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СООБЩЕНИЯ

SEED DORMANCY AND GERMINATION BIOLOGY IN *DIOSCOREA VILLOSA* AND *DIOSCOREA DELTOIDEA* (DIOSCOREACEAE)

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The research focused on clarifying the phenomenon of seed dormancy and the analyzing the relationships between seed dormancy and germination in two species of *Dioscorea* L., specifically *D. deltoidea* and *D. villosa*. The experiment employed various regimes of stratification: a constant 15°C and 22°C; 10°C → 22°C; 2°C → 22°C. Monitoring of embryo growth indicated that post-development in both species occurred prior to the start of germination. Heterogeneity of seeds with respect to type and depth of dormancy was revealed, with *D. villosa* exhibiting pronounced heterogeneity; nearly 40% of the seeds displayed morphological dormancy, while the remainder had morpho-physiological dormancy. The majority of *D. deltoidea* seeds exhibited morphological dormancy, with a minor proportion (4–5%) demonstrating morpho-physiological dormancy. The reliance of germination on higher temperatures in *D. deltoidea* led to a predominance of cotyledon growth within the seed during germination, whereas the reliance on lower temperatures in *D. villosa* resulted in the preeminence of root growth, subsequently facilitating earlier development of the root system.

Keywords: *Dioscorea deltoidea* Wall. ex Griseb., *Dioscorea villosa* L., seed dormancy, embryo post-development, germination pattern, seedling morphogenesis

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The challenge of achieving successful germination, attributable to the phenomenon of seed dormancy, is crucial for the conservation and sexual propagation of rare and economically significant plant species, including those within the genus *Dioscorea* L. The tropical tuberous species of this genus, commonly known as yams, serve as an important food source in many regions worldwide. Additionally, rhizomatous species in subtropical and temperate climates hold considerable economic value due to their production of steroidal and phenolic compounds, which exhibit various biological effects, including antioxidant activity, antitumor, antimutagenic, and antibacterial properties (Asiedu, Sartie, 2010; Shah, Lele, 2012; Price et al., 2016; Padhan, Panda, 2020; Bano et al., 2021, etc.).

The examination of diverse aspects of reproductive biology in rhizomatous species has revealed significant discrepancies among them regarding various attributes of their reproductive organs (Burkill, 1960; Chung, Chung, 2015; Vinogradova et al., 2022, et al.). Furthermore, the analysis of seed dormancy types was enhanced through experiments investigating seed germination under different temperature regimes (Gerasimenko, Tropova, 1966; Okagami, 1986; Terui, Okagami, 1993; Titova, Torshilova, 2013; Langhu, Deb, 2014; Bano et al., 2021, etc.).

The examination of the seeds of *Dioscorea* species has unveiled a spectrum of seed dormancy types, as categorized by M.G. Nikolaeva (1977) or the kinds (classes), as categorized by J.M. Baskin and C.C. Baskin (2004, 2021) including the morphological dormancy (MD), characterized by the presence of underdeveloped embryos within mature seeds, as well as the physiological dormancy (PD) and the morpho-physiological dormancy (MPD) (Pozdova et al., 2005; Albrecht, McCarthy, 2006; Titova, Torshilova, 2013). However, other authors (Okagami, Kawai, 1982; Terui, Okagami, 1993), who studied *Dioscorea* species, excluded the presence of morphological dormancy in them not considering the degree of embryo development in mature seed and its growth during post-development. They proposed a criterion for evaluating the type of seed dormancy based on the seeds' response to cold stratification and chilling, although their experiments revealed that cold positively influenced *Dioscorea* species exhibiting various types of seed dormancy, extending beyond just those classified with MPD and PD to include those with MD.

It should be noted that the presence of a morphological type of seed dormancy can only be determined by monitoring the embryo growth within the seed, and its presence has been confirmed only in studies on *D. nipponica*, in which the length of the embryo was measured at certain intervals.

Investigations of the seed dormancy in *Dioscorea* species, alongside other plant taxa exhibiting this phenomenon, have typically culminated in the mere documentation of seed germination, neglecting the subsequent development of seedlings. Noteworthy, the studies have addressed these processes within the context of *Dioscorea*, although they did so without establishing connections to patterns of seed dormancy and germination (Smith, 1916; Sharma, 1976; Zhong et al., 2002).

In our previous studies on the seed dormancy in the species of Ranunculaceae family (Butuzova et al., 1997; Butuzova, 2018), we uncovered distinct characteristics of embryogenesis and their interrelation with the nuances of seed formation both prior to and following dissemination. These features were found to depend on the type of seed dormancy exhibited. Consequently, in our current investigation, we have chosen to extend our focus beyond dissemination to explore the processes involved in seedling establishment. We propose that there exists a close relatioship between the processes occurring within the seed post-shedding, during germination, and throughout seedling development, all of which are linked to the specific variety of seed dormancy manifested in *Dioscorea* species. Such comprehensive investigations have yet to be undertaken.

In the present study, two contrasting *Dioscorea* species were selected, distinguished by the types of seed dormancy: *D. villosa* L., prevalent across the eastern region of North America and presumably exhibiting morpho-physiological dormancy (Terui, Okagami, 1993), and *D. deltoidea* Wall. ex Griseb., which thrives in the subtropical foothills of the Himalayas and is thought to manifest morphological dormancy (Gerasimenko, Tropova, 1966).

The objectives of this investigation were threefold: to ascertain the presence of embryo post-development following dissemination, to elucidate the type of seed dormancy, and to examine the intricacies of seed germination and seedling formation during their early stages, thereby uncovering the correlations between these processes and the type of seed dormancy.

MATERIALS AND METHODS

Seeds of *Dioscorea deltoidea* and *D. villosa* were collected at maturity from 11- to 12-year-old plants introduced into the outdoor collection at the Botanical Garden of the Komarov Botanical Institute of the Russian Academy of Sciences (Saint Petersburg, Russia). The experiments utilized seeds from both species, freshly collected in 2023 and those from 2022, which had been stored for one year at 2°C.

The seeds were soaked in water on filter paper in Petri dishes for 24 hours at room temperature (20 ± ± 2°C) and subsequently subjected to various regimes in the dark. The experiment was designed as a completely randomized design (CRD) with three replications for each treatment.

To examine the effects of different temperatures on embryo post-development and seed germination, the following regimes were employed: for *D. deltoidea*: 1) constant 22°C, 2) 10 days at 10°C followed by transfer to 22 °C, 3) 10 days at 2°C followed by transfer to 22°C; for *D. villosa*: 1) constant 15°C (only for seeds from 2022), 2) constant 22°C, 3) 30 days at 10°C followed by transfer to 22°C, 4) 30 days at 2°C followed by transfer to 22°C. The duration of pre-treatment was determined based on the anticipated type of seed dormancy for each species. Due to a poor harvest year for *D. deltoidea* in 2023, only a single treatment (10 days at 10 °C followed by transfer to 22°C) was conducted.

Each experimental treatment comprised 60 seeds distributed across three Petri dishes. Germination was meticulously monitored over a span of 40 days for *D. deltoidea* and 60 days for *D. villosa*, continuing until the germination percentages attained their peak values.

Upon the commencement of germination, the Petri dishes housing the seeds were exposed to light. The seeds, embryos, and seedlings were systematically examined every 2 to 3 days utilizing a Stemy 2010 Zeiss stereoscopic microscope in conjunction with the Image-Pro Insight 8.0 software package.

Data concerning embryo post-development, seed germination, and seedling formation were consistently collected. Each embryo was measured every two days for a duration of 40 days following its extraction from the seed.

Germination was recorded when the tip of the radicle emerged through the seed coat, while germination energy was noted on the fourth day subsequent to the start of germination. The calculation of germination energy and the final percentage for each treatment was based on the cumulative sum of seeds from the three dishes, and the data underwent regular statistical processing.

RESULTS

Embryo post-development

Upon seed dispersal from the maternal plant, the embryo of *Dioscorea deltoidea* and *D. villosa* exhibited differentiation, characterized by a broad, flat cotyledon with a petiole, a shoot apex with a leaf organ, and a short hypocotyl-root axis. The embryo length of *D. deltoidea* was measured at 1.4 ± 0.07 mm, which is 4.5–5 times shorter than that of the endosperm, registering 6.1 ± 0.52 mm along the embryo's axis. In contrast, *D. villosa* presented an embryo length of 1.2 ± 0.03 mm, while its endosperm measured 4.3 ± 0.10 mm, rendering the embryo 3.5–4 times smaller (Fig. 1, a; 2, a).

In the initial 6–8 days following stratification, no alterations in embryo size were observed in either

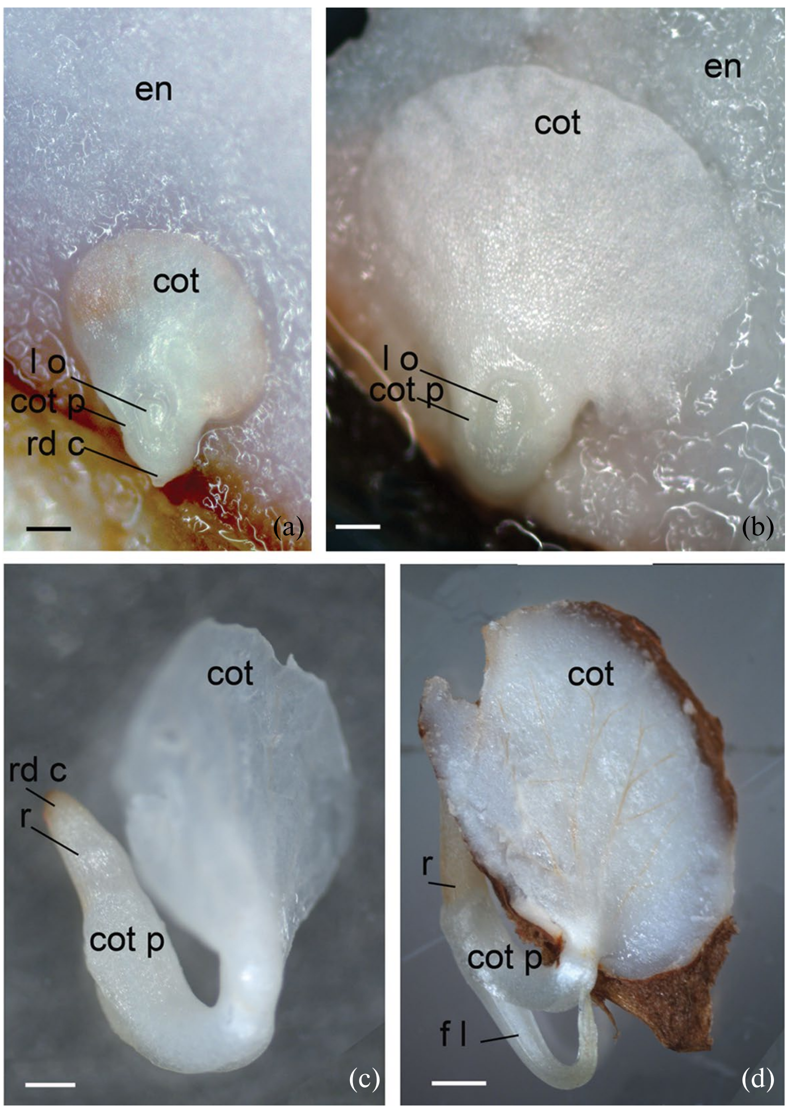

Fig. 1. The structure of embryo in *Dioscorea deltoidea* during post-development and germination: a – embryo structure at dissemination and germination, b – embryo structure at post-development completing, c, d – the structure of seedling on the 11th and 13th days of experiment, respectively; cot – cotyledon, cot p – cotyledon petiole, en – endosperm, rd – radicle, f l – first leaf, l o – leaf organ, r – root, rd c – radicle cap.

Scale: a, b – 0.2, c – 0.5, d – 1 mm.

Рис. 1. Строение зародыша у *Dioscorea deltoidea* в ходе доразвития: а – строение зародыша на момент диссеминации, b – строение зародыша на момент окончания доразвития, c, d – строение проростка на 11 и 13 дни эксперимента соответственно; cot – семядоля, cot p – черешок семядоли, en – эндосперм, f l – первый лист, l o – листовой орган, r – корень, rd c – корневой чехлик.

Шкала: a, b – 0.2, c – 0.5, d – 1 мм.

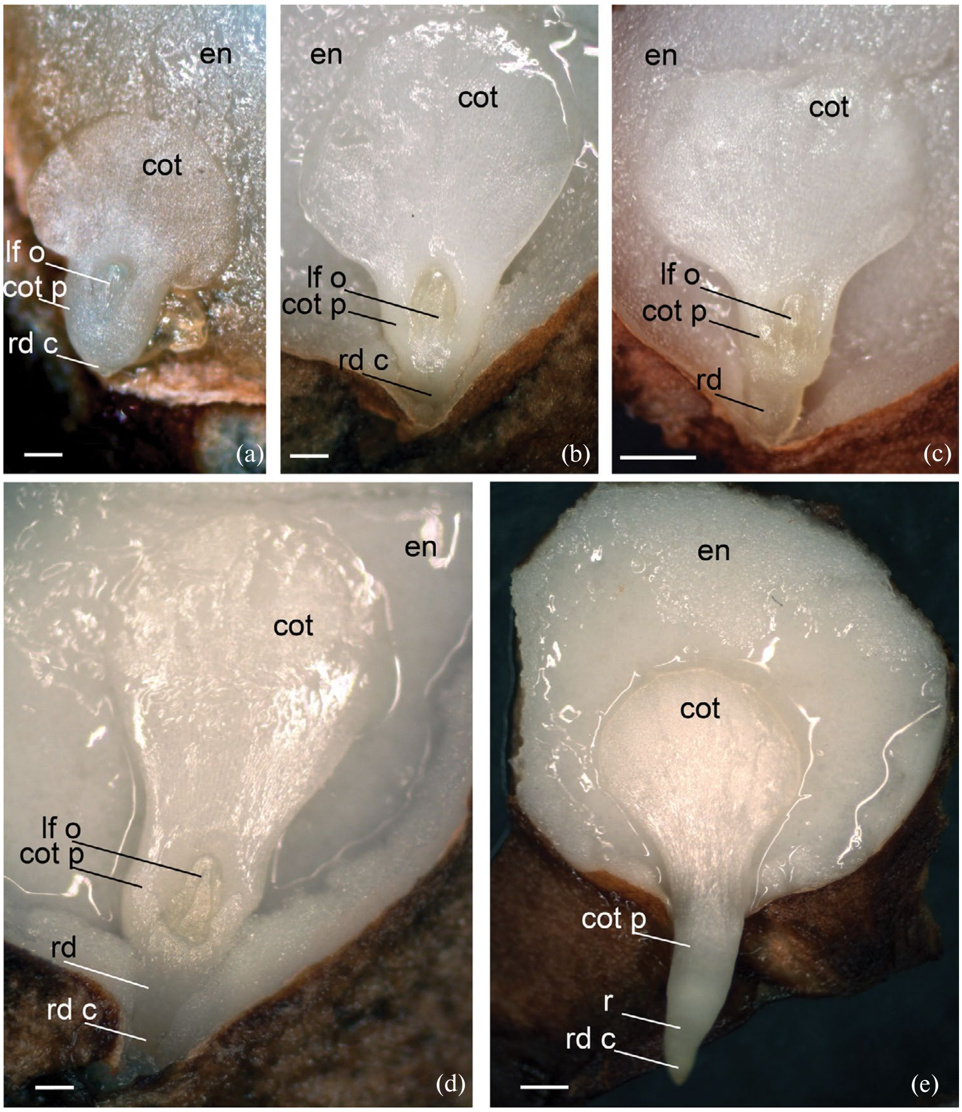

Fig. 2. The structure of embryo in *Dioscorea villosa* during post-development: a – embryo structure at dissemination, b – embryo structure at post-development completing on the 11th day, c, d – the structure of embryo before and at the moment of germination on the 13th day, e – a section of germinated seed on the 14th day of experiment; cot – cotyledon, cot p – cotyledon petiole, rd – radicle, en – endosperm, lf o – first leaf, r – root, l o – leaf organ, rd c – radicle cap.

Scale: a, b, d – 0.2, c, e – 0.5 mm.

Рис. 2. Строение зародыша у *Dioscorea villosa* в ходе доразвития: a – строение зародыша на момент диссеминации, b – строение зародыша на 11 день, c, d – строение зародыша до и на момент прорастания на 13 день, e – срез прорастающего семпени на 14 день эксперимента; cot – семядоля, cot p – черешок семядоли, en – эндосперм, lf o – листовой орган, r – корень, rd – зародышевый корень, rd c – чехлик запродышевого корня.

Шкала: a, b, d – 0.2, c, e – 0.5 mm.

species upon their extraction from the seeds. However, post 8 days of stratification at 22°C for *D. deltoidea* and 10 days for *D. villosa*, a notable enhancement in embryo development was recorded, primarily attributed to the growth of the cotyledon. No changes in the embryos were noted under alternative temperature conditions.

On the 10th day of stratification, the embryo length in *D. deltoidea* increased to 2.2 ± 0.10 mm, reflecting a growth of 1.5–1.7 times (Fig. 1, b). By the 12th day, the length of the *D. villosa* embryo had more than doubled, reaching 2.5 ± 0.19 mm (Fig. 2, b). During post-development in both species, the differentiation of embryo organs, excluding the radicle, coincided with an increase in embryo size. This process resulted in the branching of cotyledon vascular bundles and a heightened differentiation of the leaf organ primordium.

Germination of seeds

As the radicle emerged from the micropyle, the cotyledon were still growing. By the 11th day in *D. deltoidea*, the cotyledon attained a length of 4.1 ± 0.15 mm and a width of 5 mm. The petiole became distinctly differentiated, the cotyledon plate filled the endosperm cavity, while its edges taking on a thinner and more irregular appearance. The vascular bundles within the cotyledon were well-defined. In *D. villosa*, by the 13th day of germination, the cotyledon also increased in length, albeit to a lesser extent, reaching only 3.3 ± 0.14 mm; its width remained virtually unchanged, and the edges retained a smooth contour (Fig. 1, c, d; Fig. 2, d, e).

The species under study exhibited distinct differences in their germination mechanisms. In *D. deltoidea*, the radicle emerged from the micropyle primarily due to the elongation of the cotyledon petiole, which pushed the radicle and shoot apex outward from the seed; wherein the radicle grew very slowly (Fig. 1, c, d). Furthermore, throughout the germination process, the cotyledon plate continued to expand, occupying the entire volume of the endosperm (Fig. 1, c, d).

Conversely, in *D. villosa*, it was predominantly the radicle that developed; it began to grow within the seed, separating from the hypocotyl (Fig. 2, c). After the radicle successfully passed through the micropyle, emerging from the seed (Fig. 2, d), the petiole of the cotyledon commenced its elongation, exiting the seed alongside the shoot apex (Fig. 2, d, e), herewith the cotyledon plate hardly increased in size.

The seeds of *D. deltoidea* germinated under warm conditions in the dark. The onset of seed germination for the 2022 harvest was observed on the 10th day following seed soaking, with a high germination energy of 83.7%. The final germination percentage at 22°C reached 96.2% by the 26th day (Fig. 3).

The seeds collected in 2023 (solid line) and 2022 year (broken lines) were exposed to constant 22°C for 28 days in the dark and with preliminary stratification at 2 and 10°C for 10 days before transfer to 22°C.

Preliminary stratification of *D. deltoidea* seeds at 10°C for 10 days, followed by a transfer to 22°C, delayed the onset of germination in warm conditions to 18 days, reduced germination energy to 70%, and lowered the final germination percentage to 92.5%.

Pre-stratification at 2°C for 10 days also inhibited the onset of germination upon transfer to 22°C, significantly diminishing germination energy to 30%, while increasing the final percentage of germinated seeds to 100%.

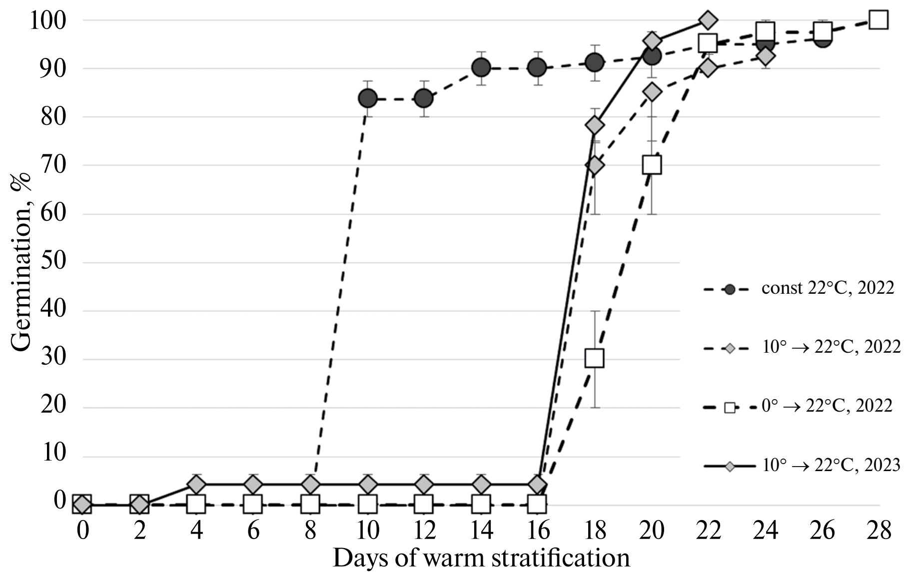

Fig. 3. The effect of temperature and storage on the seed germination in *Dioscorea deltoidea*.

Рис. 3. Влияние температуры и сроков хранения на прорастание семян *Dioscorea deltoidea*.

The experiment with *D. deltoidea* seeds from the 2023 harvest, pre-stratified at 10°C, revealed that fresh seeds germinated significantly better than those stored from 2022. Singular germination was noted as early as the 4th day after the transfer to warm conditions, with mass germination occurring on the 18th day and a germination energy of 78.3%. By the 22nd day, 100% seed germination was recorded (Fig. 3).

Fresh seeds of *D. villosa* harvested in 2023 germinated in the dark at a constant temperature of 22°C on the 12th day post-soaking. The germination energy was recorded at a modest 18%. By the 24th day, the proportion of germinated seeds ascended to 41.6%, with no further germination observed at this temperature (Fig. 4). Seeds that had been stored for a year exhibited even poorer germination performance, initiating the process on the 14th day, with energy remaining at 18% and achieving a final germination rate of only 20%.

The seeds collected in 2023 (solid lines) and 2022 year (broken lines) were exposed to constant 15 and 22°C for 38 days in the dark and also with preliminary stratification at 2 and 10°C for 30 days before transfer to 22°C.

Treatment at 15°C positively influenced the germination of *D. villosa* seeds, although the process was considerably prolonged, spanning 36 days. Germination commenced on the 20th day, with energy surpassing 23% and culminating in a final germination of 87.4% (Fig. 4).

Stratification at 10°C for one month, followed by a transfer to warm conditions, markedly enhanced germination rates in both fresh and stored seeds of *D. villosa* (Fig. 4). Germination was observed as early as the 4th day after the transition to warm, achieving germination energy levels of 71.6% for the 2023 harvest and 40% for the 2022 harvest. Final germination percentages were relatively high, reaching 96.6% for fresh seeds and 93.3% for stored seeds. In this scenario, the germination process required 16 days for fresh seeds and a mere 10 days for those that were stored.

Conversely, pre-stratifying *D. villosa* seeds at 2°C for one month adversely affected their germination capabilities. Germination was delayed, commencing only 10 days after being transferred to warm conditions. The germination energy recorded was 41.6% for fresh seeds and 60% for stored seeds (Fig. 4). The duration of the germination process was extended, tak-

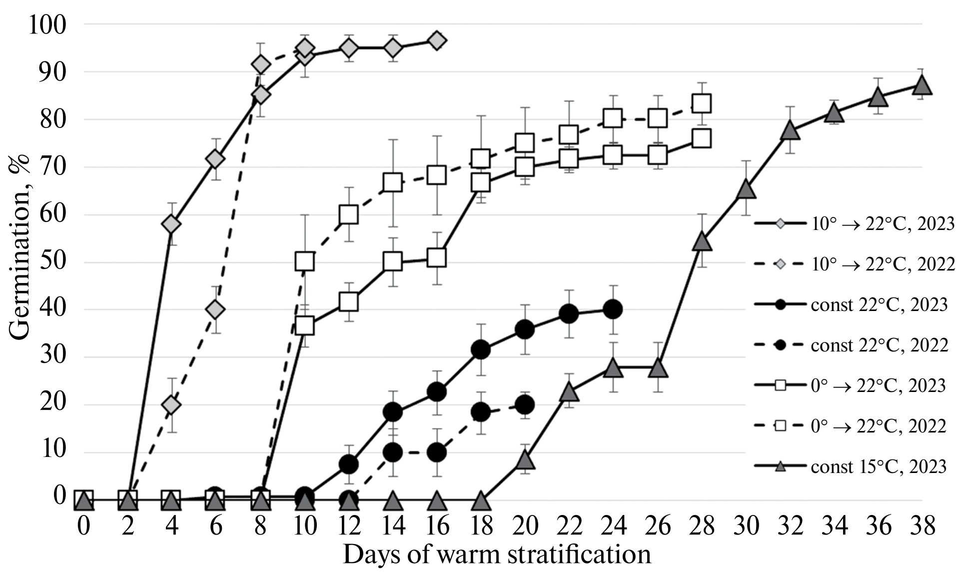

Fig. 4. The effect of temperature and storage on the seed germination in *Dioscorea villosa*. The seeds collected in 2023 (solid lines) and 2022 year (broken lines) were exposed to constant 15 and 22°C for 38 days in the dark and also with preliminary stratification at 2 and 10°C for 30 days before transfer to 22°C.

Рис. 4. Влияние температуры и сроков хранения на прорастание семян *Dioscorea villosa*. Семена, собранные в 2023 г. (сплошные линии) и 2022 г. (пунктирные линии), стратифицированные при постоянных 15 и 22°C в течение 38 дней в темноте, а также с предварительной стратификацией при 2 и 10°C в течение 30 дней перед переносом на 2 в течение 30 дней перед переносом на 22°C.

ing 28 days across both treatments, ultimately yielding germination percentages of 75.8% for the 2023 seeds and 83.3% for those harvested in 2022.

Morphogenesis of seedlings

The morphology of seedlings arising from the seeds of *D. deltoidea* and *D. villosa* showed notable similarities. Germination commenced on the 10th day following the soaking of *D. deltoidea* seeds, while it transpired on the 12th day for *D. villosa*. This hypogeal type of germination initiated with the emergence of the embryo's hypocotyl-root axis from the micropyle, with the cotyledon plate remaining within the seed and serving a haustorial function.

Between the 11th and 13th days from the experiment onset for *D. deltoidea* (Fig. 5, a–c), and from the 13th

Fig. 5. The structure of seedling in *Dioscorea deltoidea*: a – the seed with embryo root at germination beginning, on the 10th day from the experiment starts; b–d – the seed with basal part of the seedling on the 11th, 13th and 15th days from the experiment starts, respectively; e–i – the structure of seedling perts on the 19th, 23rd, 28th, 30 and 35th days from the experiment starts, respectively; ad r – adventive root, cot p – cotyledon petiole, f l – first leaf, h – hypocotyl, fl lsh – first leaf of the lateral shoot, r – root, s – seed, s l – scale leaf.

Scale: a–d, f – 1, e – 2, g–i – 0.5 mm.

Рис. 5. Строение проростка *Dioscorea deltoidea*: a – семя с зародышевым корнем в начале прорастания, на 10 день от начала эксперимента; b–d – семя с базальной частью проростка на 11, 13 и 15 день от начала эксперимента соответственно; e–i – строение частей проростка на 19, 23, 28, 30 и 35 день от начала эксперимента, соответственно; ad r – адвентивный корень, cot p – черешок семядоли, f l – первый лист, h – гипокотиль, fl lsh – первый лист латеральногот побега, r – корень, s – семя, s l – чешуевидный лист.

Шкала: a–d, f – 1, e – 2, g–i – 0.5 mm.

to 15th days for *D. villosa* (Fig. 6, a–c), the development of the shoot apex with the first leaf was observed. The dorsal side of the petiole was protuberant, while the ventral side acquired the appearance of a deep channel. The first leaf began to develop rapidly, with its differentiation into a morphologically distinct plate

Fig. 6. The structure of seedling in *Dioscorea villosa*: a – the seed with embryo root at germination beginning, on the 12th day from the experiment starts in warm; b–d – the seed with the basal part of the seedling on the 13th, 16th, 18th days from the experiment starts in warm, respectively; c–h – the structure of seedling parts on the 21st, 24th, 28th and 35th days from the experiment starts in warm, respectively; i – the seedling on the 35th day from the experiment starts in warm; ab z – absorption zone, ad r – adventive root, cot p – cotyledon petiole, f l – first leaf, h – hypocotyl, fl lsh – first leaf of the lateral shoot, r – root, s – seed, s l – scale leaf.

Scale: a, c, d – 1, b, h – 2, f, g – 0.5, e – 0.2 mm, i – 1 cm.

Рис. 6. Строение проростка Dioscorea villosa: a – семя с зародышевым корнем в начале прорастания, на 12 день от начала эксперимента в тепле; b–d – семя с базальной частью проростка на 13, 16 и 18 день от начала эксперимента в тепле, соответственно; c–h – строение частей проростка на 21, 24, 28 и 35 день от начала экспериментав тепле, соответственно; i – проросток на 35 день от начала эксперимента в тепле; ab z – зона абсорбции, ad r – адвентивный корень, cot p – черешок семядоли, f l – первый лист, h – гипокотиль, fl lsh – первый лист латерального побега, r – корень, s – семя, s l – чешуевидный лист.

Шкала: a, c, d – 1, b, h – 2, f, g – 0.5, e – 0.2 mm, i – 1 cm.

and petiole being distinctly noted on the 15th day in *D. deltoidea* (Fig. 5, d) and on the 18th day for *D. villosa* (Fig. 6, d).

The hypocotyl displayed no discernible changes, while root growth was predominantly observed in *D. villosa*. An absorption zone manifested at the junction with the hypocotyl, characterized by a slender band of short root hairs that expanded as the root elongated (Fig. 6, b). In contrast, root growth in *D. deltoidea* was minimal, devoid of absorption hairs, and progressed at a slower pace than in *D. villosa*. Here, the growth of the seedling's basal part primarily resulted from the elongation of the cotyledon petiole (Fig. 5, b, c). The emergence of an absorption zone with short hairs was observed later, coinciding with the unfolding of the first leaf plate.

During the period from the 15th to the 19th days in *D. deltoidea* and the 18th to the 21st days in *D. villosa*, intensive growth of the petiole of the first leaf was observed, accompanied by the unfolding of its plate. At this stage, along with the main meristem on the shoot apex, the meristem of the lateral shoot is formed (according to Sharma, 1976), from which a primordium of a scale leaf was developed (Fig. 5, e; 6, e). The emergence of lateral roots from the main embryonic root was noted in both species, with *D. villosa* exhibiting the longest ones. Furthermore, a region comprising absorption hairs was observed in *D. deltoidea*.

Between the 19th and 23rd days in *D. deltoidea* and the 21st and 24th days in *D. villosa*, a substantial increase in seedling size was noted. This period was marked not only by vigorous growth the petiole in the first leaf but also the active formation of its green photosynthetic plate. The base of scale leaf thickened considerably, mainly due to the development of rhizome initial. The seedlings of *D. villosa* produced a considerable number of lateral roots, which were significantly longer than those of the *D. deltoidea* seedlings (Fig. 5, f; 6, f).

From the 23rd to the 28th days in *D. deltoidea* and the 24th to the 28th days in *D. villosa*, the formation of the true leaf occurred from the meristem of the lateral shoot, which emerged through a cleft in the apical part of the scale leaf (Fig. 5, g, h; 6, f, g). Similar to the first leaf, this leaf from the lateral shoot continued to grow, and by the 35th day in both species, it manifested as a broad green plate with a lengthy petiole (Fig. 5, i; 6, h, i).

DISCUSSION

The findings regarding temperature regimes for seed germination in the examined *Dioscorea* species largely consistent with existing literature. Nonetheless, we offer an alternative interpretation of certain results.

Upon shedding from the maternal plant, the seeds of both species contained an underdeveloped embryo continued to grow within the seed prior to the onset of germination. Notably, the length of the embryo increased by a factor of 1.7 in *D. deltoidea* and doubled in *D. villosa*, with their morphological differentiation becoming increasingly pronounced.

Monitoring of embryo post-development within the seeds of both species revealed that this process was not incremental, contrary to findings in *D. caucasica* (Titova, Torshilova, 2013). It was only shortly prior to germination—occurring within a mere 2 to 3 days – that a rapid expansion in embryo size was recorded, culminating in the initiation of radicle development.

Indeed, the embryo post-development in *Dioscorea* species appears to be intricately linked with other developmental processes, particularly root dormancy. It seems that embryo growth commences only after the break of root dormancy, suggesting that root development serve as a signal for the initiation of these subsequent processes.

While previous authors investigating seed germination in *D. villosa* have acknowledged the existence of an underdeveloped embryo in mature seeds, they did not closely follow the post-development process in their studies (Smith, 1916; Albrecht et al., 2006). Similar patterns of embryo development have not been documented in other *Dioscorea* species, such as *D. nipponica* and *D. caucasica*, where monitoring data suggest that post-development process is gradual (Gerasimenko, Tropova, 1966; Pozdova et al., 2005; Titova, Torshilova, 2013). The limited data may be due to the methodological challenges in observing embryo growth within the seed.

Consequently, the occurrence of the embryo post-development indicates the presence of morphological dormancy, as defined by the classifications of M.G. Nikolaeva (1977). However, in the classification of J.M. Baskin and C.C. Baskin (2004, 2021), the species exhibiting similar embryo growth characteristics were not mentioned. A comparable nature of embryo post-development, occurring immediately prior to seed germination, has been observed in *Dyckia* *goehringii* of the Bromeliaceae family (Duarte et al., 2009) and in several *Nymphaea* species (Dalziell et al., 2019). Nonetheless, J.M. Baskin and C.C. Baskin contend that embryo growth in these instances constitutes part of the germination process itself, thereby suggesting that such seeds do not exhibit dormancy in any form.

Germination, in its generally accepted definition, refers to the emergence of the radicle from the seed, which was not observed during embryo growth in the species examined. Moreover, a combination of various factors such as the small size of the embryos in mature seeds, the presence of an endosperm cavity facilitating their growth, the occurrence of similar post-development patterns in several other genera, support the suggestion that the growth of the embryo within the seed constitutes its post-development.

Nonetheless, within both *Dioscorea* species analyzed, we observed a clear distinction between the processes of embryo post-development and root growth, with embryonic growth preceding germination. This embryonic growth primarily manifests through an expansion of the cotyledon plate, during which the differentiation of the leaf organ primordium also becomes increasingly pronounced intensifies. The conductive system of both the cotyledon and leaf organ developed, while no significant changes in the hypocotyl-root region were detected.

The seeds of *Dioscorea deltoidea* are presumed to exhibit solely morphological dormancy. However, a comparison of germination at 22°C (97.5%) versus pre-stratification at 2 and 10°C (100%) suggests the potential presence of a small fraction of seeds exhibiting morpho-physiological dormancy. Such heterogeneity in seed dormancy within the same plant has been previously documented in other species, such as *Conium* (Baskin, Baskin, 1990) and *Aristolochia* (Adams et al., 2005).

A notable degree of plasticity in seed dormancy is observed across the other genera. The existence of several levels in depth of complex morphophysiological dormancy within a single seed has been demonstrated in *Sanicula* species (Hawkins et al., 2010). Furthermore, the depth of seed dormancy can vary among plants within the same population and across populations of the same species located in different latitudinal and altitudinal ranges (Wang et al., 2011; Gremer et al., 2020; Klupczyńska, Pawłowski, 2021).

In the majority of *D. villosa* seeds, morphological dormancy is accompanied by physiological dormancy, which can be alleviated through preliminary stratification at 10 and 2°C. However, up to 40% of seeds may exhibit morphological dormancy and germinate in warm conditions without pre-treatment. Prolonged stratification at 2–10°C increased the germination percentage, likely indicating variations in seeds regarding the depth of morpho-physiological dormancy. When stratified at a constant temperature of 15°C, the germination percentage of *D. villosa* seeds was notably high (87%), although the process itself was most advanced. Lower temperatures facilitated germination, suggesting the presence of non-deep dormancy in the majority of seeds.

Thus, the implementation of different germination regimes revealed a greater degree of seed heterogeneity in both the depth and type of seed dormancy for both species. This dormancy heterogeneity likely accounts for the conflicting opinions regarding seed germination of these species found in the literature.

The heterogeneity in seeds dormancy types is believed to provide a reserve, enabling a population to adapt to changing climate conditions and establish a seed bank in the soil (Willis et al., 2014). Our experiment revealed that the heterogeneity in seed dormancy is more pronounced in *D. villosa*, allowing this species to possess a significantly broader distribution area, encompassing various climatic zones.

Mass germination of fresh seeds of *D. deltoidea* in the warm without pre-sowing treatment has been previously documented (Gerasimenko, Tropova, 1966; Tyagi et al., 1973; Kumar, 2017). Preliminary stratification of seeds collected from introduced plants (for a month at 1–8°C) expedited the onset of germination (on the 6th day) and significantly enhanced germination energy (59%), although it did not affect the final germination percentage, which reached 96%. Seeds collected from the species' natural habitats began germination on the 4th day after being transferred from 3–4°C to warm conditions, achieving a germination percentage of 92% (Bano et al., 2021).

The slow germination in warm conditions and the necessity of cold stratification for the germination of *D. villosa* seeds align with our data. Seeds obtained from introduced plants also demonstrated low germination at a constant 23°C (30%), and cold stratification for 5 months increased the germination percentage to almost 100% (Terui, Okagami, 1993). Pre-treatment at 4°C for 50 days resulted in the germination of 71% of seeds after transfer to warm conditions (Gerasimenko, Tropova, 1966). Seeds of *D. villosa* collected from natural habitats failed to germinate in warm conditions, and with long-term cold stratification (12 weeks at 5°C → 4 weeks at 15/6°C → 4 weeks at 20/10°C), germination reached up to 100% (Albrecht, McCarthy,2006). The absence of morphological dormancy in *D. villosa* seeds collected from natural habitats may indicate that the depth and even type of dormancy have changed following their introduction.

Dry storage is known to promote seed germination, especially in species characterized by physiological seed dormancy (Viana, Felippe, 1990; Baskin, Baskin, 2020; Peng et al., 2021). However, in our experience, dry cold storage reduced germination rates across both species in all treatments. It was only after stratification at 2°C that the stored *D. villosa* seeds exhibited a higher germination percentage compared to fresh seeds (83% for stored seeds versus 78% for fresh seeds).

Seeds harvested in different years demonstrated varying germination capabilities in other *Dioscorea* species (Okagami, Kawai, 1982). Further clarification is required to ascertain whether the discrepancies observed in these experiments stemmed from germination loss during storage or the influence of climatic conditions on seed quality during maturation.

We found that the variations in dormancy types among both *Dioscorea* species influenced the germination pattern, particularly regarding root emergence, which is crucial for the subsequent development of the seedling.

In *D. deltoidea*, the initiation of germination was primarily linked to the development of the cotyledon, particularly the vigorous growth of its petiole and plate. The elongating petiole did carry the hypocotyl-root axis out of the seed, while the radicle exhibited slower growth. Throughout the germination process, the cotyledon plate continued to expand, occupying the entirety of the endosperm's volume.

In contrast, germination in *D. villosa* was distinctly associated with the growth of the radicle, which initially developed within the seed before emerging through the micropyle. Subsequently, the cotyledon's petiole elongated, elevating the shoot apex, while the cotyledon plate underwent minimal expansion during germination. This disparity may stem from the fact that cotyledon development generally thrives in warm conditions, whereas the radicle requires cold treatment as a stimulus to initiate growth within the seed.

The development of the seedlings largely aligned with existing literature on *Dioscorea* species (Smith, 1916; Gerasimenko, Tropova, 1966; Sharma, 1976; Hori, Oshima, 1986; Pozdova et al., 2005; Norman et al., 2021).

The germination of *D. villosa* seeds in warmer conditions began 2 to 4 days later than that of *D. deltoidea*; however, seedling development progressed at a slightly faster pace in the latter species. By the 30 to 35-day mark, both species exhibited an equivalent number of leaves, with the initial leaves of the seedlings and lateral shoots differentiating into distinct petioles and broad plates. Furthermore, *D. villosa* seedlings displayed a more advanced development in root system, characterized by the emergence of the absorption zone with root hairs and the early branching of lateral roots, occurring significantly sooner than in *D. deltoidea*.

The observed disparities in the correlation of processes during seedling formation among the studied species can be attributed to the dominance of specific types of seed dormancy and the varying temperature conditions required for its alleviation. Higher temperatures (22°C) effectively eliminate the morphological dormancy predominant in *D. deltoidea*, fostering robust development of the cotyledon and shoot apex under warm conditions. Conversely, lower temperatures (2–15°C) are requisite for the dissipation of the physiological dormancy in *D. villosa*, resulting in vigorous growth of the root system. Such temperature-dependent variations in the development of different organs during seedling formation have been documented in another *Dioscorea* species, *D. tokoro* (Zhong et al., 2002), thereby providing empirical support for our conclusions.

The issue of dormancy within *Dioscorea* species is notably intriguing due to the relict nature of this genus. Its species have evolved during the period, roughly coinciding with the establishment of physiological dormancy in seeds that necessitates cold exposure for its removal (Martínez-Berdeja et al., 2020). It has been suggested that the increased seed germination observed in subtropical *Dioscorea* species following cold exposure is a trait inherited from the glaciation period (Okagami, Kawai, 1982).

The features of embryo post-development in *Dioscorea* species show a close association with the germination process. Our findings indicate a possible synchronization between seedling development and the type of seed dormancy. Consequently, it can be surmised that all processes occurring within the seed post-shedding, at the moment of germination, and during the initial stages of seedling formation are intricately interconnected and influenced by the type of seed dormancy.

In light of the economic importance of *Dioscorea* species, our results could offer significant insights into hybridization strategies. The heterogeneity of seeds concerning their dormancy state, along with the temperature dependencies among various seedling parts and their correlated development, should be carefully considered when integrating new species into cultivation and employing advanced breeding technologies (Qin et al., 2023). At interspecific hybridization within *Dioscorea*, emphasis must be placed on the nature of seed dormancy to produce cultivars that ensure high germination rates and seedling viability.

CONCLUSION

1. In two *Dioscorea* species, the embryo post-development following dissemination did not occur immediately and progressed gradually under favourable conditions, which is typical for species with morphological dormancy (MD); it transpired just prior to the onset of germination, likely after the removal of root dormancy.

2. Heterogeneity of seeds by the type and depth of dormancy was revealed in both species. This was particularly pronounced in *D. villosa*, where a part of the seeds (40%) exhibited MD, the others – morphophysiological dormancy (MPD) of different depths; herewith non-deep MPD was overcome by stratification at 10°C, whereas deeper one was alleviated at 2°C. In *D. deltoidea*, the majority of seeds exhibited MD, with only a small fraction (4–5%) displaying MPD.

3. Seed dormancy deepened with the extension of the period of seed dry storage in *Dioscorea* species. The transition from MD to MPD was also facilitated by exposure to low temperatures during storage.

4. The associations between the type of seed dormancy and the features of germination and further seedling development have been found. The dependence of germination on warm temperatures led to a predominance of cotyledon growth during germination, while reliance on cold temperatures resulted in the dominant growth of the embryo root, subsequently promoting earlier development of the root system.

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ПОКОЙ СЕМЯН И БИОЛОГИЯ ПРОРАСТАНИЯ У *DIOSCOREA VILLOSA* И *DIOSCOREA DELTOIDEA* (DIOSCOREACEAE)

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Зрелые семена многих видов *Dioscorea* L. обладают покоем разной степени выраженности, что значительно затрудняет воспроизводство этих редких и хозяйственно ценных видов растений. Данные по выявлению оптимальных условий проращивания семян у видов диоскореи во многом противоречивы из-за разных точек зрения на наличие доразвития зародыша после диссеминации. На двух видах *Dioscorea*, контрастирующих по типу покоя, *Dioscorea villosa* L. и *D. deltoidea* Wall. ex Griseb, мы поставили цель уточнить тип покоя и установить его связь с прорастанием семени и формированием проростка. В эксперименте использовались разные режимы стратификации: постоянные 15 и 22°C; 10 → 22°C; 2 → 22°C. Измерения длины зародыша производили при его извлечении из семени. Наблюдения показали, что у обоих видов имеет место доразвитие зародыша после опадения семени, однако рост его начинался не сразу, а лишь перед самым прорастанием, вероятно, после снятия покоя корня. У обоих видов выявлена гетерогенность семян по типу и глубине покоя, наиболее выраженная у *D. villosa*, у которой почти 40% семян имели морфологический покой (МП), а остальные – морфофизиологический (МФП). У *D. deltoidea* большинство семян имели МП, лишь небольшая их часть (4–5%) обладала МФП. Выявлено, что с увеличением срока сухого хранения семян этих видов покой становится более глубоким. Возможен также переход от МП к МФП на фоне воздействия низких температур при хранении. Обнаружено, что имеет место корреляция между типом покоя и прорастанием, а также дальнейшим развитием проростка. Зависимость прорастания от повышенных температур ведет к преимущественному росту семядоли при прорастании, а зависимость от холода — к преимущественному развитию зародышевого корня и впоследствии более раннему развитию корневой системы.

Ключевые слова: *Dioscorea deltoidea* Wall. ex Griseb., *Dioscorea villosa* L., покой семян, доразвитие зародыша, специфика прорастания, морфогенез проростка

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