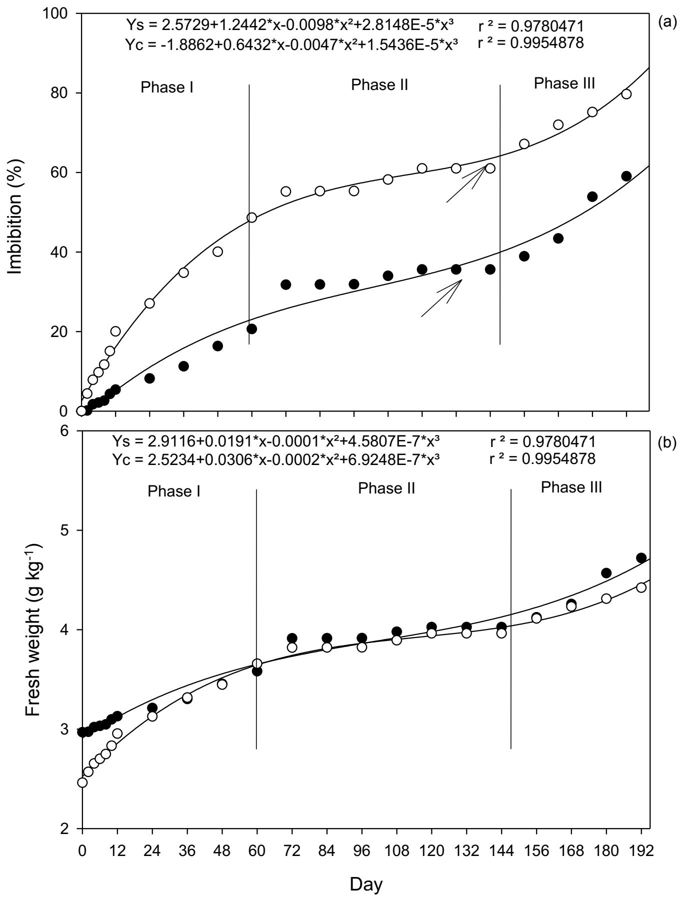

Figure 1 (a) Imbibition curve and (b) fresh seed mass of wild (●) and cultivated (○) *Hevea brasiliensis* seeds; arrows indicate the emergence of root primordia

for activation of metabolism in seeds, the reorganisation of cells and beginning of embryo growth, and the establishment of seedlings. Loureiro et al. (2013) verified the three-phase pattern of imbibition for *Jatropha curcas*, which belongs to the same family as *H. brasiliensis*. Cultivated seeds of *H. brasiliensis*, being smaller compared with wild seeds, absorbed water faster than wild seeds, a finding similar to the report by Duarte et al. (2010).

Seed morphology, germination, and seedling emergence

Wild seeds exhibit dark brown colouration, with irregular light brown streaks; globoid shape; thin testa; smooth and shiny surface; thin raphe, forming a straight line, connecting the hilum to the micropyle; fibrous tegmen of a beige colour, with presence of reddish-brown striae (Figure 2a). On the other hand, the cultivated seeds are beige, with dark brown layering; globoid; thin testa a smooth surface with a shiny and translucent appearance; thin raphe, with the presence of dark brown striae (Figure 2b). The reserve tissue of both seeds is the endosperm, with a firm and whitish consistency surrounding the embryo by the hypocotyl-radicular axis, which is short, cylindrical and straight; the cotyledons are foliaceous, white and oblong (Figure 2c). These morphological characteristics are also observed in other species of the same family, i.e. Euphorbiaceae. In *Cnidoscolus quercifolius* and *Jatropha curcas*, the raphe is well marked longitudinally (Loureiro et al. 2013, Widyarani et al. 2014). *Jatropha elliptica*, *C. quercifolius* and *J. curcas* also have whitish endosperms. The integument of *J. curcas* is composed of a tegmen (Loureiro et al. 2013). The cotyledons have visible ribs in both the adaxial and abaxial regions.

The emergence of hypocotyl in both genotypes (wild and cultivated) occurred between 1 and 2 days (Figure 2d and e). The elongation of the roots, adventitious (1.1–1.5 cm) and primary (1.2–1.7 cm), occurred between days 2 and 5. The adventitious roots lengthened before the primary roots, presenting pinkish hypocotyls of 0.7–0.9 cm, with white spots (Figure 2f). Between

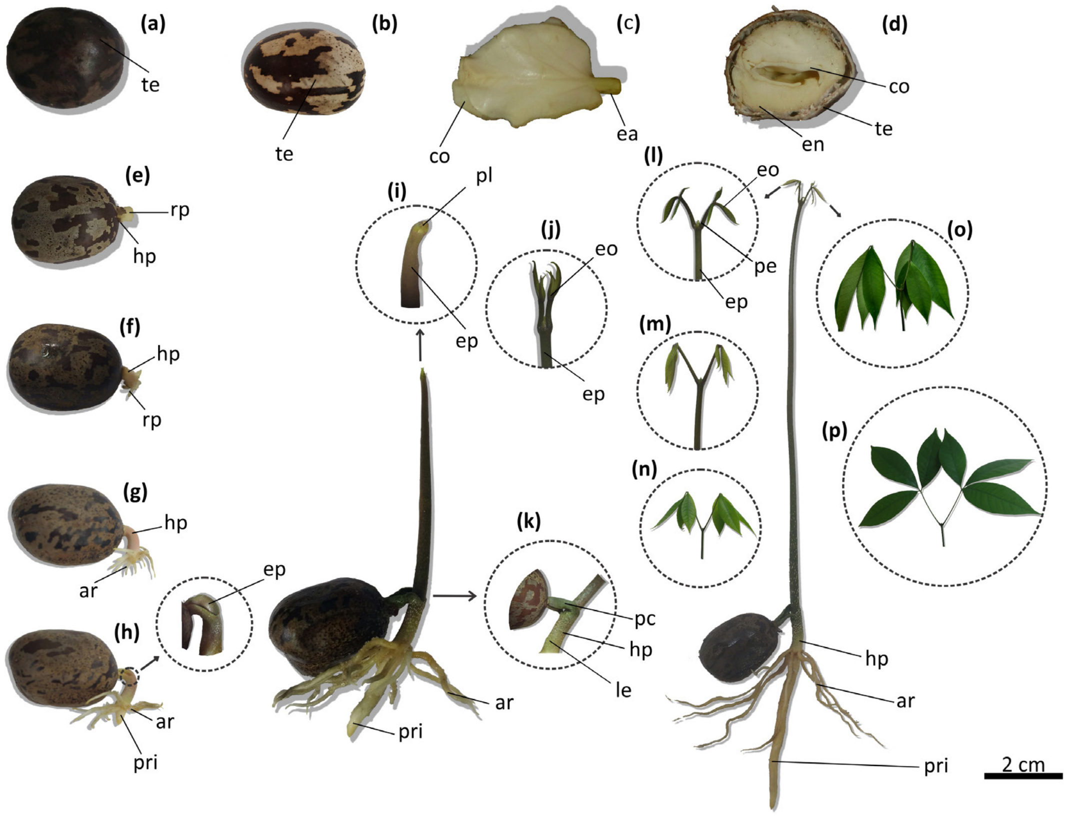

Figure 2 Morphology of wild seeds and seedlings of *Hevea brasiliensis*; (a) the outside of the wild seed, (b) the outside of the cultivated seed, (c) embryo (cotyledons and embryonic axis), (d) longitudinal cutting of the seed, endosperm, (e) emergence of hypocotyl and root primordia, (f) emergence of adventitious root, (g) elongation of adventitious root, (h) emergence of primary root, (i) emergence of secondary root, epicotyl, cotyledons petioles and plumula, (j) emergence of eophylls; te = testa, ea = embryonic axis, en = endosperm, co = cotyledon, hp = hypocotyl, rp = root primordia, ar = adventitious root, pri = primary root, pl = plumula, ep = epicotyl, cp = cotyledon petiole, eo = eophyll, pe = petiole, le = lenticels

days 5 and 8, we observed the appearance of the epicotyl, 0.7–1.0 cm, purplish colour with greenish tones, which arose from the cleavage between the hypocotyls and the cotyledon petioles (Figure 2g). Between 8 and 9 days after sowing, the elongation of the epicotyl was 3.0–3.2 cm), and secondary roots emerged (Figure 2h). At 9 and 10 days, it was possible to observe the appearance of the greenish coloured apical bud, which gave rise to the eophylls (Figure 2i). From 10 to 16 days, light green eophylls (0.6 cm) emerged (Figures 2j and k), followed by the beginning of the expansion of dark green eophylls (2.0–8.0 cm) at 18 days (Figure 2l, m and n). The total expansion of the eophylls (12.0–13.0 cm) took 20–25 days (Figure 2o).

The wild *Hevea* genotype showed distinct characteristics of colour and size similar to the studies by Oliveira and Pereira (1987) and Camargo et al. (2008). The cultivated species had hypogeal, cryptocotyledonous and unipolar germination, with lateral axis to the cotyledons, slightly elongated hypocotyl (1.0–1.5 cm) and reddish-brown, unreserved cotyledons, which remained in the seed, with only its petioles being visible (Camargo et al. 2008). Knowing the types of germination, growth and the establishment of seedlings is useful in understanding the biological cycle of the species, as well as the type of natural regeneration.

Seedling emergence and initial growth

The wild seeds of *H. brasiliensis* germinated at day 5 and the cultivated at day 7. At day 20, their germination percentages were 75 and 65% respectively (Figure 3a). Germination speed indices were 2.8 and 1.6 while average germination times were 12 and 18 days for the wild and the cultivated seeds respectively

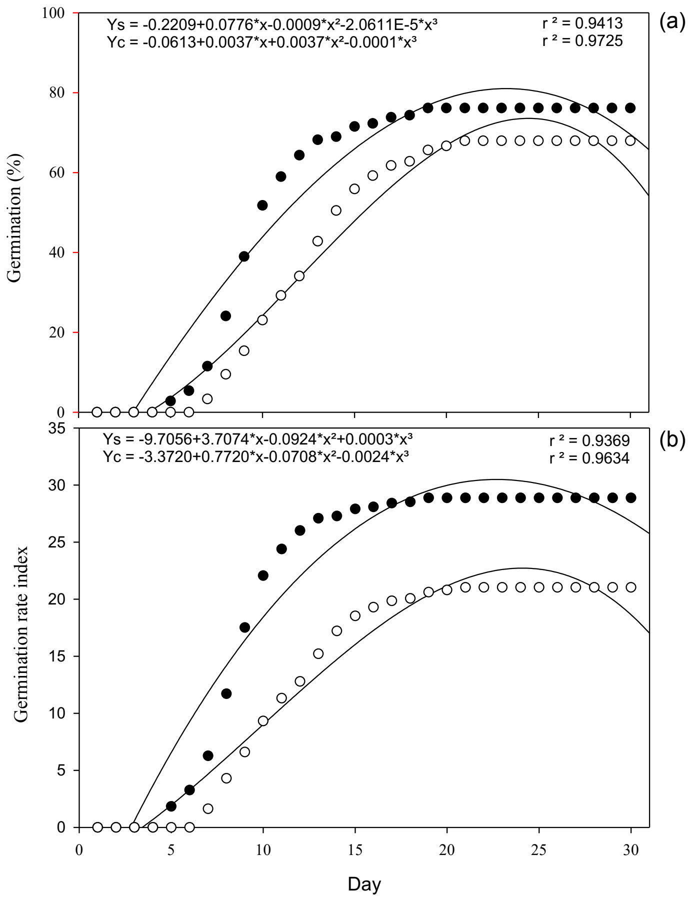

Figure 3 (a) Germination percentage and (b) seed germination speed index of the wild (●) and the cultivated (○) seeds

(Figure 3b). The longer average germination time for cultivated seeds may be associated with their higher degree of moisture and consequently greater seed viability. Zhou et al. (2015) reported lower seed germination rates in natural forests (11.7%) than in rubber plantations (44.3%). The authors suggested that soil temperature, which was 21 °C in natural forests and 23 °C in plantations, influenced the germination rate. Garcia and Vieira (1994) evaluated some of the aspects related to the germination of *H. brasiliensis*, and reported germination speed indices values of 1.31 and 2.13 with germination times of 20 days at 30 and 35 °C respectively. These data corroborated the results obtained in the present study.

The biometric parameters used in the evaluation of the development and initial growth of seedlings of the wild and cultivated *H. brasiliensis* showed significant differences ($p < 0.001$) in the height and diameter of the plant (Figures 4a and b). As for the number of leaves, differences were observed only after 30 days ($p < 0.001$) (Figure 4c). Wild seedlings had higher values of height and diameter during all evaluated periods (Figure 4a and b).

Differences in the size of the seedlings might be related to the size of the seeds, since, it was observed that the seed of wild *H. brasiliensis* was larger than that of the cultivar PB260. Seeds of larger size or with greater density are potentially the most vigorous (Carvalho & Nakagawa 2000). Seed size is an important physical indicator of seed quality that directly affects seedling emergence and growth, as well as the performance of seedlings in the field (Adebisi et al. 2013). Initial seedling growth and seed size have positive correlations with germination rate as well as seedling growth (Ambika et al. 2014, Pereira & Ferreira 2017, Nascimento et al. 2019). Studies of rubber seeds are very important for the production of rootstocks for seedlings, which mainly aims at the implantation and/or replacement of commercial rubber plants. The production of quality seedlings is key in making vigorous seed selection (Silva et al. 2020b).

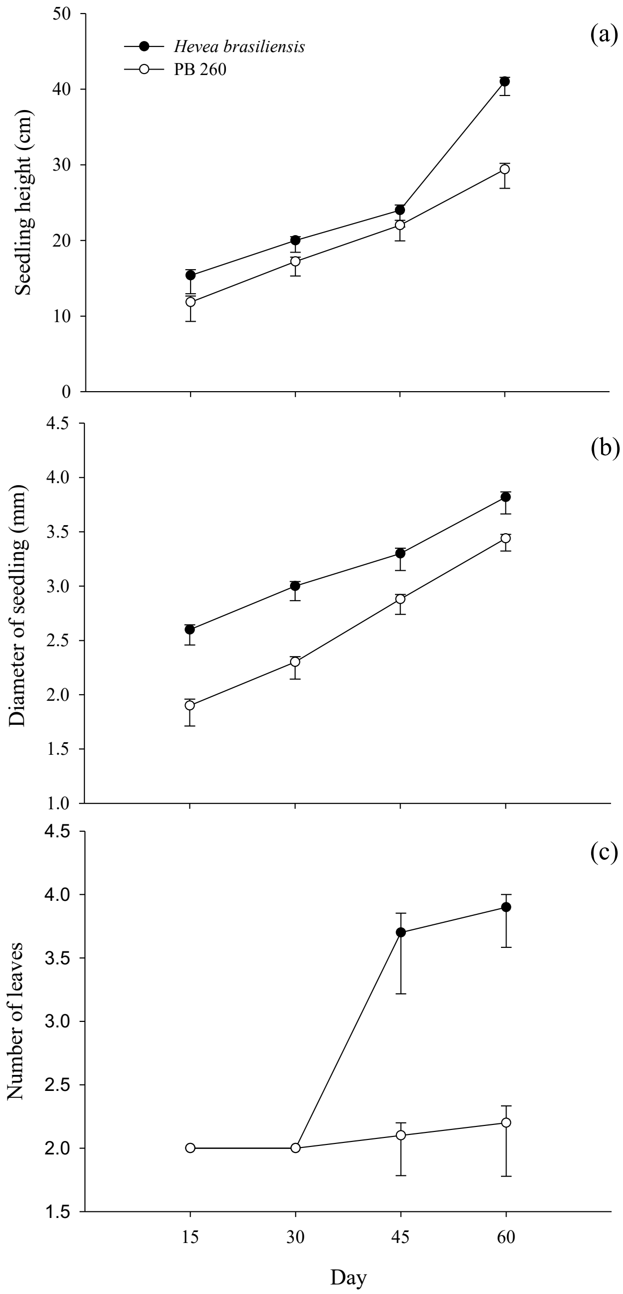

Figure 4 Biometric measurements of seedlings of two genotypes of *Hevea brasiliensis,* (a) seedling height (cm), (b) stem diameter, and (c) number of leaves

CONCLUSION

The results of this study provide an integrated understanding of the process of rubber tree seed germination and early post-germination events. Wild and cultivated *H. brasiliensis* seeds showed different behaviour patterns during the experiment. *Hevea brasiliensis* seeds of different genotypes can be differentiated by the morphological characteristics associated with the size and colour of the seeds. The wild genotype showed better performance in the growth of seedling. These morphophysiological variables in seeds and seedlings can be taken into account when selecting seeds for *H. brasiliensis* breeding programmes. We underline the importance of wild seeds in this kind of approach because unique characteristics in wild genotypes have the potential to be incorporated into clones currently used in rubber tree plantations.

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REFERENCES

Adebisi MA, Kehinde T, Salau AW et al. 2013. Influence of different seed size fractions on seed germination, seedling emergence and seed yield characters in tropical soybean (*Glycine max* L. Merrill). *International Journal of Agricultural Research* 8: 26–33. https: //doi.org/10.3923/ijar.2013.26.33

Alencar NLM, Innecco R, Gomes-Filho E et al. 2012. Seed reserve composition and mobilization during germination and early seedling establishment of *Cereus jamacaru* D.C. ssp. *jamacaru* (Cactaceae). *Anais da Academia Brasileira de Ciências* 84: 823–832. https://doi.org/10.1590/S0001-37652012000300024.

Ambika S, Manonmani V & Somasundaram G. 2014. Review on effect of seed size on seedling vigour and seed yield. *Research Journal of Seed Science* 7: 31–38. https://doi.org/10.3923/rjss.2014.31.38

Anonymous. 2009. *Regras Para Análise de Sementes.* Ministério da Agricultura, Pecuária e Abastecimento, Brasil.

Berjak P & Pammenter ENW. 2013. Implications of the lack of desiccation tolerance in recalcitrant seeds. *Frontiers Plant Science* 4: Article 478. https://doi.org/10.3389/fpls.2013.00478

Bewley JD, Bradford KJ, Hilhorst HWM & Nonogaki H. 2013. *Seeds: Physiology of Development, Germination and Dormancy.* Third edition. Springer, New York.

Bezerra FTC, Andrade LA, Bezerra MAF, Silva MLM, Nunes RCR & Costa E. 2014. Biometria de frutos e sementes e tratamentos pré-germinativos em *Cassia fistula* L. (Fabaceae Caesalpinioideae). *Semina: Ciências Agrárias* 4: 2273–2286. doi: 10.5433/1679-0359.2014v35n4Suplp2273

Bonome LTS, Moreira SAF, Oliveira LEM & Sotero AJ. 2011. Metabolism of carbohydrates during the development of seeds of the Brazilian rubber tree [*Hevea brasiliensis* (Willd. Ex Adr. de Juss) Muell. Arg.]. *Acta Physiologiae Plantarum* 33: 211–219. https://doi.org/10.1007/s11738-010-0540-8

Camargo JLC, Ferraz IDK, Mesquita MR, Santos BA & Brum HD. 2008. *Guia de Propágulos e Plantas da Amazônia.* Instituto Nacional de Pesquisas da Amazônia, Manaus.

Carvalho NM & Nakagawa J. 2000. *Sementes: Ciência, Tecnologia e Produção.* FUNEP, Jaboticabal.

Chin HF, Aziz M, Ang BB & Hamzah S. 1981. The effect of moisture and temperature on the ultrastructure and viability of seeds of *Hevea brasiliensis. Seed Science & Technology* 2: 411–422.

De Carvalho JC, De Carvalho Gonçalves, JF, Fernandes AV et al. 2022. Reserve mobilization and the role of primary metabolites during the germination and initial seedling growth of rubber tree genotypes. *Acta Physiologiae Plantarum* 44: 80. https://doi.org/10.1007/s11738-022-03415-5

Duarte EF, Carneiro IF, Silva Da & Guimarães NNR. 2010. Características físicas e germinação de sementes de *Dyckia goehringii* Gross & Rauh (Bromeliaceae) sob diferentes temperaturas. *Pesquisa Agropecuária Tropical* 40: 422–429. https://doi.org/10.1590/S1983-40632010000400011

Dutra LAS, Pereira JCS & Mendonça SR. 2019. Viabilidade das sementes de seringueira em função do tempo de armazenamento. *Ipê Agronomic Journal* 3: 97–107. https://doi.org/10.37951/2595-6906.2019v3i1.4332

Field A. *Descobrindo a Estatística Usando o SPSS.* Second edition. Artmed, Porto Alegre.

Garcia A & Vieira DR. 1994. Germinação, armazenamento e tratamento fungicida de sementes de seringueira (*Hevea brasiliensis* Muell. Arg.). *Revista Brasileira de Sementes* 16: 128–133. doi: 10.17801/0101-3122/rbs.v16n2p128-133

Genaina AS, Denise CFSD, Thaline MP et al. 2018. Morpho-anatomical, physiological and biochemical changes in rubber tree seeds. *Anais da Academia Brasileira de Ciências* 90: 1625–1641. https://doi.org/10.1590/0001-3765201820170340

Henning AA. 1994. *Patologia de Sementes. Documentos n° 90.* EMBRAPA/CNPSo, Londrina.

Hytönen J, Kaakkurivaara N, Kaakkurivaara T & Nurmi J. 2018. Biomass equations for rubber tree (*Hevea brasiliensis*) components in Southern Thailand. *Journal of Tropical Forest Science* 30: 588–596. https://doi.org/10.26525/jtfs2018.30.4.588596

Igeleke CL & Omorusi VI. 2007. Review of post-harvest deterioration of rubber seeds. *Journal of Agricultural and Social Research* 7: 11–19. doi:org/10.4314/jasr.v7i2.2852

Krickl S, Touraud D & Kunz W. 2017. Investigation of ethanolamine stabilized natural rubber latex from *Taraxacum kok-saghyz* and from *Hevea brasiliensis* using zeta-potential and dynamic light scattering measurements. *Industrial Crops & Products* 103: 169–174. https://doi.org/10.1016/j.indcrop.2017.03.046

Labouriau LG & Valadares MEB. 1976. On the germination of seeds *Calotropis procera* (Ait.) Ait.f. *Anais da Academia Brasileira de Ciências* 48: 263–284.

Liu H, Wei Y, Deng Z, Yang H, Dai L & Li D. 2019. Involvement of HbMC1-mediated cell death in tapping panel dryness of rubber tree (*Hevea brasiliensis*). *Tree Physiology* 39: 391–403. https://doi.org/10.1093/treephys/tpy125

Loureiro MB, Teles CAS, Colares CCA, Araújo BRN, Fernandez LG & Castro RD. 2013. Caracterização morfoanatomica e fisiológica de sementes e plântulas de *Jatropha curcas* L. (Euphorbiaceae). *Revista Árvore* 37: 1093–1101. https://doi.org/10.1590/S0100-67622013000600011

Maguire JD. 1962. Speed of germination: aid in selection and evaluation for seedling emergence and vigour. *Crop Science* 2: 176–177. https://doi.org/10.2135/cropsci1962.0011183X000200020033x

Martins NA, Suguino E, Gazola E, Gonçalves OS, Scaloppi Júnior EJ & Silva JK. 2020. Germinação de sementes clonais de seringueira (*Hevea brasiliensis* Muell. Arg.) para produção de porta-enxertos. Brazilian *Journal of Animal and Environmental Research* 3: 884–891. https://doi.org/884-891. 10.34188/bjaerv3n3-008

Miranda MJS, Marcolan LG, Miranda KS, Carvalho JC & Souza EB. 2021. Soil attributes and vegetative performance of *Acosmium nitens* Vog.Yakovlev (Itaubarana) with organic compost. *Brazilian Journal of Development* 7: 17984–18002. doi:10.34117/bjdv7n2-439

Nascimento CR, Chagas EA, Smiderle OJ, Souza AA & Chagas PC. 2019. Biometric characteristics of camu-camu seeds from native populations in the State of Roraima, classified by size class. *Advence in Horticultural Science* 33: 359–364. https://doi.org/10.13128/ahs-23992

Nogueira RDF, Cordeiro SA, Márcio A, Leite P, Luiza M & Binoti S. 2015. Mercado de borracha natural e viabilidade econômica do cultivo da seringueira no Brasil. *Nativa* 3: 143–149. https://doi.org/10.14583/2318-7670.v03n02a12

Oliveira EC & Pereira TS. 1987. Euphorbiaceae, morfologia da germinação de algumas espécies I. *Revista Brasileira de Sementes* 9: 9–29.

Oliveira AB & Bosco MRO. 2013. Biometria, determinação da curva de absorção de água em sementes e emergência inicial de plântulas de *Copernicia hospita* Martius. *Revista Brasileira de Agroecologia* 8: 66–74.

Paula NF, Borges EEL, Borges RCG & Paula RC. 1997. Alterações fisiológicas em sementes de seringueira (*Hevea brasiliensis* Muell. Arg.) durante o armazenamento. *Revista Brasileira de Sementes* 19: 326–333.

Pereira SA & Ferreira San. 2017. Fruit and seed biometry and seedling morphology of *Parkia discolor* (Spruce ex Benth.). *Revista Árvore* 41. https://doi.org/10.1590/1806-90882017000200006

Priyadarshan PM. 2017. *Biology of Hevea Rubber*. Springer, Cham. https://doi.org/10.1007/978-3-319-54506-6

Sant'anna IC, Gouvêa LRL, Spitti AMDS, Martins ALM & Gonçalves PS. 2020. Relationships between yield and some anatomical and morphological traits in rubber tree progenies. *Industrial Crops and Products* 147: 112221. https://doi.org/10.1016/j.indcrop.2020.112221

Sant'anna IC, Gouvêa LRL, Martins MA, Scaloppi Junior EJ, Freitas RS & Gonçalves PS. 2021. Genetic diversity associated with natural rubber quality in elite genotypes of the rubber tree. *Scientific Reporters* 11: 1081. https://doi.org/10.1038/s41598-020-80110-w

Silva LMM, Matos VP, Pereira DD & Lima AA. 1995. Morfologia de frutos, sementes e plântulas de *Luetzelburgia auriculata* Ducke (pau-serrote) e *Pterogyne nitens* Tul. (madeira-nova-do-brejo)—Leguminosae. *Revista Brasileira de Sementes* 17: 154-159.

Silva KB, Alves EU, Bruno RLA & Matos VP. 2012. Caracterização morfológica de frutos, sementes e germinação de *Sideroxylon obtusifolium* (Roem. e Schult.) Penn. (Sapotaceae). *Revista Árvore* 36: 59–64. https://doi.org/10.1590/S0100-67622012000100007

Silva AG, Costa E, Pereira TCC, Binotti FFS, Scaloppi Junior EJ & Zoz T. 2020a. Quality of rubber tree rootstock seedlings grown in protected environments and alternative substrates. *Acta Scientiarum Agronomy* 42: e43469. https://doi.org/10.4025/actasciagron.v42i1.43469

Silva GP, Sales JF, Nascimento KJT, Rodrigues AA, Camelo GN & Borges EEDL. 2020b. Biochemical and physiological changes in *Dipteryx alata* Vog. seeds during germination and accelerated aging. *South African Journal of Botany* 131: 84–92. https://doi.org/10.1016/j.sajb.2020.02.007

Theodoro GF & Batista TS. 2014. Detection of fungi in rubber tree (*Hevea brasiliensis*) seeds harvested in northeast of Mato Grosso do Sul, Brazil. *Revista Agrarian* 7: 365–368.

Vu VT, Lai VL, Le MT et al. 2020. Population genetic structure of a thousand rubber tree accessions from wild Rondônia populations conserved in Vietnam. *Genetic Resources and Crop Evolution* 67: 475–487. https://doi.org/10.1007/s10722-019-00843-0

Widyarani, Ratnaningsih E, Sanders JPM & Bruinsa ME. 2014. Biorefinery methods for separation of protein and oil fractions from rubber seed kernel. *Industrial Crops and Products* 62: 323–332. https://doi.org/10.1016/j.indcrop.2014.09.005

Zhou Y, Fan M, Chen L & Zhuang J. 2015. Lignocellulosic fibre mediated rubber composites: an overview. *Composites Part B: Engineering* 76: 180–191. https://doi.org/10.1016/j.compositesb.2015.02.028