# Maturation and quality of seeds of an endangered tropical palm species (Euterpe edulis Martius) assessed by imaging and X-ray densitometry

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**Research Article**

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# Abstract

*Euterpe edulis* Martius is an endangered palm species that grows in the Atlantic Forest and the Cerrado of South America. Economic exploitation of its antioxidant-rich fruits could ensure the sustainable management of this species. However, this relies on the rapid selection of high-quality seeds from which to derive seedlings. The objective of this study was to investigate the maturation of *E. edulis* seeds using image analysis and X-ray densitometry. Fruits were harvested from ten matrices at different stages of maturation, from 94 days after anthesis (DAA) to 290 DAA. Seed dry mass, water content, germination, vigor, and density were quantified at each stage. At the same time, seeds were analyzed by GroundEye® imaging, radiography, scanning electron microscopy (SEM), and energy dispersive spectroscopy (EDS). The highest dry mass was detected 255 DAA (0.83 g), *in vitro* germination began 115 DAA and achieved 100% from 150 DAA, and maximum vigor was observed 164 DAA, whereby 100% of seedlings appeared normal. X-ray imaging revealed dehydrated seeds and small mechanical damage, such as cracking of the pericarp. X-ray densitometry revealed that seed density increased considerably 185 DAA. SEM/EDS detected changes between maturation stages, such as the accumulation of K and Si, in the mesocarp and endocarp. Overall, *E. edulis* seeds presented maximum *in vitro* germination, vigor, percentage of normal seedlings, and physicochemical qualities 164 DAA (green epicarp), which corresponds to 126 days earlier compared with the fruits harvested 290 DAA (black epicarp) for *ex vitro* germination.


# Introduction

*Euterpe edulis* Martius is a dominant palm species in the understory of the Atlantic Forest and the Cerrado of South America. Repeated cycles of exploitation aimed at extracting the palm heart, whose quality and flavor are superior to those of other *Euterpe* species, have led to a decline in this species' population (Borges et al. 2011; Rother et al. 2016; Barroso et al. 2019; Lugon et al. 2021). Given that *E. edulis* is a key species for the structure and dynamics of the Atlantic Forest, conservation efforts should focus on a more sustainable use of this resource (Rother et al. 2016; Souza and Prevedello 2020).

The valorization of fruits is one of the main strategies for sustainable species management (Farinazzo et al. 2020). *E edulis* fruits are harvested annually and are composed of a single seed covered by a thin pulp (Inada et al. 2015). The pulp's dark purple to black color is indicative of a high concentration of anthocyanins, mainly cyanidin-3-rutinoside (449 g $mol^{-1}$) and cyanidin-3-glucoside (595 g $mol^{-1}$) (Vieira et al. 2018), which boosts the nutritional value of *E. edulis* compared to *Euterpe oleracea* Martius or *Euterpe precatoria* Martius (Borges et al. 2013). Accordingly, the harvesting of *E. edulis* fruits could be more profitable than the extraction of palm hearts.

Fruits represent the only natural means of propagation for *E. edulis* (Schulz et al. 2016). However, little is known about seed maturation and its correlation with germinal potential and vigor.

Traditionally, seed quality is determined by destructive, labor-intensive, and time-consuming methods. In recent years, new and refined technologies based on computerized procedures have allowed a detailed

diagnosis of the external and internal characteristics, as well as the structural integrity of seeds (Arkhipov et al. 2019). Because they can automate laboratory analyses, these techniques enable a faster, less subjective, and non-invasive assessment of seed quality (Bianchini et al. 2021).

The use of imaging coupled with customized analytical hardware and software simplifies the estimation of various seed characteristics, such as their external and internal morphology, mechanical or insect-derived damage, physiological potential, as well as the relationship between integrity and morphology (Colmer et al. 2020). The objective of this study was to investigate the maturation of *E. edulis* seeds using image analysis and X-ray densitometry.

# Materials And Methods

*E. edulis* fruits were collected from mother plants located in the district of Pedra Menina, in the municipality of Dores do Rio Preto and Espera Feliz, Espírito Santo/Minas Gerais, Brazil, close to the Caparaó National Park (Fig. 1a, b). The maximum temperature during the study period was 28.0°C in September, and the minimum was 14.9°C in June, with maximum rainfall of 514.4 mm in January and maximum relative humidity of 92% through May–June (Fig. 1c, d).

The fruit harvest started 94 days after anthesis (DAA) and lasted until 290 DAA, at intervals of seven days and totaling 29 stages. The fruits were picked manually and sent to the Laboratory of Forest Seeds and Plant Tissue Culture of the Center for Agricultural Sciences and Engineering, Federal University of Espírito Santo, where moisture (%) was evaluated in an oven at 105 ± 3°C (Brasil 2009), along with fresh fruit mass (g), germination, and vigor.

*In vitro* germination

In the laboratory, the fruits were cleaned, the tegument was removed, and the fruits were immersed in 2% ascorbic acid to prevent phenolic oxidation of plant tissues.

Using a laminar flow chamber, *E. edulis* seeds were immersed in 70% ethyl alcohol for 1 min, 2.5% sodium hypochlorite (Candura) for 10 min, and 3 g $L^{-1}$ amoxicillin solution (Germed) for 10 min. Three washes with distilled and autoclaved water were carried out after each step. Following disinfection, the seeds were placed in test tubes (25 × 150 mm) containing 10 mL MS culture medium (MURASHIGE and SKOOG, 1962) (Sigma), supplemented with 1 g $L^{-1}$ polyvinylpyrrolidone (Synth), 30 g $L^{-1}$ sucrose (Dinâmica), 0.1 g $L^{-1}$ myo-inositol (Sigma), and 6 g $L^{-1}$ agar (Kasvi). The pH of the medium was adjusted to 5.7 ± 0.1 with KOH (Alphatec) and/or 1.0 N HCl (Vetec) before autoclaving.

The explants were kept in a growth room at 27 ± 2°C, 8/16-h light/dark photoperiod, and light intensity of 30 µmol $m^{-2}$ $s^{-1}$ until seedlings were obtained. During this period, the germination percentage (%) (Brasil 2009), germination speed index (Maguire 1962), and average germination time (days) (Labouriau 1983) were obtained. After 150 days, normal seedlings (%), seedling height (cm), and root length (cm) were measured.

GroundEye® and X-ray analysis

Seed imaging was performed using the GroundEye® system, version S800 (Elbit Systems). Briefly, a high-resolution professional camera captured the information and combined it with software-generated results obtained from the percentage of colors present at each stage of maturation. Radiographic images were generated using a Faxitron MX 20 X-ray unit operated at an intensity of 24 kV for 20 s.

X-ray densitometry

The density profile was determined using X-ray densitometry. The seeds were arranged between two wood samples of 5 × 5 × 0.5 cm and placed in holders with the capacity for six samples within the internal compartment of the X-ray microdensitometer (DAX 6000; GreCon). Density values were determined at 20-µm steps, 33-kV voltage, current between 0 and 1 mA, radiation angle of 11°, and initial and final beam collimations of 100 and 50 µm, respectively. The average, maximum, minimum, and gravimetric densities were determined.

Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS)

Five maturation stages (94, 115, 164, 241, and 290 DAA) were selected. The seeds were dehydrated in an ethanol series to absolute alcohol, and dried at a critical point with $CO_2$ (Autosamdri 815; Tousimis). Samples of the seeds' external surface (epicarp) and internal surface (mesocarp and endocarp) (Fig. 2) were sectioned and placed in stubs, which were then subjected to metallic gold deposition by sputtering (Desk V; Denton Vacuum).

Analyses and photodocumentation were performed using a scanning electron microscope (JSM-6610LV; Jeol) in combination with EDS (XFlash 6–10 Detector; Bruker). Measurements were carried out at the Carlos Alberto Redins Cellular Ultrastructure Laboratory, Federal University of Espírito Santo, Vitória, ES.

Experimental design

The design was completely randomized and consisted of 29 treatments (maturation stages) and four replications with 10 seeds each. Samples were analyzed for biometric properties, *in vitro* germination, and vigor. Four replications with one seed were subjected to physicochemical analyses. Data were submitted to the Scott-Knott mean cluster test using R software (R Core Team 2020).

# Results

The highest dry mass of *E. edulis* seeds (0.83 g) was documented 255 DAA, just prior to detachment (290 DAA) from the mother plant (Fig. 3a). Seed moisture decreased throughout maturation, from 77% (94 DAA) to 45% (290 DAA) (Fig. 3b). The seeds started germinating 115 DAA and achieved 100% germination 150 DAA, with minor fluctuations past 206 DAA (Fig. 3c). The seeds attained maximum vigor 164 DAA (germination speed index = 1.42; average germination time = 7 days), with 100% germination

and normal seedlings (Fig. 3d–f). The highest mean seedling height was obtained 220–227 DAA, and the highest mean root length was attained 227 DAA (Fig. 3g, h).

In the initial stages of seed maturation and up to 227 DAA, the fruits were predominantly green (96.7–88.0%) (Figs. 4 and 5a). Then, 234 DAA, the fruits began to ripen and change color, with 53.8% now classified as green and 42.9% as black. After this point, a reversal of the initial situation was observed, with 2.5% green fruits and > 97.5% black fruits 290 DAA (Figs. 4 and 5a).

X-ray densitometry of *E. edulis* seeds (Fig. 6a–c) revealed that the average density increased linearly throughout seed maturation, but with a particularly sharp acceleration around 164–185 DAA (Fig. 6d, e).

Chemical analysis of the seeds' epicarp detected the presence of C, O, K, and Si (Fig. 7a–e). C was the most abundant element, increasing linearly with seed maturation (72.42–76.60%); whereas O decreased from 27.23–23.09% (Fig. 7f, g). K was adjusted to a quadratic model with a maximum value of 0.24% 210 DAA (Fig. 7h). Although Si appeared in the spectrum, its percentage at the different stages of maturation remained below detection.

SEM of the epicarp revealed swelling of the fruit pulp during ripening (108–241 DAA) and its dehydration in the last stage (290 DAA) (Fig. 8). A map of the chemical composition highlighted the points on the surface of the epicarp, in which each element was detected, thereby providing an estimate of its fluctuation during maturation (Fig. 8c, f, i, l, o).

Chemical analysis of the seeds' mesocarp and endocarp revealed the presence of C, O, K, and Si (Fig. 9a-e). However, unlike the epicarp, the percentage of C in the mesocarp decreased linearly with seed maturation (70.24–59.79%); whereas O, K, and Si increased from 29.43–39.29%, 0.15–0.37%, and 0.18–0.57%, respectively (Fig. 9f-i).

Finally, SEM/EDS was used to detect the main changes occurring during maturation in the mesocarp and endocarp of *E. edulis* seeds (Fig. 10). SEM imaging revealed few mesocarp laminae 94 DAA, which rendered the fibers from the endocarp more visible (Fig. 10a, b). As determined by EDS, this pattern coincided with lower Si deposition (Fig. 10c, d). As maturation progressed, mesocarp sheets covering the fibers emerging from the endocarp became more visible, together with greater Si deposition (Fig. 10e–t).

# Discussion

The dry mass of *E. edulis* seeds increased gradually throughout maturation until reaching maximum 255 DAA and decreased thereafter. Moisture, instead, exhibited the opposite trend and decreased to 45% 283–290 DAA. Dry mass accumulated during seed development as a result of rapid cell division and expansion. This, in turn, depended on high water content during early development, which allowed for metabolic activity and maintenance of seed growth (Kok et al. 2013). With the progressive linear accumulation of storage reserves, composed mainly of lipids and fibers (Mello et al. 2021), water became displaced from maturing *E. edulis* seeds.

Maturation comprises a series of morphological and physiological transformations in the fertilized egg until the physiological maturity point is reached, whereby seeds attain maximum dry mass, germination, and vigor. Here, *E. edulis* seeds reached maximum germination, vigor, and percentage of normal seedlings 164 DAA *in vitro*. For *ex vitro* germination, the fruits are generally harvested 290 DAA, which means 126 days (4.2 months) after they have attained maturity, according to our findings. In fact, our *in vitro* results show that immature fruits (characterized by a green pericarp) can be used in the production of quality seedlings, for conservation purposes in the form of *in vitro* germplasm banks, and as juvenile explants in somatic embryogenesis within breeding programs.

Monitoring of *E. edulis* seeds enables a more accurate determination of the changes, including in water content, that occur during the maturation process. In the present work, X-rays revealed fissures in the fruits' pericarp and a reduction in moisture with advancing maturation. Fissures are likely sites of infection by microorganisms, which is extremely undesirable in plant tissue culture, and further exacerbate water loss. Therefore, such alterations should be monitored to prevent desiccation of the embryo.

Diagnostic imaging studies on *Jatropha curcas* L. used integrated density (expressed in gray mm pixel$^{-1}$) to describe tissue integrity, with non-germinated seeds assigned the lowest average gray levels based on seedling vigor (Medeiros et al. 2020a). The IJCropSeed tool for the interpretation of X-ray images in ImageJ uses numerous descriptors of tissue integrity from *Crambe abyssinica* Hochst. ex R.E.Fr. Specifically, the integrated density parameter assigns higher tissue density, reflected in higher gray values on radiographs, to high-quality seeds, which generate normal seedlings (Medeiros et al. 2020b). In the seeds of *Capsicum chinense* Jacq., a significantly positive correlation was found between tissue density parameters, evaluated by analysis of X-ray images in ImageJ, and the percentage/speed of germination, as well as seed viability; whereas a negative correlation was observed with dormancy (Medeiros et al. 2019). In the seeds of *Lecythis pisonis* Cambess., the densities derived from X-ray images and interpreted in ArcGIS® differed by only 4% from those obtained in the laboratory, which validated the use of this methodology (Rosa et al. 2020). Therefore, these non-destructive methods are reliable for internal evaluation of plant tissues, including the identification of structures (endosperm and embryo), mechanical damage, invading organisms, and deterioration. Crucially, they allow the processing of large batches of seeds, which gives them a strong advantage over traditional laboratory methods.

Here, X-ray densitometry revealed that the average density increased with seed maturation. X-ray densitometry is widely used in the quantitative analysis of wood from different forest species and wood products; however, it has not been applied to seed analysis. The present study shows that it may be a promising, non-destructive technique for quantifying seed density and internal morphology, as it can identify empty seeds, partially empty seeds, and those with defects in the endosperm (Gomes-Junior et al. 2012).

Chemical elements, such as Si and K, were detected in the pericarp, mesocarp, and endocarp of *E. edulis* seeds. SEM/EDS indicated that the highest concentration of Si was found in the mesocarp and endocarp

290 DAA (0.63%). Si affects the physical and mechanical properties of the seeds, promoting resistance to the penetration of water and other compounds. Lima et al. (2021) detected 2% (w/w) Si in samples of *E. oleracea* seed fiber. The observed 1.63% difference in Si concentration between *E. edulis* and *E. oleracea*, implies a 3.17-times lower mechanical resistance in the former compared to the latter, which can considerably influence seed imbibition.

The increase in fruit biomass is accompanied by the accumulation of K, a macronutrient present in seeds, whose function is to harden the seedling. This benefits seed acclimatization upon subsequent planting in the field. In addition, it is important in fruit formation, the transport of phloem assimilates, and the activation of enzymes involved in the synthesis of organic compounds, including soluble sugars produced during fruit maturation (Lima et al. 2014).

# Conclusions

In the present study, maximum germination, vigor, and percentage of normal seedlings of *E. edulis* were obtained from *in vitro*-matured fruits harvested 164 DAA (green epicarp). This finding anticipates the possible harvesting of these fruits by 126 days (4.2 months) compared to what is done normally for *ex vitro* seed germination, whereby fruits are picked 290 DAA (black epicarp).

Moreover, we show the feasibility of using X-ray densitometry for the non-destructive, high-throughput assessment of *E. edulis* seed quality. In this manner, it becomes possible to identify denser seeds during maturation and select them for breeding programs.

Finally, we report an increase in Si and K in the mesocarp and endocarp of *E. edulis* fruits with advancing maturation. This result, achieved by SEM/EDS, implies increased water absorption by the seed and hardening of seedlings, respectively, which should thus be monitored more carefully to ensure optimal plant growth.

# Declarations


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### Declaration of Competing Interest

The authors declare no conflict of interest.

### Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

### Author contribution statement

TM: investigation, methodology, writing – review, and editing. TÉCZ, YST, IMS, JOB, FGG, and CENH: supervision, writing – review, and editing. ERS and AF: formal analysis. HOS, JCL, and WCO: supervision, writing original draft, writing – reviewed, and editing. RSA: conceptualization, supervision, project administration, funding acquisition, writing – original draft, writing – review, and editing.

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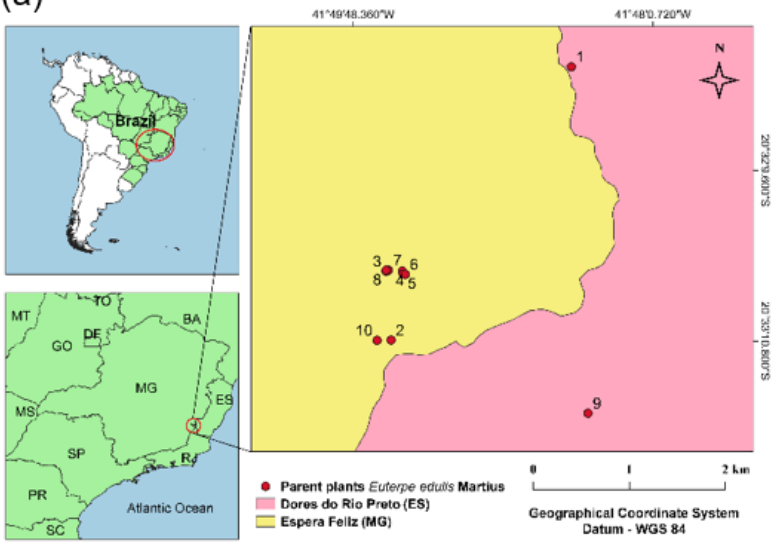
# Figures

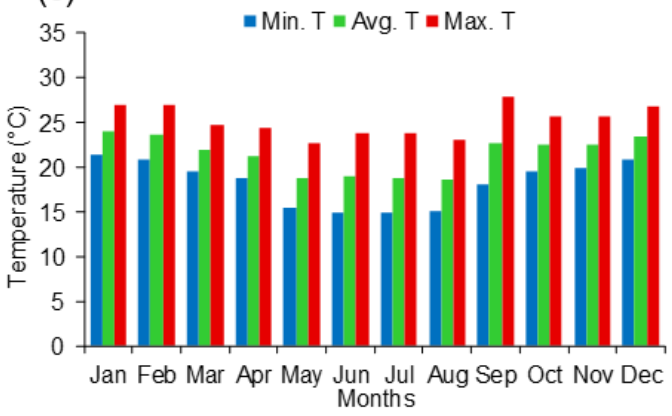
(a)



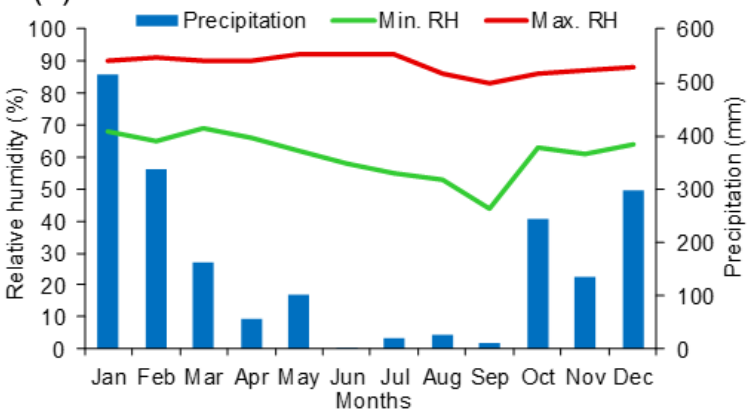

(b)

| Parent plants | Altitude (m) | DBH* (cm) | Height (m) |
|---|---|---|---|
| 1 | 1164 | 15.4 | 11.56 |
| 2 | 928 | 18.1 | 7.11 |
| 3 | 957 | 18.2 | 9.14 |
| 4 | 954 | 17.8 | 8.29 |
| 5 | 954 | 14.8 | 6.71 |
| 6 | 954 | 19.6 | 8.24 |
| 7 | 951 | 13.4 | 6.70 |
| 8 | 951 | 16.8 | 8.39 |
| 9 | 1210 | 26.0 | 11.43 |
| 10 | 916 | 24.9 | 5.50 |

(c)

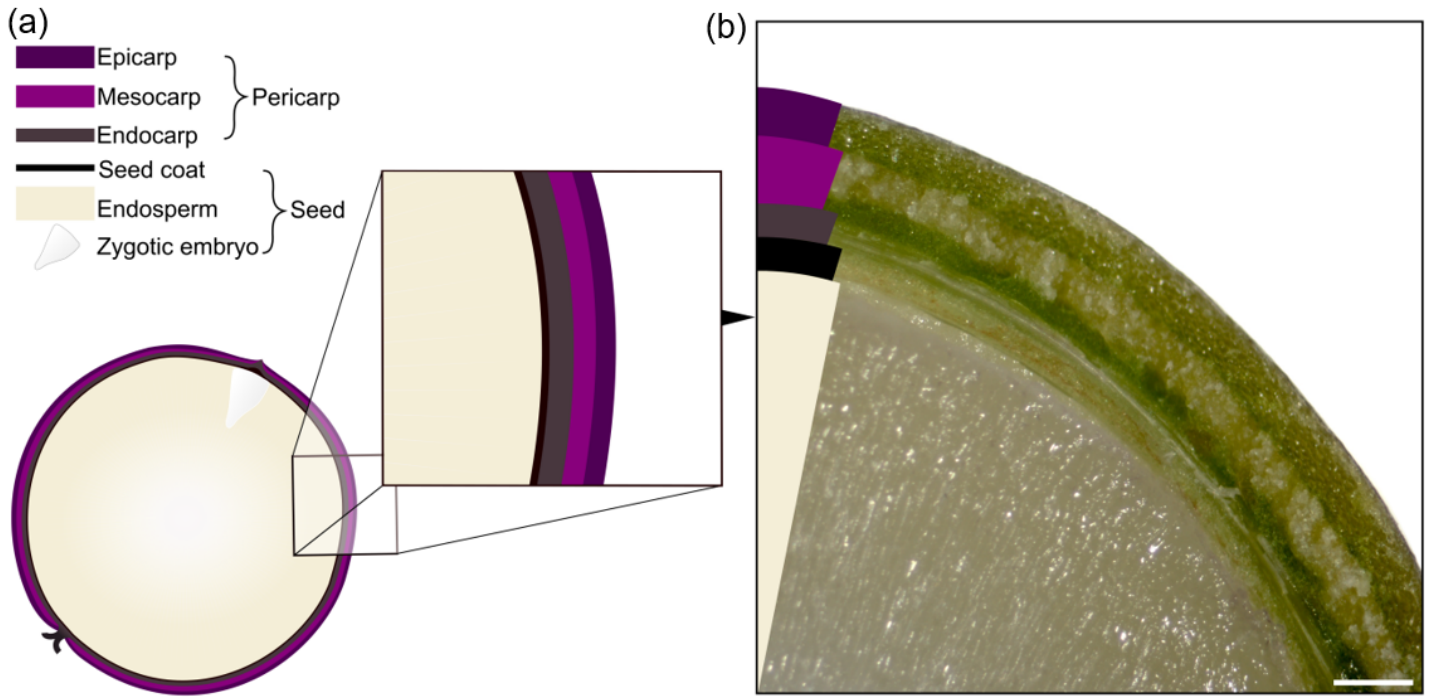



(d)



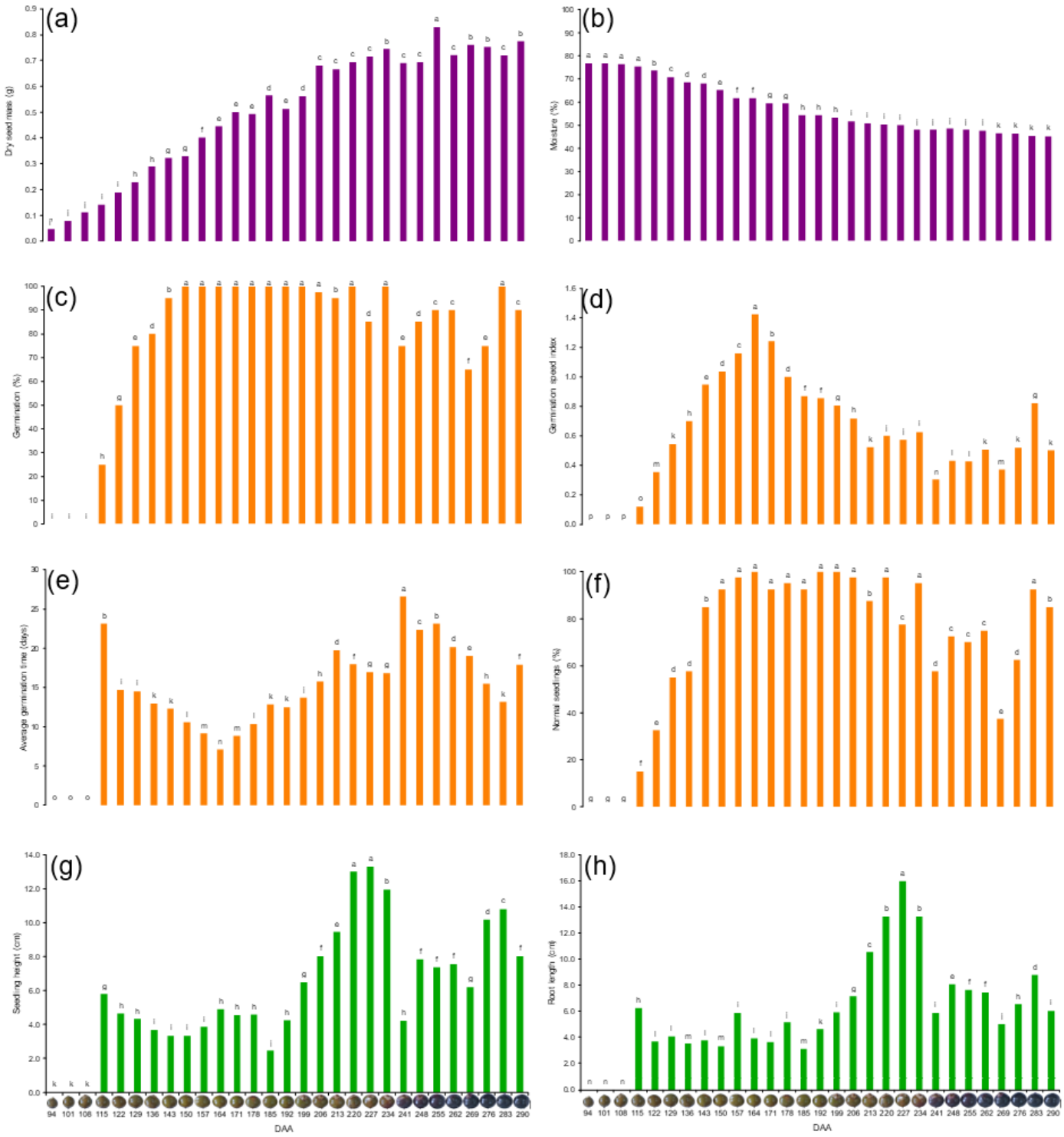

## Figure 1

Location and physical properties of parent plants, as well as geographical characteristics of their growth area. (a) Coordinates of the sampling area. (b) Characteristics of parent plants: altitude at which the plant grew, diameter at breast height (DBH; 1.30 m), and plant height. (c) Temperature in the sampling area: maximum (Max. T), average (Avg. T), and minimum (Min. T) temperature. (d) Humidity in the sampling area: maximum relative humidity (Max. RH), minimum relative humidity (Min. RH), and precipitation from the beginning of flowering to the end of fruit harvesting in 2020. Data were provided by the Instituto Nacional de Meteorologia, Brazil.



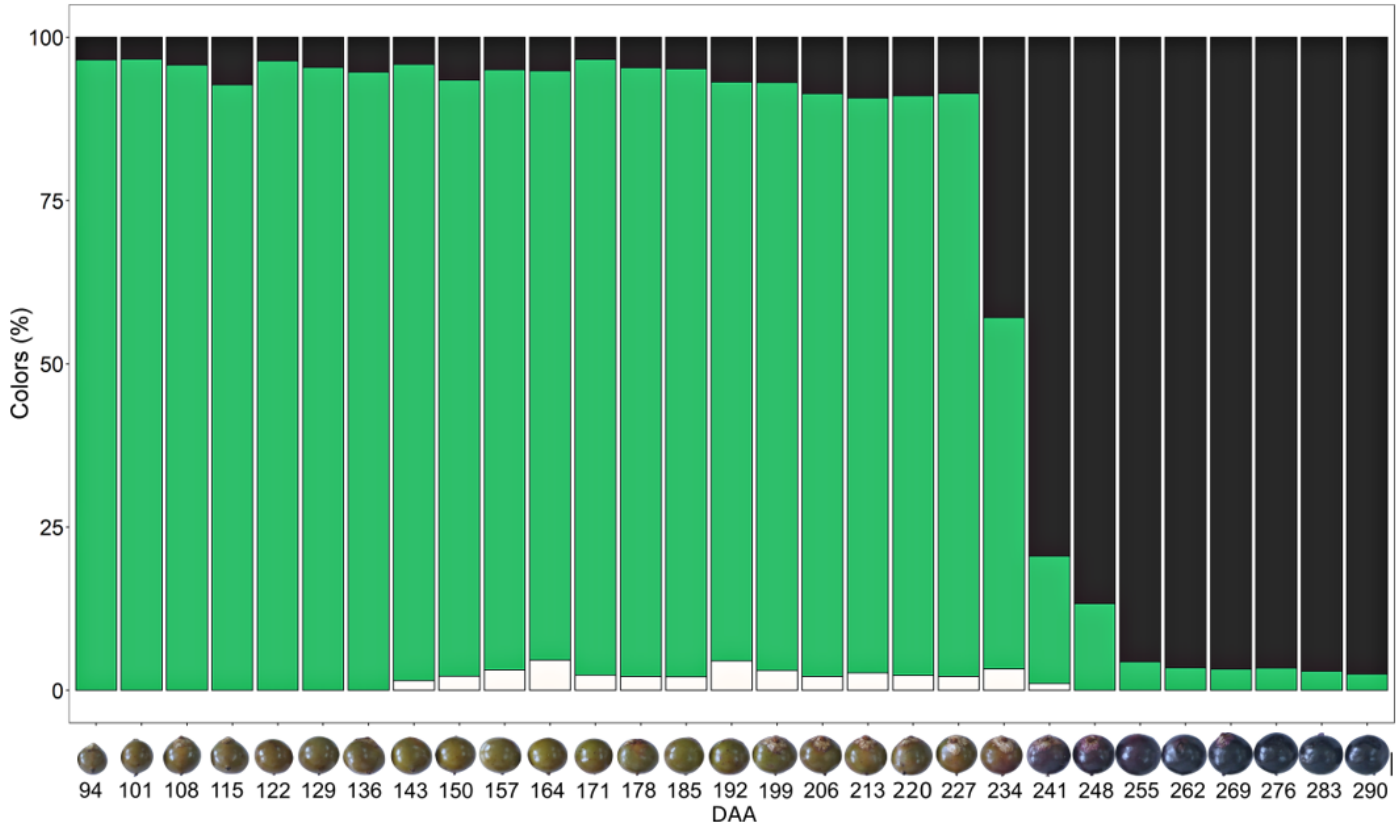

## Figure 2

Illustration of surface layers in *E. edulis* fruits. (a) Pericarp layers: epicarp, mesocarp, and endocarp. (b) Section of an immature fruit. Bar: 500 μm.



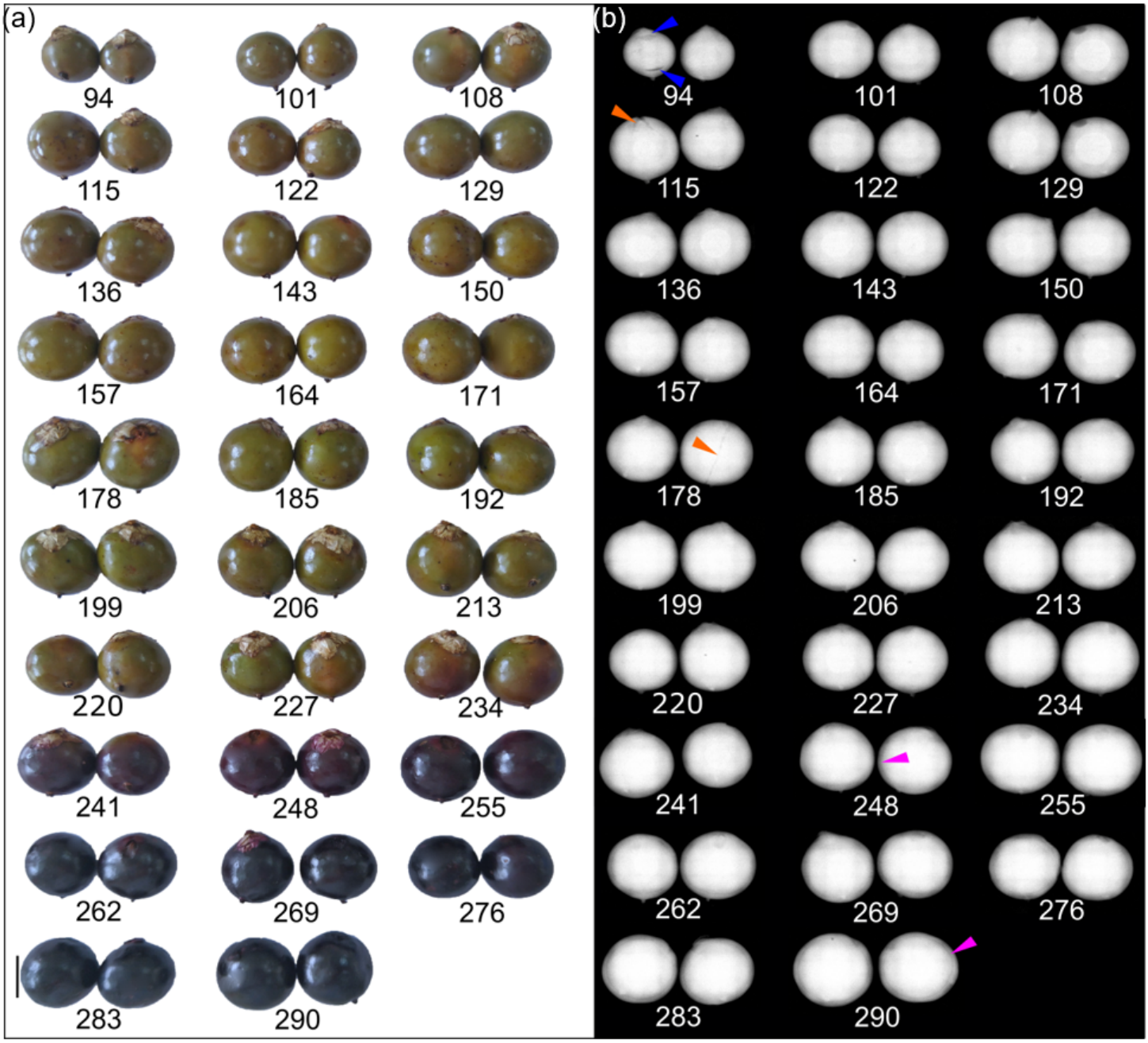

## Figure 3

*E. edulis* seed parameters during maturation expressed in days after anthesis (DAA). (a) Seed dry mass. (b) Seed moisture. (c) Percentage of germination. (d) Germination speed index. (e) Average germination time. (f) Percentage of normal seedlings. (g) Seedling height. (h) Root length. [1]Means not followed by the same letter differ from each other by the Scott-Knott mean cluster test ($P < 0.05$). Fruit scale bar: 1 cm.



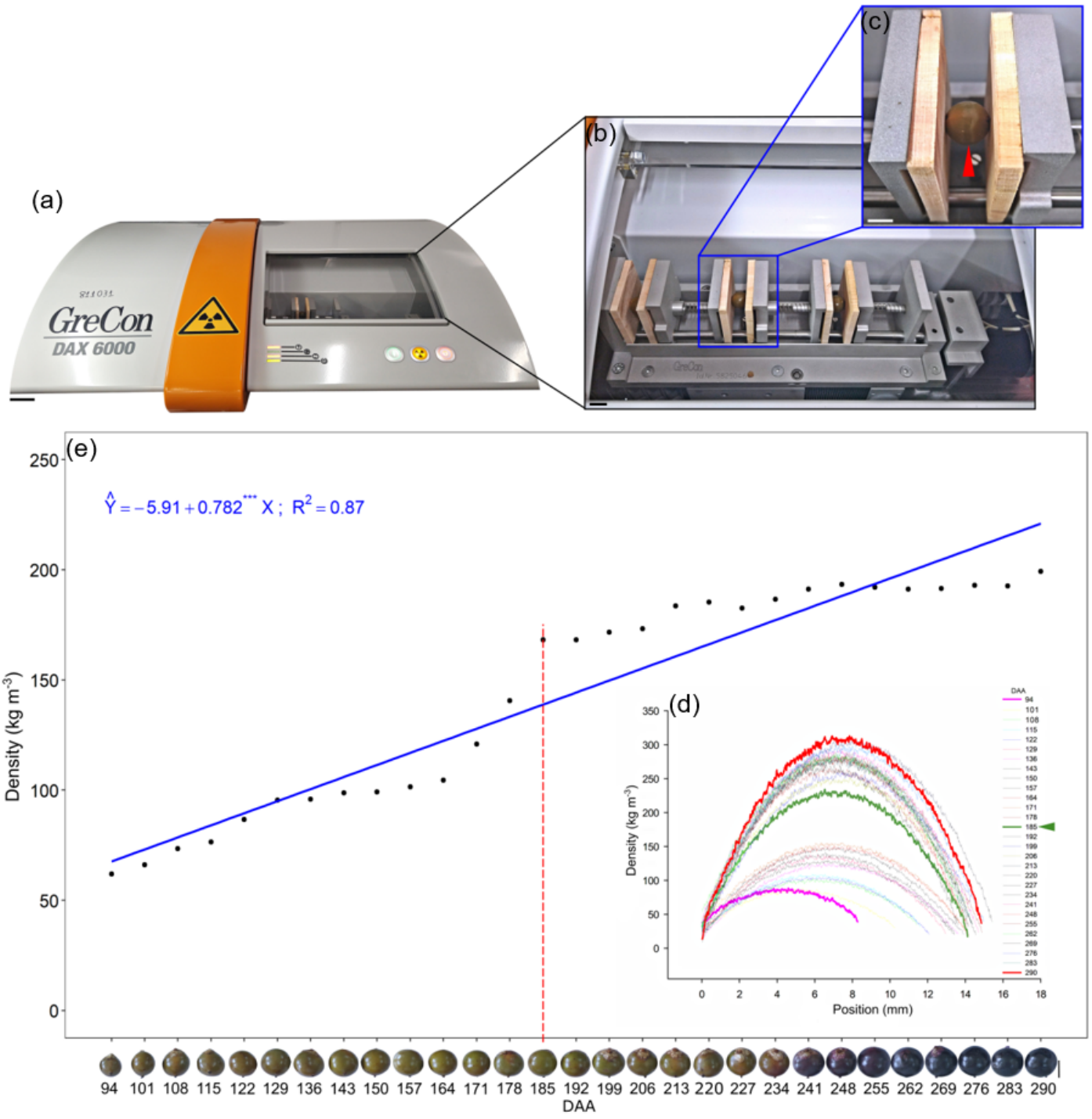

## Figure 4

Average pericarp color of *E. edulis* seeds at each maturation stage expressed in days after anthesis (DAA). Fruit scale bar: 1 cm.



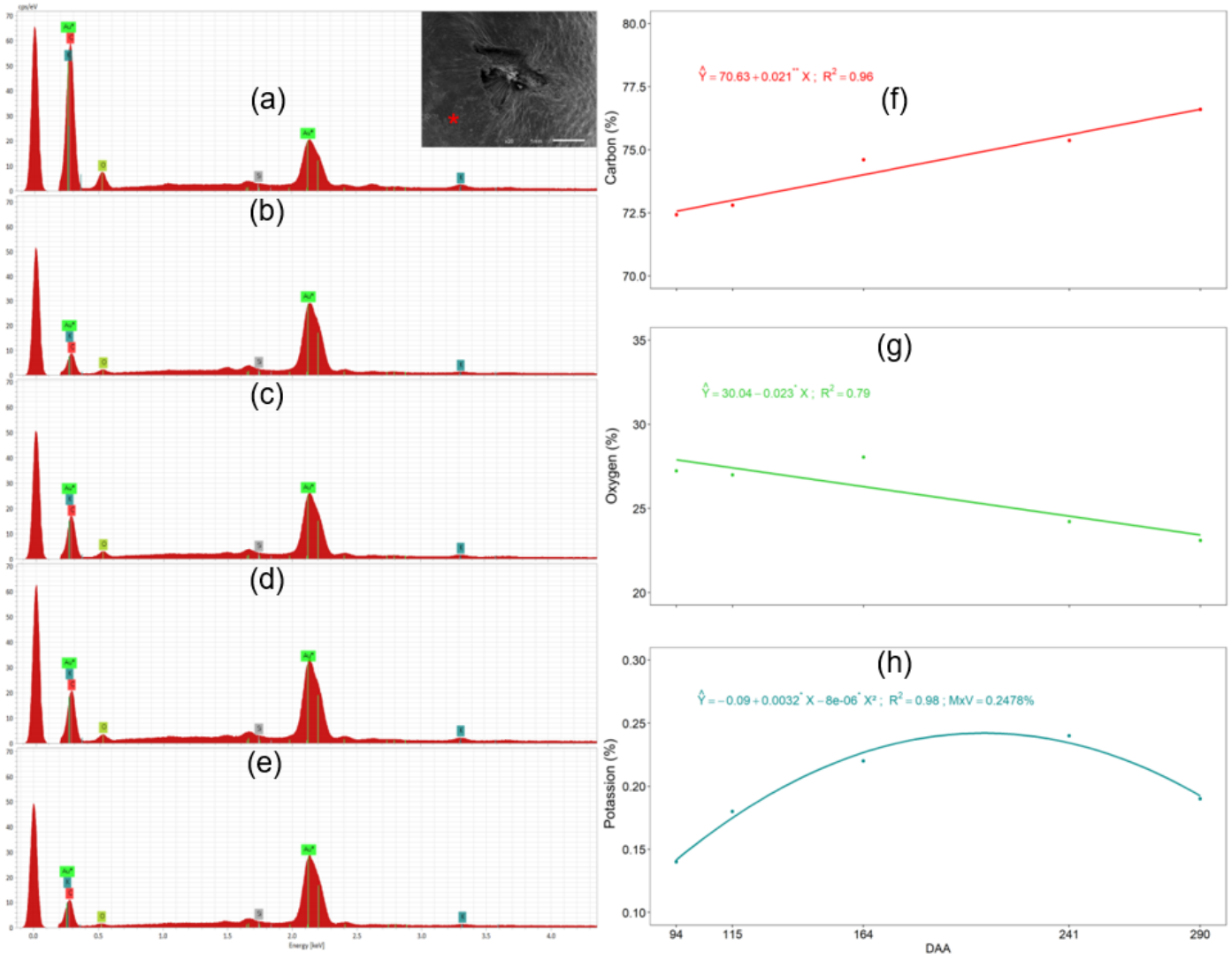

## Figure 5

Morphology of *E. edulis* fruits during maturation at different days after anthesis (94–290). (a) Macroscopic appearance. (b) X-ray images of the seeds in panel *a*, highlighting dehydration damage (blue arrows), mechanical damage to the pericarp (orange arrows), and the natural drop in seed moisture (pink arrows). Bar: 1 cm.



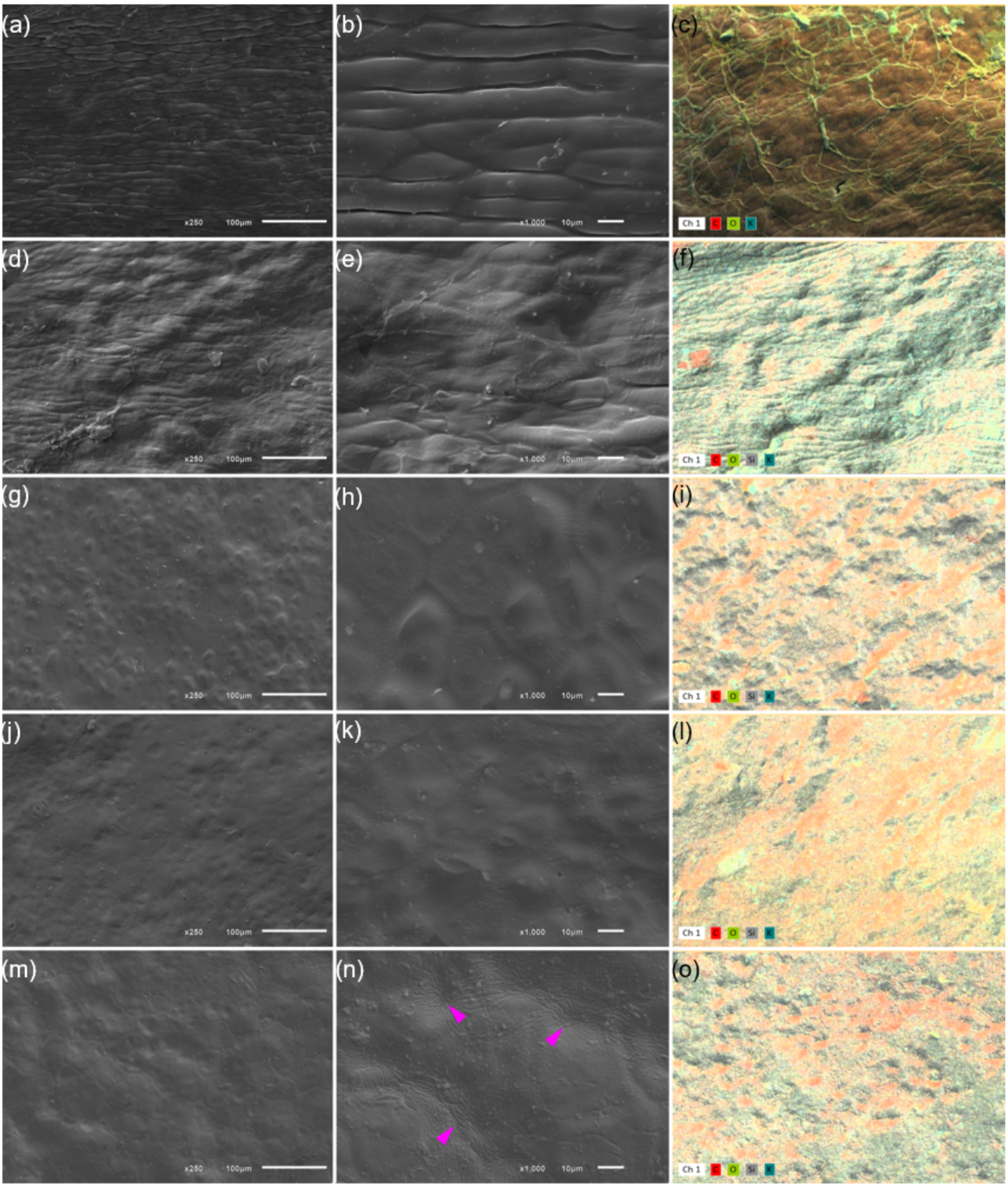

## Figure 6

X-ray densitometry measurements of *E. edulis* seeds. (a) X-ray microdensitometer. (b) Internal sample compartment. (c) Sample holder containing a seed under analysis (red arrow). (d) Graphic representation of the density reading (kg $m^{-3}$) of a seed during maturation from 94 to 290 days after anthesis (DAA). (e) Average seed density throughout maturation. ***P < 0.001 (*t*-test). Bar: 5 cm (a), 1 cm (b, e), 0.5 cm (c).



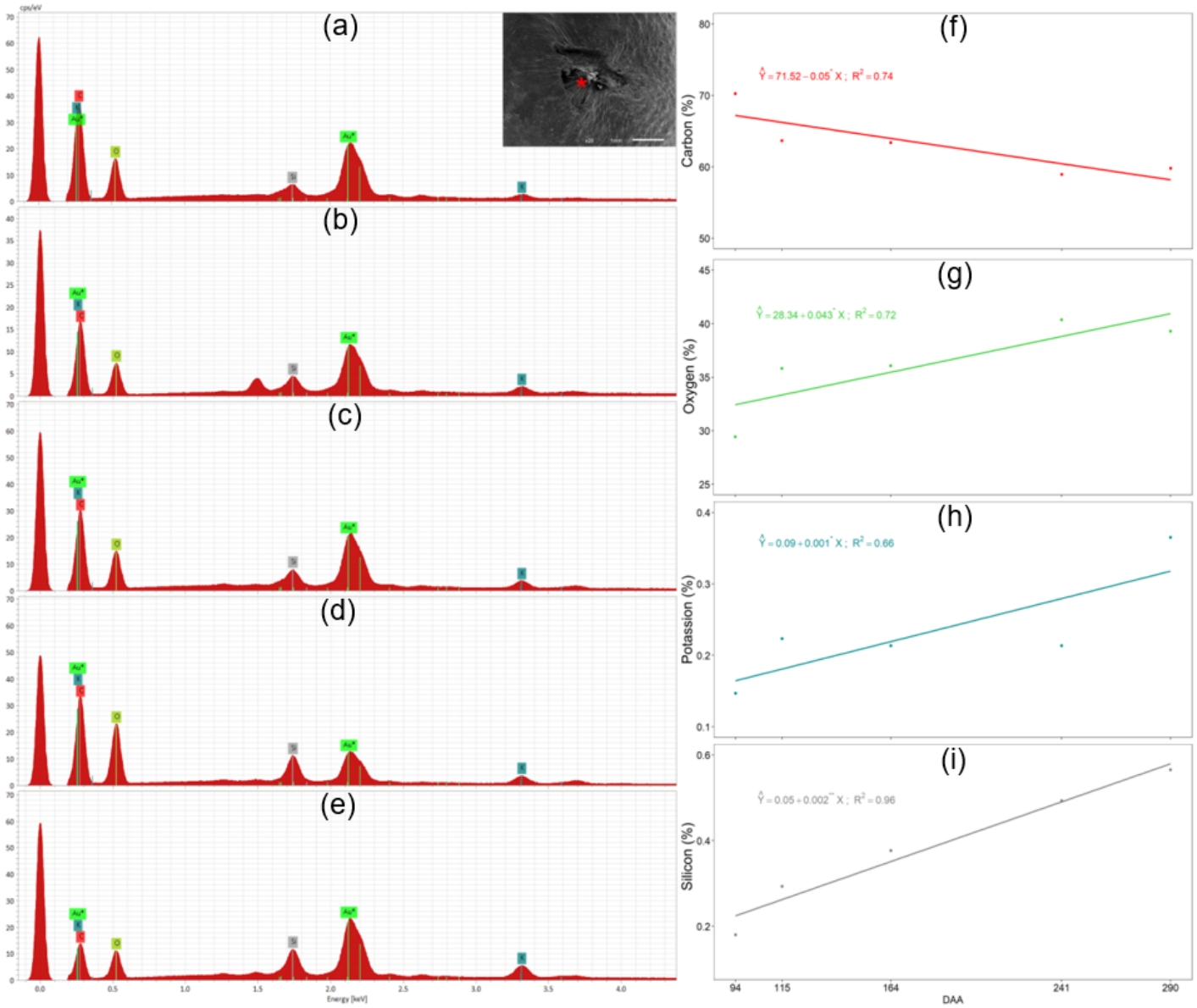

## Figure 7

Chemical composition of the fruits during maturation. (a–e) Microanalysis of the chemical elements present in the epicarp (red asterisk) of *E. edulis*seeds 94 (a), 115 (b), 164 (c), 241 (d), and 290 (e) days after anthesis (DAA). (f–h) Averaged percentage of each element present in the epicarp during maturation: C (f), O (g), and K (h). ***P < 0.01* and **P < 0.05*(*t*-test). MxV, maximum value; Au, sample gold bath.




## Figure 8

SEM/EDS micrograph with map of chemical elements present in the epicarp of *E. edulis* seeds at different stages of maturation: (a–c) 94 days after anthesis (DAA), (d–f) 115 DAA, (g–i) 164 DAA, (j–l) 241 DAA, and (m–o) 290 DAA. Pink arrows denote pulp dehydration points (n).




## Figure 9

Chemical composition of the fruits during maturation. (a–e) Microanalysis of chemical elements present in the mesocarp and endocarp (red asterisk) of *E. edulis* seeds 94 (a), 115 (b), 164 (c), 241 (d), and 290 (e) days after anthesis (DAA). (f–i) Averaged percentage of each element present in the mesocarp and endocarp during maturation: C (f), O (g), K (h), and Si (i). ***P < 0.01* and **P < 0.05* (*t*-test). Au, sample gold bath.

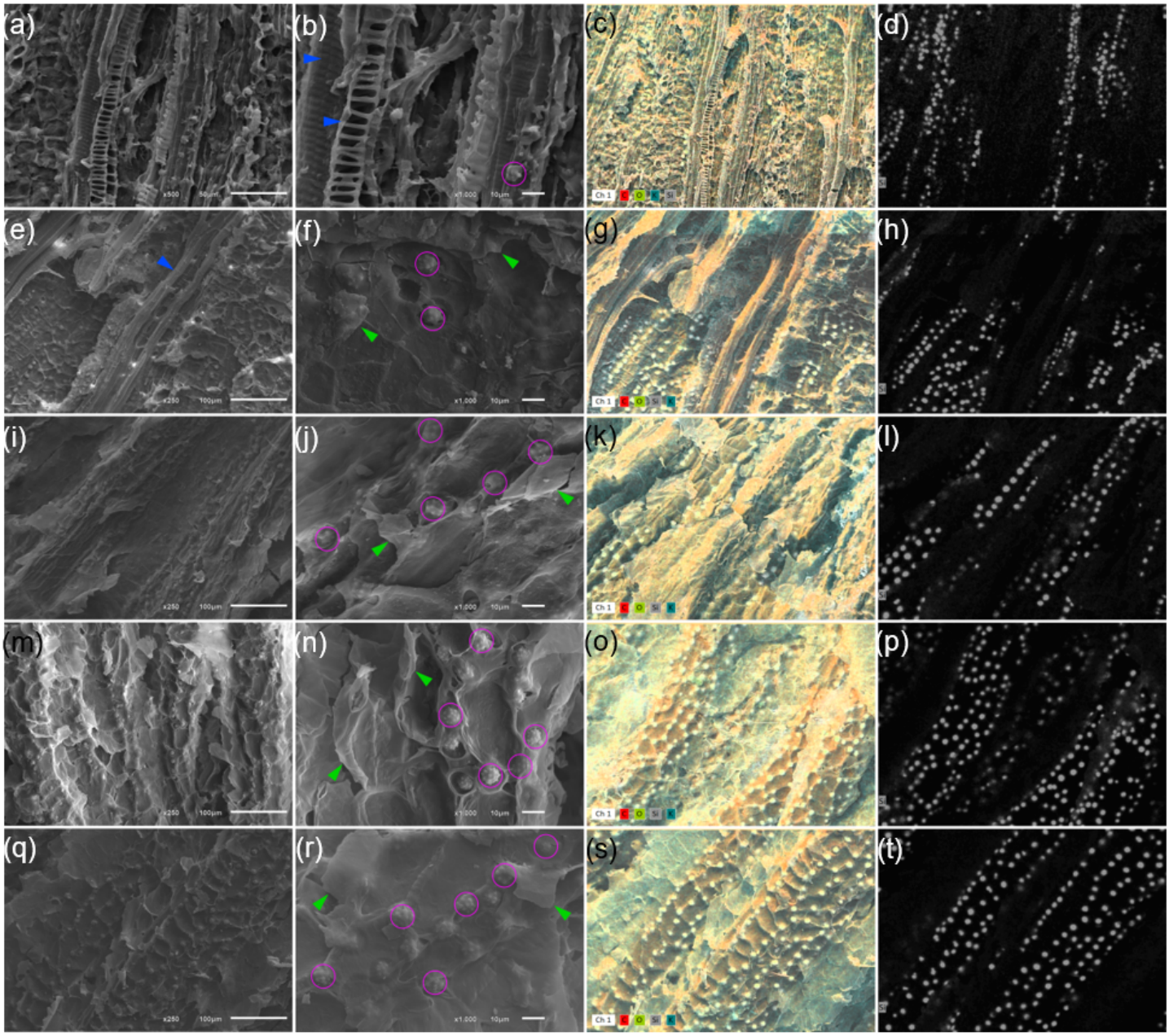



**Figure 10**

SEM/EDS micrograph with map of chemical elements present in the mesocarp and endocarp of *E. edulis* seeds at different stages of maturation: (a–d) 94 days after anthesis (DAA), (e–h) 115 DAA, (i–l) 164 DAA, (m–p) 241 DAA, and (q–t) 291 DAA. Blue arrows denote fibers from the endocarp (b, e), green arrows denote mesocarp laminae (f, j, n, r), pink circles denote Si deposition in SEM images (b, f, j, n, r), and grey dots correspond to Si deposition in EDS images (d, h, l, p, t).

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- floatimage1.png