

Short Communication

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First evidence of polyembryony in black mangrove *Avicennia germinans*

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Abstract: We provide the first documented case of the black mangrove, *Avicennia germinans*, producing multiple seedlings from a single propagule, a phenomenon called polyembryony, on several occasions. There is a lack of knowledge about polyembryony in mangroves, as it is an extremely rare phenomenon previously observed in only three other mangrove species. A higher weight of mature propagules may indicate polyembryony, thus enabling early detection. *A. germinans* may make use of mixed reproductive strategies to ensure the continued survival of the species, or to increase its expansion under favourable environmental conditions. Potential causes and implications of polyembryony in *A. germinans* are discussed.

Keywords: *Avicennia*; mangrove; polyembryony; propagule; seedling.

The black mangrove (*Avicennia germinans* (L.) L., angiosperm, Acanthaceae) is a tropical mangrove species native to the American continent and West Africa (Dodd et al. 2002) that grows into a tree or shrub and reproduces by cryptovivipary (Alleman and Hester 2011). As a common reproductive strategy in mangroves, *A. germinans* produces propagules that, once detached from the parent plant, are aquatically dispersed until they take root in

sediment (Alleman and Hester 2011). Once established, propagules develop into saplings, as shown in Figure 1.

Polyembryony in plants is the result of multiple sexually or asexually produced embryos in one seed, resulting in twin (or multiple) seedlings (Batygina and Vinogradova 2007). We collected propagules from a captive mangrove tree from the mangrove ecodisplay at Royal Burgers' Zoo Arnhem, The Netherlands in two consecutive years, 2020 and 2021. Polyembryony was observed in one out of 46 propagules in 2021 (July 28) and 1 out of 15 propagules in 2020 (November 27). Polyembryos were first recognised by the identification of two hypocotyls, one from each seedling, and two independent pairs of cotyledons (Figure 2).

The parent *A. germinans* tree of our polyembryonic propagules was collected in Cuba as a sapling, and grew into an adult tree in captivity. At Royal Burger's Zoo, the parent tree grows alongside another *A. germinans* individual blooming simultaneously, therefore our polyembryonic propagules could have resulted from either cross- or self-pollination. Resembling standard habitat conditions, humidity levels range between 60 and 85%, and water and air temperature are kept constant at 25 °C all year round. Water salinity in the mangrove semi-diurnal tidal basin is 33. Nutrient analyses in the mangrove basin indicate a concentration of 0.018 mg l⁻¹ of phosphate and 0.46 mg l⁻¹ of nitrate in seawater.

The visual appearance of the 2021 polyembryonic, dry mature propagule before sprouting did not differ from the rest in any morphological parameter (Figure 2A). However, the polyembryonic propagule's initial weight on the day of collection was 2.2 g, 27.5% higher than the average of propagules in a similar development stage (1.7 g, $n = 46$). This suggests that a higher weight of mature propagules could indicate the presence of multiple embryos, which may enable early detection of polyembryony. Propagules were grown in a greenhouse on waterlogged quartz sand, at 26 °C, salinity of 20, and under a 12-h light regime of 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. All collected propagules sprouted after 48 h from the time of collection. After five days, hypocotyls were visible and the pericarp had decayed, exposing the cotyledons. In the case of

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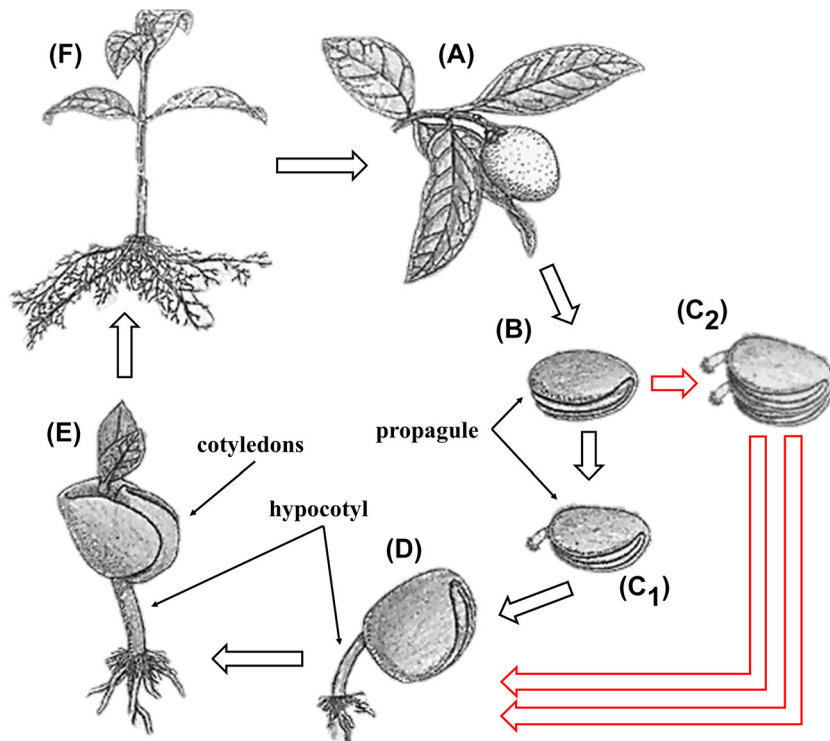


Figure 1: Reproductive cycle of *Avicennia germinans*. Propagules are produced by cryptovivipary thus remaining attached to the parent plant until germination (A and B). Propagules emerge by first developing an hypocotyl (C) and then roots (D). Epicotyl and first leaves emerge from the cotyledons (E), and subsequently the seedling develops into a sapling (F). Red arrows indicate deviations from the standard cycle as a result of polyembryony. Visible differences are the presence of two hypocotyls and a thicker, heavier mature propagule. Adapted from *The mangrove ecosystem—adaptations* (available at: <https://themangroveecosystem.weebly.com/adaptations.html> (Accessed: 8 September 2021)).

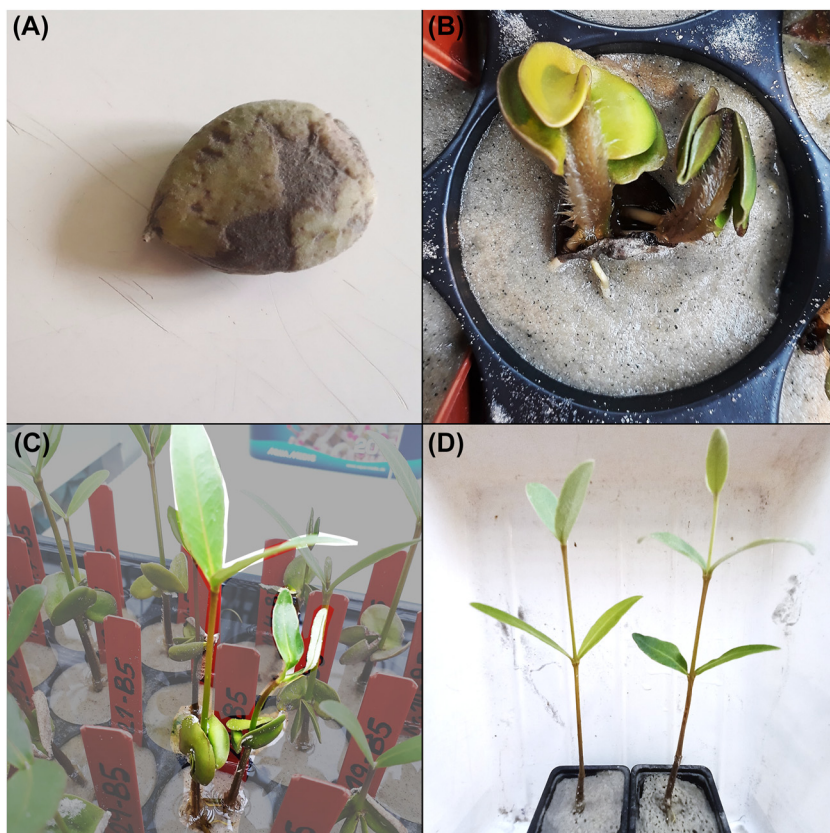


Figure 2: *Avicennia germinans*: polyembryonic, mature propagule (A) and different developmental stages of seedlings at (B) 5 days, (C) 6 weeks, and (D) 19 weeks after germination.

our polyembryonic propagule, each seedling had their own pair of cotyledons and separate, independent hypocotyls from which more roots developed (Figure 2B). These four cotyledons presented an abnormal asymmetrical shape, whereas in normal seedlings it is usually symmetrical. This deviation is due to a limited amount of space under the seed coat that, under normal conditions, only accommodates one pair of cotyledons. This deformity was particularly visible in one of the seedlings (Figure 2C, right). One of the polyembryonic seedlings showed a delayed growth as no epicotyl or leaves had developed from the cotyledons as opposed to its polyembryonic counterpart. Six weeks after germination, an abnormal growth of the epicotyl and deformed leaves became evident in only one of the seedlings (Figure 2C, right). Over time, the abnormally-shaped seedling overgrew its original deformities and developed into a mangrove sapling (Figure 2D).

Polyembryony is an extremely rare phenomenon in mangroves that has only been observed in a few previous occasions and only three other mangrove species. Bowman (1917) reported the observation of polyembryony in *Rhizophora mangle* by other authors in the West Indies and Fiji and mentioned the existence of a heredity factor in certain trees of those regions to develop polyembryony. This phenomenon has also been reported in *Rhizophora mucronata* (Kumar and Joshi 1942) and *Lumnitzera racemosa* (Lakshmanan and Narmatha Bai 1986). To our knowledge, there are no published reports of polyembryony in the mangrove species *A. germinans*.

The genotype of our polyembryonic seedlings of *A. germinans* is unknown. In almond seeds, a slow initial growth and poor development was observed in non-diploid organisms (Martínez-Gómez and Gradziel 2003), however there could be several other causes for the stunting of mangrove seedlings. The fact that our observation corresponds to a propagule produced in captivity raises the question whether the production of polyembryos was due to chance or induced by the more favourable growing conditions provided in the zoo. In citrus fruits, the frequency of polyembryony was found to be increased by environmental factors such as plant nutrition, air temperature, environmental and soil humidity, and wind speed (Andrade-Rodríguez et al. 2005). We cannot completely rule out the possibility that these cases of polyembryony simply resulted from the anomalous development of the original cells. However, our multi-year observations indicate that polyembryony in *A. germinans* could be a genetically regulated character resulting in a deviation from the normal reproduction process. It cannot be determined whether this

deviation is an artifact of captivity, but the fact that polyembryony has previously been observed in several natural mangrove communities (Bowman 1917; Kumar and Joshi 1942; Lakshmanan and Narmatha Bai 1986) provides evidence against the artifact hypothesis. In other words, *A. germinans* may make use of mixed reproductive strategies to ensure the continued survival of the species, or to increase its expansion under favourable environmental conditions.

The type of polyembryony determines the genetic diversity of the seeds (Batygina and Vinogradova 2007; Michel et al. 2017). Lower genetic diversity can, in turn, determine the vulnerability of an ecological population to disturbance (Wernberg et al. 2018). Ample environmental tolerance boundaries in highly genetically diverse populations of mangroves could possibly provide an additional source of ecological resilience against the impacts of climate change. Among these impacts, increased evaporation and salinity, water temperature and sea level rise are determining factors for the survival of mangroves (Ward et al. 2016). Therefore, polyembryony could play an important role in the adaptive capacity of *A. germinans* to new, rapidly changing environmental conditions.

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