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*Short Communication*

# Evaluation of doubled haploid culture conditions and regeneration of an *indica* rice hybrid

**Anjanabha Bhattacharya,* Venugopal Mikkilineni, Laxmikant Verma, Bhavesh Palan, Kishor Mali and Bharat Char**

Mahyco Research Centre, Dawalwadi, Maharashtra Hybrid Seeds Company Limited, Aurangabad Road, Jalna 431203, Maharashtra

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**Abstract**

**A study was undertaken to develop an effective protocol to generate doubled haploid plants from a proprietary Mahyco hybrid MRP5401. A total of 20,300 anthers were plated on four different DH media (basal N6 + phytohormones) and obtained a callus induction success rate of 1.52%, of which 0.48% developed into green plants. A total of 232 lines were obtained of which, 98 lines showed homozygosity in $DH_{2-3}$ generations. Well established DH lines from MRP5401 in $DH_3$ generation had early flowering and yield gain.**

**Key words:** Rice, doubled haploid (DH), anther culture, hybrid, callus induction, plant regeneration, genetic analysis

As the human race continues to rapidly march towards the 9 billion mark by 2050, rice production needs to catch up with the decreasing land and water resources coupled with unpredictable weather patterns. Conventional breeding has played a crucial role in increasing rice productivity but takes long time and is labor and resource-intensive [1]. In order to hasten the process of incorporation of different traits, plant regeneration through double haploidy provides a rapid and stable method for achieving homozygosity. Several high yielding rice varieties were developed by this technique in a shorter time as this method permits for the selection of best lines in the early generation of the breeding program [2]. Many factors like genotype, culture medium, plant growth regulators, age of pollen and sucrose/maltose content in the medium influence the plant regeneration from anther culture, thereby restricting its exploitation in breeding [3]. In Rice, *indica* genotypes are known to be typically recalcitrant yielding limited number of green plantlets. Various protocols have been proposed to improve anther culture efficiency but green plant regeneration remained low especially for *indica* rice. Present study, therefore, was initiated with an aim of evaluating various pre-treatment, media culture conditions and other factors to improve the regeneration efficiency of *indica* rice varieties.

Two *indica* elite lines (MRP5401 and BR29) and one *japonica* variety (Taipei309) from Mahyco Rice Research Station, Kallakal, Hyderabad, India were used in this study. The panicles were collected and given two sets of treatments namely with or without cold exposure. Panicles were cold pretreated at $8^0$C for 8 days. Panicles were surface sterilized with 0.1% $HgCl_2$ for 10 minutes. Further, anthers were cultured on N6 medium supplemented with different phytohormones and exposed in the dark for callus induction. Subsequently calli (1-2mm size) were transferred to proliferation and regeneration media (modified MS media) with exposure in white light for 16h at $28\pm2^0$C until shoots development in about 3-6 weeks. Well grown shoots were transferred to MS basal media for rooting. The developed plantlets were acclimatized and transplanted in green house for further

*Corresponding author's e-mail: anjanabha.bhattacharya@mahyco.com
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growth, seeding and evaluation. Ploidy level of the regenerated DH plants were evaluated either by one of the following methods such as chromosome counting method, Partec ploidy analyzer, segregation analysis in the field, and guard cell length measurement.

A total of 20,300 MRP5401 anthers were cultured on N6 medium, typically used for *japonica* anther culture, with some modifications as 5 mg/l 2, 4-D; 1 mg/l NAA; 0.5 mg/l Kinetin or 0.5 mg/l BAP; 5mg/l $AgNO_3$ and 50 g/l Maltose. The callus induction frequency (0.75 to 5.15 %) in the present study was found nearly equal to the observed callus induction frequency (0 to 4.35%) on the N6 medium [4]. The *indica* hybrid MRP5401 and BR29 showed lower i.e. 0.75±0.5% and 1.52±0.5% callus induction respectively as compared to *japonica* cultivar Taipei309 (5.15±0.5%). Further, the callus tissues of controlled genotypes BR29 and Taipei309 were not taken up further for plant regeneration. The higher response of *japonica* variety for callus induction corroborates the previous findings [3, 5]. The varied callus induction frequency clearly indicates that anther culture response is highly genotype dependant in rice. Maltose was found four times more effective than sucrose for androgenesis in anther culture of *indica* rice cultivar IR 43 [6]. Hence, the maltose sugar was used in place of sucrose in the study.

The green plant regeneration (Fig. 1) frequency was observed to be 0.48% in this study (Table 1), which was consistent with earlier reports (0.03 to 0.61%) [7]. Albino plantlets were also observed during regeneration. The spontaneous chromosome doubling was reported in various species with varying frequencies such as barley (70-90%), wheat (25-70%) including rice (50-60%) [8]. It means colchicine treatment is needed for those species with low doubling percentage [8] and not necessary in case of above species but advocated for increased DH development. To reduce chances of mixoploids and polyploids, colchicine treatment was avoided in the present study.

**Fig. 1. Regenerated rice plantlet**

**Table1.** Details of rice anther culture experiment on DH medium

| Genotype | Total anther cultured | Callus induction (%) | Green plant regeneration (%) |
|---|---|---|---|
| MRP 5401 | 20,300 | 1.52 ± 0.5 | 0.48 |
| Taipei 309 (*Japonica*) | 3800 | 5.15 ± 0.5 | - |
| BR29 (*Indica*) | 2700 | 0.75 ± 0.5 | - |

Note: Callus tissue of Taipei 309 and BR 29 was not proceeded for plant regeneration

Segregation analysis was done in subsequent generations, $DH_{2-3}$. DH lines showed uniformity in subsequent generations along with the stable characters, which came from pollen cells. Whereas those coming from somatic cells tend to segregate as they are false DH plants. Further in more than 90% cases of anther culture, homozygous and stable plants progeny were observed from generation to generation [9] Further, chromosome counting and ploidy analysis were conducted for the selection of double haploid lines. Chromosome counting can distinguish between double haploid and haploid plants. Chromosome counting combined with segregation analysis can distinguish diploids, haploids and double haploids plants. Many researchers have preferred cold pre-treated panicles and observed anther culture response for callus induction and plant regeneration as compared to the control [5]. Contradictory to this, we observed the highest frequency of callus induction and plant regeneration when the fresh spikes were used for anther culture without cold pre-treatment. This may be due to the fact that cold treated panicles might have undergone some stress response, which might have altered biochemical and hormonal pathways leading to reduced callusing response.

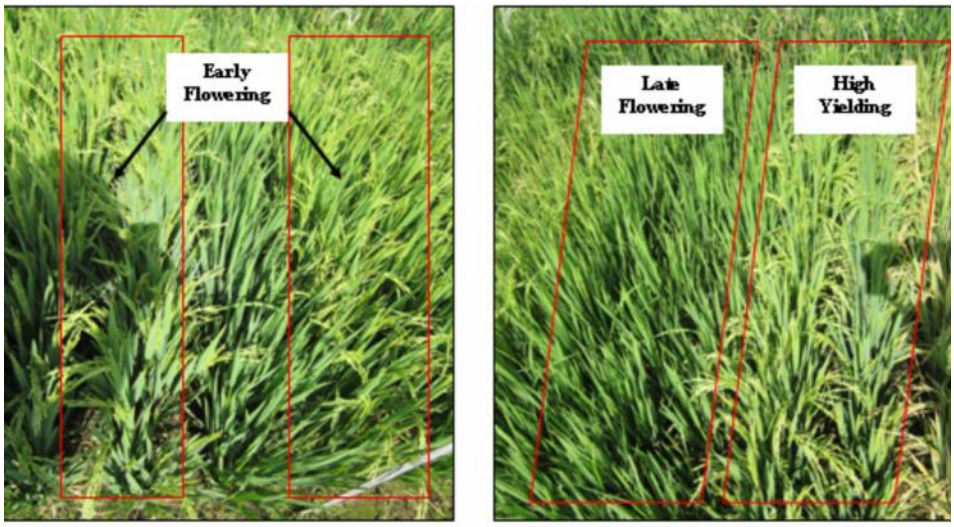


**Fig. 2. Developed DH lines of MRP 5401**

Out of a total of 232 lines of MRP5401, 98 lines were found to be double haploid lines. True double haploid lines showed uniform characters with novel traits such as early or late flowering, dwarf height, high yield, which will be beneficial to serve different market segments (Fig. 2). The anther culture technique with modified N6 medium was successfully used to regenerate green plants in *indica* rice hybrid MRP 5401. However, efficiency of cultured anthers to produce green plant on this medium was poor. This could be overcome by further optimization of media components and culture conditions to improve green plant production and reduce the frequency of albino plants. In essence, the protocol developed in this study is suitable for the development of double haploid lines in *indica* rice cultivars through anther culture.

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