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Microspore staging for haploid cell culture in *Oryza sativa* sub species *indica* (rice)

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Microspore staging is a necessary adjunct in developing a successful anther culture protocol for haploid plant production because *in vitro* response depends to a large extent on the stage of pollen development at explant inoculation. This requires a staining procedure to identify correctly the stages of pollen most suitable for culture and a correlation between the pollen stage and an easily observable marker to facilitate the selection of explants at required maturity. The objective of this study was to test the validity of such a correlation between the stage of microspore development and that of a visual marker in rice. This was tested using iron –alum haematoxylin stain on pollen of different developmental stages from three *indica* rice varieties, Bw 267-3, Bw 361 and Ld 3-12-36. Panicles, to obtain pollen, were harvested based on the indicator which was the measured distance from the flag leaf auricle to the penultimate leaf auricle, when this distance was 2.5, 5.0, 6.0, 7.0, 8.0, and 9.0 cm.

Uni- and bi- nucleate pollen stages were clearly recognized with the haematoxylin stain. Uni-nucleate pollen was present in anthers of panicles that were harvested when the indicator distances were 2.5 – 7.0 cm but absent from the anthers of those panicles that were harvested when this distance was ≥ 8 cm. This indicated that at these latter stages of maturity, pollen development had progressed beyond the uni- nucleate stage in all rice varieties examined. This idea was further supported by the observation that in these panicles (of ≥ 8 cm), only bi- nucleate stage pollen was present. Although bi- nucleate pollen could also be observed in panicles of 7 cm length difference between flag and penultimate leaf auricles in all three rice varieties, and in panicles when this distance was 6 cm in rice varieties Bw 361 and Ld 3-12-36, these were present generally at a lower frequency (3-11%) in comparison to the frequency of uni nucleate pollen (16-19%). A large percentage of cells that were analyzed could not be categorized as being of uni- or bi- nucleate type and possible improvements to the staining technique needs to be examined further. However, irrespective of the cells that could not be staged, the identified stages clearly demonstrated that uni- nucleate stage microspores that are the most suitable for *in vitro* culture for haploid plant production in rice could be obtained from panicles that corresponded to a distance 2.5 – 7.0 cm between flag leaf and penultimate leaf auricles, with more mature panicles within this range containing a higher frequency of uni- nucleate pollen.

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