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Improved nuclear staining for accurate determination of pollen development stages in rice

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The maturity stage of pollen is a key factor which regulates the response of anthers during anther culture. The most appropriate stage of pollen is determined by examining the culture response of anthers with pollen at different development stages. This requires effective staining of pollen nuclei. Haematoxylin stain used previously on pollen of a few local rice varieties produced limited success. Many pollen cells could not be scored due to poor staining of nuclei. The present investigation reports on improved haematoxylin staining of pollen nuclei for accurate identification of the pollen development stages in rice variety Kurulu thuda.

Pollen from six stages of panicle development based on the distance between the flag leaf and the penultimate leaf collars (3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 cm) of the enclosed panicle was examined. Nuclei could be clearly distinguished following staining with haematoxylin and uni- and bi-nucleate stages were identified in nearly all the pollen examined. Staining for 5 min. was effective for pollen from panicles harvested when the distance of flag leaf to penultimate leaf collar of the plant was 3 cm. However staining duration had to be increased to 140 min. to observe nuclei of more mature pollen. Cytological features related to pollen ontogeny were also observed, where positioning of the nuclei in the cytoplasm was found to vary depending on the stage of pollen development. Morphological index, the distance between the collars of flag leaf and penultimate leaf of the tiller, that was used to identify the maturity stage of panicles, correlated well with the pollen developmental stages within the panicle. Uninucleate pollen were confined to panicles relating to the marker distance 3 – 4 cm, and the transition from uni- to binucleate pollen occurred in panicles relative to 5 – 6 cm distance. Binucleate cells were exclusively present in panicles of the 7- 8 cm distance range. While the distance between the collars of flag leaf and penultimate leaf serves as a reliable morphological guide to pollen maturity, correlations between the length and stage of pollen development may vary from one variety of rice to another, and have to be worked out for a particular variety of interest beforehand.

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