

Surface Plant Opals of *Setaria viridis* Seeds Correlate with Seed Dormancy

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ABSTRACT

Background and Objective: Plant opals, formed by the deposition of soluble silicic acid in plant cells, serve structural and defensive functions in leaves and stems. However, their role on seed surfaces remains unclear. This study investigates the structure and potential function of plant opals on the seeds of *Setaria viridis*, a species exhibiting seed dormancy. The objective was to determine whether these surface opals are associated with dormancy regulation. **Materials and Methods:** Seed surface morphology was analyzed using scanning electron microscopy. Comparative observations were made between *S. viridis* and *S. italica* (a non-dormant cultivated form). A standard cold and moist stratification treatment was applied to assess dormancy release. Observations focused on changes in seed coat structure, particularly the detachment or cracking of plant opals. No additional statistical tests were required for this morphological comparison. **Results:** A distinct parallel arrangement of plant opals was observed on the outer seed coat of *S. viridis*. These opals detached following dormancy-breaking treatment, initiating cracks in the seed coat. In contrast, *S. italica* exhibited pre-damaged or missing opal structures and lacked dormancy. These findings suggest a correlation between intact opal structures and seed dormancy, implying that opal detachment may facilitate water permeability and germination. **Conclusion:** The plant opals on the seed surface of *S. viridis* may contribute to seed dormancy by forming a physical barrier. Their structural disruption during dormancy-breaking treatments supports a role in regulating seed coat permeability. Further research is necessary to clarify the biochemical and mechanical processes involved.

KEYWORDS

Plant opals, seed dormancy, *Setaria viridis*, *Setaria* spp., seed coat permeability, silica bodies, electron microscopy

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INTRODUCTION

Setaria viridis is an annual Poaceae plant that performs C₄ photosynthesis, similar to other major global crops such as maize, sorghum, and sugarcane. These major C₄ crops are large and not suitable for cultivation in laboratories. However, *S. viridis* has the same NADP-ME type of photosynthesis subtype and excellent characteristics such as a small plant and genome size, making it suitable for use as a model C₄ plant^{1,2}. However, the seeds of *S. viridis* show deep dormancy and do not germinate uniformly, making it difficult to use *S. viridis* as a model plant^{3,4}. Although chemical treatments, such as gibberellin and liquid smoke, are effective^{4,5}, it remains challenging to achieve stable germination⁵. Thus, developing seed dormancy-breaking methods is important to utilize *S. viridis* as a model plant⁶⁻¹¹.



One of the factors contributing to seed dormancy in *S. viridis* is the water-repellent structure of the outer coat¹². In this study, the surface structure of the seed coat of *S. viridis* was carefully observed using an electron microscope, and the presence of plant opals on the seed surface was confirmed. Plant opals are characterized by the accumulation of soluble silicic acid in plant cells. In leaves and stems, plant opals function as skeletal tissue and protect the plant from grazing and disease^{13,14}. By contrast, the role of plant opals on seeds is unknown. This study shows that plant opals on the seed surface may play a role in dormancy.

MATERIALS AND METHODS

Plant material: *Setaria viridis* seeds were collected from the Regional Field Science Research Center, Faculty of Agriculture, Shizuoka University, (6334.9052333N, 138.2721461E, Kariyado, Fujieda, Japan) in October, 2023. No germination was observed immediately after collection, confirming dormancy. Germinated seeds broken from dormancy by cold and wet treatment at 5°C for 4 weeks were prepared.

For comparison, prepared *Setaria italica*, a cultivated species derived from *S. viridis*, and confirmed that *S. italica* seeds were not dormant and had a high germination rate.

Electron microscope: A scanning electron microscope (Miniscope®TM3030Plus, Hitachi High-Tech Corporation Tokyo) was used to observe the fine structure. Twenty dormant seeds, 20 germinated seeds of *S. viridis*, and 20 ungerminated seeds of *S. italica* were observed using electron microscopy.

Statistical analysis: The germination rates were compared using the Bell Curve for Excel 5.0 (Social Survey Research Information Co., Ltd.) with Fisher's exact test.

RESULTS AND DISCUSSION

Immediately after the seed harvest, plant opals were observed as dumbbell-shaped and arranged in parallel on the outer coats of all dormant *S. viridis* seeds (Fig. 1).

However, after the stratification treatment, the plant opals on the outer coat of the seeds peeled off. Cracks were observed to form on the outer coat of the seeds, starting from the peeled-off points in all germinated seeds (Fig. 2a-b); this suggests that the plant opals on the outer coat of the seeds affect the impermeability of the seed coat and the disappearance or damage of plant opals may trigger the breaking of dormancy.

Furthermore, in the case of *S. italica*, even in untreated seeds that had not undergone stratification, holes, cracks, and the loss of plant opals were observed in all ungerminated seeds. Unlike *S. viridis*, cultivated *S. italica* does not exhibit deep dormancy; it is speculated that this is because the outer coat shrinks during cultivation, causing the structure of the plant opals to be lost.

Recently, *S. viridis* has garnered attention as a model C₄ plant¹. However, deep seed dormancy of *S. viridis* limits its use as a model plant. Hence, to use *S. viridis* as a model plant, it is essential to develop a stable method to break dormancy⁶⁻¹¹.

In general, storing *Setaria* seeds in the dark under refrigerated conditions is effective for breaking their dormancy^{5,13}. However, this treatment requires several months to induce germination. Therefore, chemical treatment methods, such as liquid smoke treatment, gibberellic acid treatment^{14,15}, and KNO₃ treatment⁴, have been proposed to induce germination in a shorter period. However, it has been reported that the smoke liquid treatment harms seeds, resulting in a low germination rate⁵. In addition, the effect of any chemical treatments on seed germination requires several weeks and is unstable, and with these treatments⁴, it is relatively difficult to achieve consistent and uniform germination⁵.

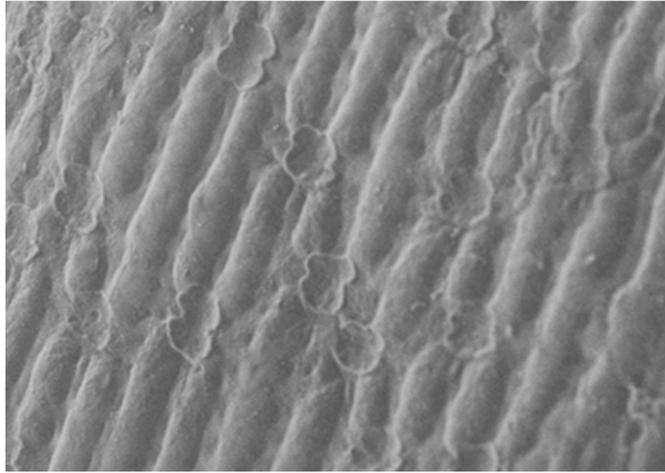


Fig. 1: Dumbbell-shaped plant opals seen on the outer skin of *Setaria viridis* seeds (1500×) in SEM analysis

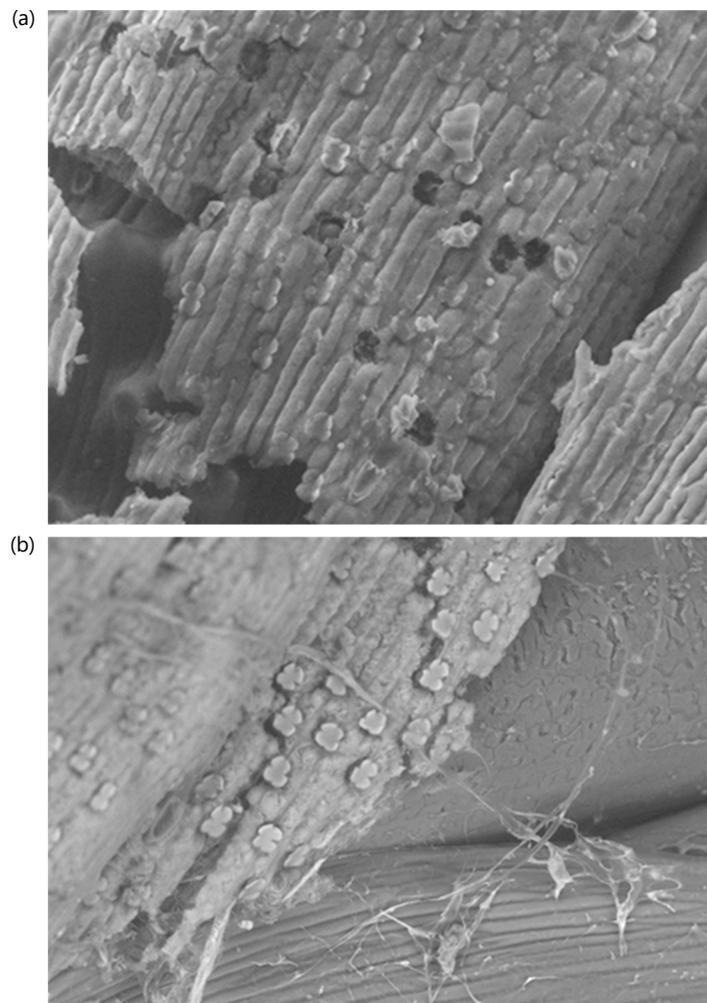


Fig. 2(a-b): Outer skin of *Setaria viridis* seeds after stratification in SEM analysis, (a) Missing plant opals in a *Setaria viridis* seedling and (b) Cracks starting from where the opal plant peeled off

Meanwhile, it has been suggested that the water-repellent structure of the outer skin may be related to dormancy in *S. viridis*^{16,17}. The study demonstrated the possibility that plant opal acts as a water tap, destroying the water-repellent seed coat structure and triggering the breaking of dormancy. Current observations support the hypothesis that dormancy in *S. viridis* depends on the water-repellent structure of the seed coat.

Conversely, plant opals are characterized by the accumulation of soluble silicic acid in plant cells. Plant opals are physically stable and remain in the soil as microfossils even after the plant body dies. Therefore, plant opal analyses are mainly conducted in the field of archaeology, such as in vegetation surveys of ruins^{16,17}. However, the role of opals in living plants remains unknown. It is thought that plant opals present in leaves and stems function as plant skeletal tissue and protection from feeding and disease. By contrast, the role of plant opals observed on seeds is unknown. In response to this issue, this report is the first to show that the phytoplankton on the seed surface may act as a water tap and be involved in breaking dormancy. In the future, a deeper understanding of the seed surface structure of *S. viridis*, particularly the plant opals on the seed coat, could provide an approach to breaking dormancy.

CONCLUSION

The plant opals on the outer coat of *S. viridis* seeds may act as water faucets, affecting seed dormancy. Their structural disruption during dormancy-breaking treatments plays a role in regulating seed coat permeability. Further research is needed to examine the role of plant opals by observing and analyzing the microstructure of the seed surface of *S. viridis* in three dimensions.

SIGNIFICANCE STATEMENT

There are many unknowns regarding the role of phytoliths in seeds. The results of this study revealed that the phytoliths on the seed surface of *S. viridis* act as a physical water tap, and the destruction of this tap may contribute to seed dormancy. The deep seed dormancy in *S. viridis* is considered a constraint when using it as a model plant, and the findings of this study may solve this problem. Furthermore, the observations on the role of the phytoliths in *S. viridis* in this study provide important insights into the role of phytoliths in seeds.

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