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博士（環境科学）

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学 位 論 文 題 名/Title of Dissertation

Development of systems for evaluating *Paeonia suffruticosa* pollen viability using
deep learning techniques

(深層学習を用いた*Paeonia suffruticosa*の花粉稔性の評価システムの開発)

Paeonia suffruticosa, commonly known as the tree peony, is a popular ornamental shrub. Until now, various tree peony cultivars have been produced by cross-pollination and artificial selection. Breeding new tree peony cultivars typically involves hybridization and selecting seedlings from cross-pollination. A critical factor in successful hybrid breeding is pollen viability, which determines the ability of pollen grains to fertilize the ovules effectively. As seen in other plants, high pollen viability correlates with successful seed production.

Various methods are used to assess pollen viability, each with its own limitations. For instance, staining methods damage the integrity of the pollen cell, preventing subsequent analysis. The IFC method requires specialized and costly equipment. *In vitro* pollen culture is useful because it can evaluate the potential to retain pollen germination ability. However, the *in vitro* culture method combined with manual counting of pollen tubes is extremely time-consuming. To address the above issues, this study proposed two deep learning-based systems as non-invasive alternatives to these methods.

The first system was built upon the Mask region convolutional neural network (R-CNN) model. The proposed model is proficient at instance segmentation of pollen grains and tubes, allowing for the precise detection and counting of individual pollen grains and tubes in microscopic images. By adding a synthetic dataset to the training dataset, the model can handle more complex scenarios of pollen tube crossing. Additionally, the inclusion of post-processing code allows the model to skeletonize pollen tube masks, enabling it to calculate the pollen germination frequency and estimate the average length of pollen tubes. This enhancement provides deeper insights into pollen germinability. At an IoU threshold of 50%, the model achieved a mAP of 0.949. For higher IoU thresholds, the model still performed commendably, with mAP values of 0.820 for mAP₇₀ and 0.633 for mAP@ [0.5:0.95]. In terms of accuracy, the model achieved 0.997 for pollen grains and 0.903 for pollen tubes at the IoU threshold of 50%. These metrics highlight the model's effectiveness in accurately segmenting pollen grains and tubes, confirming its reliability and robustness in pollen analysis. The generalizability of the proposed Mask R-CNN model extends beyond tree peony pollen. Its robust segmentation capabilities make it applicable to other plant species. Furthermore, transfer learning can enhance this adaptability, where a model trained on tree peony pollen can be fine-tuned for pollen from other plant species with distinctly different appearances. This feature highlights the model's potential to serve as a valuable tool in diverse research, enabling researchers to assess pollen viability without requiring extensive reconfiguration.

The second system was based on the You only look once (YOLO) v7, an object detection model known for its efficiency and speed. This model was used to predict the pollen germination potential based on their hydrated morphological characteristics. By

capturing the images of fully hydrated pollen grains, the study analyzed key morphological features such as size, color, and shape to determine the viability of the pollen. Under the microscope, the pollen grains of tree peony with an area greater than $14\text{ }\mu\text{m}^2$ typically exhibit germinability, whereas pollen grains with less than $12\text{ }\mu\text{m}^2$ generally do not exhibit such ability. Correlation analysis indicates a strong positive relationship between the pollen area and their germinability ($\rho=0.81$). Additionally, pollen grains with lighter colors (higher grayscale values) often possess higher germinability, with a moderate correlation coefficient of $\rho=0.40$. The aspect ratio, which is related to shape (pollen grains closer to a circular shape have a lower aspect ratio), exhibits a moderate negative correlation with germinability ($\rho=-0.40$). Pollen grains that expand into a circular shape upon hydration typically demonstrate higher germinability. Overall, the findings suggest that the hydrated pollen grains exhibit distinct features correlated with their germinability. Pollen grains with larger areas, brighter colors, and shapes closer to circular are more likely to germinate upon hydration, highlighting the significance of these morphological characteristics as indicators of pollen viability. The proposed model effectively categorized pollen grains into two categories based on these characteristics: germinability and those that do not. The model achieved a high mAP of 0.979 and a low validation loss of 0.057 with a 50% IoU threshold after training for 800 epochs. Additionally, the precision-recall curves for both classes exhibit high precision and recall, and the AUC-PR is notably high, indicating the model's effectiveness in accurately identifying instances while minimizing false positives. These results all support the strong model performance. However, the model's specificity to the dataset from this study suggests that further training and adjustments would be necessary to apply it broadly across different plant species. The YOLOv7's speed allows for real-time processing, making it suitable for time-sensitive experiments.

Developing deep learning-based systems for assessing pollen viability is a promising advancement in plant research. These technological advancements enable the rapid assessment of the viability of tree peony or other plant pollen, facilitating agricultural research on crop production and efficient breeding. The ability to accurately assess pollen viability using deep learning techniques offers significant advantages over traditional methods, including greater accuracy, reduced time and labor, enabling subsequent research on the tested pollen, and the potential for real-time analysis. These achievements are particularly valuable in fundamental research on plant reproduction. Elucidation for the function of pollen in plant reproduction is expected to be applied to stable crop production and utilization of unused plant genetic resources for breeding. Integrating deep learning into this field may provide opportunities for further research and potentially enhance breeding strategies.