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Evaluation of Factors Impacting Agrobacterium-Mediated Indica Rice Transformation of IR58025B - a Public Maintainer Line

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In this study, we report our efforts to develop an efficient Agrobacterium-mediated transformation protocol for the transformation of Indica rice IR58025B with mannose as a selection agent. Various factors, such as basal medium, culture temperature, strains for infection, air-drying duration of calli after infection, and salt concentration of coculture medium, were systematically optimized so that the highest transformation frequency (TF) reached to 74%. About 94% of mature embryos were induced to generate calli of high quality on modified NB medium. Both callus quantity and quality were improved when callus was induced from endosperm-free embryos in comparison to intact mature seeds. Higher temperature (32°C) showed improvements when compared to lower temperatures at (25°C and 28°C) for both callus induction and transformation. Agrobacterium strains of EHA105 produced the best TF in comparison with EHA101, LBA4404 and AGL1. However, LBA4404 produced the highest single copy frequency. Prior to co-cultivation, air-drying of inoculated calli was performed in a laminar flow hood for 4-7 hours and produced a higher TF. Lower salt concentration for co-cultivation medium enhanced both DNA delivery and TF.

Rice is one of the most important cereal food crops in the world and *Indica*-type rice provides the staple food for more than half of the world's population. In spite of its large scale production, a number of abiotic and biotic factors have limited its productivity. To meet the growing demands of an ever-increasing global population, genetic transformation of *Indica* rice, particularly for public breeding materials, has become a matter of increased urgency. Many factors are known to affect the efficiency of T-DNA delivery to plant cells, such as different plant varieties, different explant types, the quality of explants and *Agrobacterium* strains, the cell density of *Agrobacterium* for infection, inoculation period, co-culture medium and desiccation stress of the tissue before or after infection.

Agrobacteriummediated method since 1994 and Indica rice in 1996. Few reports on the Agrobacterium-mediated transformation of IR58025B are available. This public cultivar is commonly applied to generate hybrids by breeders in the south Asia since it has higher combining ability and generates good hybrids. Therefore, there is a need to evaluate various factors that affect tissue culture and to develop a highly efficient Agrobacterium-mediated transformation protocol for this public cultivar. Hiei et al. reported a protocol for Group I Indica rice transformation including IR58025B using immature embryos as explants. Our study mainly focused on callus explants derived from mature seeds and systematically optimized several factors that could influence transformation frequency. These factors include the basal medium for generating high quality calli, the optimized tissue culture temperature, different strains, desiccation after infection, different salt concentration in co-cultivation (COC) media, and the size of transformation constructs. By optimizing these factors, a highly efficient and reproducible Agrobacterium-mediated transformation protocol was developed for Indica rice-IR58025B, a public maintainer line used in hybrid rice production.

Discussion

To establish an efficient Agrobacterium-mediated transformation system, high quality callus is a prerequisite and so choosing the best callus induction medium is one of the most important factors. Hiei et al. demonstrated that the induction of embryogenic calli with the potential for higher rates of cell division is an important factor in determining transformation frequency. The commonly used basal medium for tissue culture is MS, N6, B5 and NB medium. Different plant species and genotypes require different kinds and amounts of nutrients, even requiring specific nutrient elements during specific stages. The number of common medium is limited for our process; therefore, we decided to make some modifications based on the commonly used media for the specific variety.

In this study, the minor salts based on the NB medium were modified according to the report by Lin and Zhang and resulted in enhanced callus proliferation and generated more embryogenic calli for this cultivar. As for the isolated mature embryos performing better than the intact mature seeds for callus induction, Lin et al. considered that a regulation system or buffer action may exist in the mature rice seeds, which may protect the embryo from de-differentiating into calli if the endosperm was not removed. Removal of endosperms can break the buffer system so that embryos are more exposed to the culture medium, especially the growth hormone. Furthermore, improved quality and quantity of calli can be produced.

Temperature is one of the key factors which affect the growth rate of calli. A temperature of 32°C was deployed to be optimal for callus growth of this rice line. Higher temperature not only increases the callus induction and proliferation but also improves the quality of calli in comparison with lower temperature. However, temperatures higher than 32°C are not suitable for callus growth of this maintainer line. Calli of higher quality can be infected by Agrobacterium more efficiently. It can also withstand the infection, recover better and produce a higher TF. Air-drying treatment of calli after the Agrobacterium infection can improve the transformation frequency. We found that the air-drying of the calli after infection can significantly

suppress the over growth of Agrobacterium, and reduce contamination of Agrobacterium. This process may protect the calli from the injury during Agrobacterium infection. Another possible explanation for improved TF is that air-drying may cause cell plasmolysis so that the T-DNA delivery is enhanced as well.It has been reported that the use of low salt media during the infection and co-cultivation can enhance T-DNA delivery in many species such as maize, canola, and wheat. In 2012, Tie et al. reported that Agrobacterium infection appeared to have both up-regulatory and down-regulatory effects on thousands of genes. Fewer nutrients with starvation may lead to calli stress and trigger biochemical changes which can lead the plant cells to be more susceptible to Agrobacterium infection.