Using rice anther culture for the production of doubled haploid lines with increased induction frequencies

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Developing doubled haploid (DH) lines through anther or microspore culture is an important strategy for shortening breeding cycles and accelerating crop improvement. The present study aimed to enhance the regeneration of rice haploids through anther culture-induced calli and subsequently convert them into DH bacterial leaf blight (BLB)-resistant lines. Eleven lines expressing resistance against prevalent strains of *Xanthomonas oryzae* pv. *oryzae* were crossed as male parents with elite high-yielding basmati and non-Basmati varieties to develop F_1s for collecting anthers at the appropriate stage of pollen development. Optimum callus induction in 36.45% of the genotypes was obtained on N_6 medium supplemented with maltose (40 g/l), 2,4–D (2.5 mg/l), and kinetin (0.5 mg/l), and further callus proliferation was enhanced in the presence of amino acids, particularly a combination of cysteine and tryptophan. High scores of haploid regeneration frequency were obtained in MS medium augmented with sucrose (30 g/L), BAP (2.0 mg/l), kinetin (0.5 mg/l), and NAA (0.5 mg/l). Fortification of regeneration medium with tryptophan (25 mg/l) and cysteine (40 mg/l) resulted in maximum regeneration of calli. Ranbir Basmati, Saanwal Basmati, Basmati 564, Ranbir Basmati × IRBB53, Ranbir Basmati × IRBB60, Basmati 564 × IRBB57, and Basmati 370 × IRBB56 were observed to be the most responsive genotypes for haploid regeneration. An average of 76.34% DH induction frequencies were obtained in colchicine (0.1%) treatment, while 41.56% were spontaneously induced DHs. Consequently, using anther culture to develop DH lines is a promising approach for genomic resources and elite varieties in a shorter breeding period.

Keywords: Anther culture, bacterial leaf blight, callus, doubled haploids, haploids, rice.

RICE (*Oryza sativa* L.) is one important crop of the Poaceae family and is the second most important food crop after wheat, which feeds over half of the global population[1]. India is the second largest rice-growing country in the world, occupying an area of about 43.7 million hectares with a productivity of 124.37 million tons as 40% of total food grain production[2,3]. However, to meet the increasing population's demand and attain self-sufficiency, 40% of rice must be produced globally by 2050 (ref. 4). Major constraints for rice productivity are the inefficient input usage and increasing water and labour shortage, besides climate change, and its production could be increased through developing stress-resistant, high-yielding varieties[5,6].

Rice is a highly self-pollinated crop and requires 6–9 cycles of selfing after hybridisation, followed by 3–5 years of field evaluation to develop a new variety[7]. Anther culture is a suitable technique that significantly reduces the breeding period due to early fixation of homozygosity[8] as genetic recombination occurs during microgametogenesis, producing genetically unique male gametophytes. It induces haploid calluses to form double haploid (DH) embryos through spontaneous and induced genome doubling, which bypasses the inbreeding process[9], thus producing new true breeding lines with unique gene combinations, allowing better discrimination between genotypes[10]. Anther culture is often the method of choice for DH production in many crops due to its simplicity, which allows large-scale anther culture establishment and application to a wide range of genotypes[9].

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