

Development of Haploid Inducer lines in indica rice using CRISPR-Cas9 approach

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Abstract:

Doubled haploid, a novel approach provides a leading edge over traditional plant breeding techniques, hastening the development of homozygosity and maintains the developed genotypes as pure lines. Since, there are number of established haploid technologies available, androgenesis shows its potentiality in production of doubled haploids within a short period of time. Utilization of androgenesis could produce a number of rice varieties in japonica rice. However, indica rice is recalcitrant to anther culture. The most frustrating albino shoot regeneration along with the requirement of expertise skills hinders the wide spread use of DH technology through anther culture. Therefore, there is a need to identify an alternative of DH production technique which can be user friendly especially for the breeders; this could be answered by developing a haploid inducer line similar to maize; After extensive mapping efforts, a 4bp insertion in the carboxy terminus of MATRILINEAL (MATL) gene, which codes for pollen specific phospholipase was found to be causal factor of haploid induction system (Kelliher et al. 2017). Though the effect of MATL on Haploid induction was reported in rice, the induction rate is less than 6% (Yao et al. 2018). This could be enhanced by combining other related genes associated with pollen maturity. The cutting edge technology like CRISPR-Cas approach could be employed to generate HI line in rice. Keeping in view, a OsMATL gene-based marker was developed which was utilized to screen a number of indica rice genotypes like Swarna, N22, and DHs of 27P63 (M129-1), BS6444G (PA27-1, PA139) and CRHR32 (Y2-1, Y2-5, Y9-1), where the amplicon size is found to be ~400bp. Further, using the sequencing data a Cas9 guide would be generated for developing MATL knock-out lines using CRISPR-Cas.