European Journal of Biotechnology and Bioscience
ISSN: 2321-9122
Impact Factor: RJIF 5.44
Received: 15-03-2018: Accepted: 16-04-2018
www.biosciencejournals.com
Volume 6; Issue 2; March 2018; Page No. 26-30

Elucidation of the genetic basis of anther culture response and its breeding perspective in rice

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Abstract
Anther culture generates haploid plants which can be converted to pure breeding double haploids by colchicine treatment. In rice, anther culture is highly species and genotype specific. *O. glaberrima* L. responds more to callus induction and plantlet regeneration than *Oryza sativa* genotypes. In *Oryza sativa* L., japonica sub-group responds better than indica. Within indica sub-group, there is wide variation in anther culture response. Genetic basis for callus induction and green plantlet regeneration is still unclear. This limits the use of this technique for routine use in rice breeding. In this pursuit, the authors presented a detailed review of the genetic control of callusing and plant regeneration response for successful use of the anther culture technique in rice breeding.

Keywords: anther culture response, genetic basis, double haploids, breeding perspectives, rice

1. Introduction
Rice is the single most important cereal crop that feeds more than half of the world population and 70% Indians. Increasing productivity is continuing to be the top most priority to meet the food demand of expanding human population. With the alarming shrinking of natural resources (land and water) and adverse effects of climate change on agriculture, it becomes extremely difficult task to increase food production. At least 70% more food needs to be produced by 2050 to meet the food demand. Therefore, it compels rice breeders to reorient the breeding strategies and develop high yielding rice varieties.

Conventional recombination breeding normally begins with hybridization between diverse parents followed by 6-9 cycles of selfing and 3-5 years of field evaluation before a pure breeding line being released as a new variety. In contrast, anther culture seems to be a suitable alternative technique that allows early fixation of homozygosity from even F_1 [39]. Genetic recombination occurs during micro-gametogenesis producing genetically unique male gametophyte. Anther culture induces haploid callus formation and a sizeable proportion (70%) of plantlets in culture are *in vogue* haploids which either do not survive till maturity or become sterile due to abnormal meiotic behaviour during gametogenesis. About 15-20% of haploid cells in the callus usually converted to immortal true breeding double haploid (DH) embryos due to spontaneous genome doubling. Besides, the recovery of DHs can be improved by colchicines treatment. Each DH line obtained in this way would bypass the inbreeding process [11] and produce a new true breeding line with unique gene combination. This allows creation of usable genetic variability and better discrimination between genotypes [34] resulting increased selection response. Thus, anther culture technique provides fertile double haploids (DHs) with fixed derived character combination that might otherwise disappear in the course of an extended series of segregating generations in conventional breeding methods. Varieties developed through anther culture can yield as high as 10.3t/ha under moderate fertility. The technique can be utilized for developing direct one-step homozygous transgenic plants [7]. Besides, the DH lines derived from anther culture of hybrids
of genetically diverse parents are amenable for molecular mapping of valuable genes/QTLs. But, the technique poses several limitations. Early anther necrosis, recalcitrance to anther culture response, poor callus proliferation and more frequency of albino plants regeneration are the major problems in indica rice varieties [28]. At least 15,000 anthers need to be inoculated for *in vitro* culture to obtain regeneration of 150 plantlets [22]. Gueye and Ndir [13] could recover a total of 93 regenerants out of which 79 were albinos. The entire gamut of hinderance in anther culture response in rice and in particular indica rice is still unclear. Therefore, the authors reviewed the present status of rice research to unfold the genetic control of callusing and plantlet regeneration response in anther culture to help rice breeders in proper planning of double haploid breeding.

2. Anther/pollen culture in rice
In vitro production of androgenic haploidy was first made possible in Datura by Guha and Maheswari [14] and in rice by Niizeki and Oono [37]. Since then several researchers attempted such novel technique to improve callus induction and for plantlet regeneration. Nirouli and Bimb [38] reported higher callus induction frequency in N6 medium with 2.5mg/l 2, 4-D (2, 4-dichloro-phenoxyacetic acid) + 0.5mg/l Kinetin (Kn) than N6 + 4mg/l NAA (Napthalene acetic acid) + 0.5mg/l Kn; but reverse was the case for green plant regeneration. Many researchers have reported genotypic specificity within indica subspecies by using improved media [52]. The F_1 hybrids are more responsive to anther culture than

their parents. Xa and Lang [54] reported 5.13% to 9.27% callus induction and 6.17% to 14% regeneration from four crosses in MS medium with combination 1mg/L BA (6 benzyl aminopurine) + 2mg/L Kinetin + 3% sucrose. However, Dash *et al*. [8] reported a callus induction frequency of as high as 37.83 % from anther culture of a cross CRMS31B x CRMS24B when incubated at 26+1ºC for 24hr. Herath *et al*. [20] recorded highest callus induction frequency (29.4%) in N6 medium for F_1 hybrid Hu Lo Tao × BG 90-2 with green plant regeneration frequency (41.0%) in MS medium. This clearly indicates that anther culture response is cross specific. Green plant regeneration is also reported to be enhanced by six days of cold pre-treatment to the panicles [48].

3. Genetic basis of anther culture response

Genetic control of microspore response to *in vitro* culture in rice has been studied by a number of workers. It is largely species and genotype-specific [8]. *O. glaberrima* responds more to callus induction and plantlet regeneration than *Oryza sativa* genotypes. All *O. glaberrima* genotypes regenerated plants and few genotypes of *O. sativa* subgroup japonica only produced plants, whereas, indica was recalcitrant to plantlet regeneration. Indica rice is known to have a recalcitrant genetic background showing low anther culture response (1.2% callus induction), whereas japonica cultivars had more than 20-fold responsive (28.1%) to microspore embryogenesis [12]. Diverse genotype specificity of anther culture does exist within indica subspecies [52]. Temperate rice (hill rice) cultivars are *in vogue* more responsive to anther culture than tropical rice [38]. Anther culture ability is reported to be decreased in the order of japonica/japonica F_1 > japonica > indica/japonica F_1 > indica/indica F_1 > indica[57]. IR 43 produced maximum embryiods (16.13%) and green plantlets (11.88%) followed by BRRI Dhan 33, IR 54, Jaya and BR3 [28].

Albinism in regenerants is a serious problem in indica rice than japonica rice which may be due to breakage of plastid DNA [30]. Besides, the frequency of albinism is reported to be controlled by QTL on chromosome 9 and 10[56]. This problem can be improved by early transfer of calli into regeneration media [2], incubation at low temperature (<26ºC) and optimization of media for callus induction and plant regeneration [4]. Indica x indica hybrids produce more of albino plantlets compared to Indica x Japonica hybrids [43]. However, CRMS31B x CRMS24B among 4 inter-varietal crosses was highly responsive for callus induction (37.83 %) as well as shoot regeneration (41.09 %) and green plant regeneration (15.00 %).

Genes encoding certain enzymes are essential for metabolism of differentiating cells, tissues and organs. Calli with higher levels of the peroxidase enzyme have been reported to display a greater regeneration potential than those with lower levels of the enzyme producing predominantly albinos [51]. Induction of sporophytic haploidy in rice anther culture is *in vogue* controlled by haploid (gametophytic) inhibitor gene 'hap' [26] which is activated by cold pre-treatment. This reverses the gametophytic development of the pollen grain to sporophytic status. Further, Kiruchi *et al.* [29] reported that androgenesis in rice may be due to activation of a new class of Miniature Inverted-repeat Transposable Elements (MITE: mping elements) in anther derived calli. A rice genotype IR58025B with eui (25eB) gene is reported to be highly responsive for both callus induction and green plantlet regeneration among 13 genotypes [27]. Japonica/indica crosses usually result partial sterility due to interaction between the indica allele $S\text{-}5^i$ and japonica allele $S\text{-}5^j$ in chromosome 6. This causes partial abortion of female gamete containing $S\text{-}5^j$. A recent report shows that the "indica T 23" rice genetic stock with wide compatible allele $S\text{-}5^n$ (in chromosome 6) responds well to anther culture [36]. Dular-a indica variety has also the allele $S\text{-}5^n$ at S-5 locus which is widely compartible with both $S\text{-}5^i$ and $S\text{-}5^j$. However, many often segregation distortion (SD) at S-5 locus is reported and it may be due to preferential selection of gametes with $S\text{-}5^i$ indica allele for androgenesis [58]. Such genetic stock is being attempted for introgression of anther culture response into the indica genetic background by back crossing.

Callus induction from anthers and plant regeneration from the induced callus in rice are the two independent traits displaying quantitative inheritance [3]. Genetic analysis could be performed on haploid population to establish inheritance patterns. Additive effects seems to be more important than dominant effects of the genes concerned for callus induction while preponderance of dominant effects is reported for regeneration response [18]. Quimo and Zapata [42] suggested predominance of additive effects controlling both characters with japonica cultivars having higher general combining ability for green plant regeneration. However, callus induction is reported to be mainly controlled by gametic additive effects than maternal effects, while reverse is the case for green plantlet regeneration in anther culture [57]. Selection of parents with high GCA and most promising crosses revealing high SCA may pave the way for recovery of segregants with improved anther culture response. Anther culture response is reported to follow recessive inheritance [35] conditioned by block of genes (QTLs) on two specific chromosomal regions and japonica appear to be a good combiner for callus induction. A QTL on chromosome 1 control callus formation and another on chromosome 10 determine the balance between albino/green plant regeneration ability [56]. However, He *et al*. [17] identified five quantitative trait loci (QTL) on chromosomes 6, 7, 8, 10 and 12 responsible for callus induction and two QTL on chromosomes 1 and 9 for green plantlet regeneration. Besides, they identified a major QTL for albino plant differentiation on chromosome 9. Kwon *et al*. [31] mapped two candidate loci (QTLs) for green plantlet regeneration on chromosome 3 and 10 and identified molecular markers that co-segregate with these genes. Molecular markers such as RAPD [1], AFLP [16] and SSR [32] have proven to be a powerful tool for characterization of DH lines. Grewal *et al*. [12] used a set 124 DH lines for SSR marker analysis generated from the japonica cultivar (IR69428) × indica variety (IR64) that showed 1:1 ratio of indica and japonica alleles. Homozygosity was detected for all the marker loci in 124 DH lines and the genes for anther culturability were shown to be partially dominant. Besides, Hemaprabha *et al*. [19] established genetic differences among anther culture-derived stable breeding lines using RAPD and ISSR markers. Marker-assisted selection (MAS) strategy can improve anther culture ability in indica rice [5]. Recent studies

have identified several genes that are specifically expressed in calli with good regeneration ability and some have been cloned [49]. Transformation of a genotype which showed a poor response in tissue culture with a gene that putatively encodes glucose dehydrogenase has greatly enhanced the differentiation rate of calli [40].

4. Breeding perspective

Anther culture derived DH polulations provide a link between conventional plant breeding and genomics. DH populations are quite useful for chromosome mapping [10] and genetic analysis of QTLs [33]. Double haploid lines being permanent fixed genotypes [47]; minimize the environment component of variation and allow accurate measurement of quantitative traits. Such permanent mapping population offers more advantage over segregating F_2, F_3, BC_2, BC_3 populations. and is often preferred over near isogenic lines (NILs), back cross inbred lines (BILs), recombinant inbred lines (RILs), multiparent recombinant inbred lines (MPRILs), chromosomal segment substitution lines (CSSLs), multi-parent advanced generation intercrosses (MAGIC), nested association mapping (NAM) populations which require long time to develop[50]. In DH populations, the Identification of DNA markers seems to be much reliable as the gene segregates in the ratio of 1:1 (dominant homozygous: recessive homozygous) and most intermediate phenotypic expressions due to heterozygosity are excluded. Anther derived DH populations of rice have been used in QTL mapping of rice seed dormancy [15], grain quality [45], cooking quality [53], seed yield [41] and resistance to brown plant hopper[21], leaf folder[44], blast[24], drought [55] and salt stress[9]. Besides, validation of quantitative trait loci associated with reproductive-growth traits and grain yield under drought stress in a doubled haploid population of rice (*Oryza sativa* L.) was studied by Sellamuthu *et al*. [46]. Double haploid (DH) line population derived from the cross CT9993-5-10-1-M/IR62266-42-6-2 is tested for the QTL mapping of drought resistance in rice. DH breeding can be also useful for functional genomics studies and fixing a transgene while simultaneously removing unwanted selectable marker gene [25]. Jiang *et al*. [23] elucidated the scope of breeding for stay-green trait in a population of doubled haploid lines derived from an indica x japonica cross. The development of anther culture may be also used as a system for *in vitro* mutant selection [6].

5. Conclusion

Anther culture is recognized as an innovative *in vitro* culture technique for quick fixation of homozyosity after raising F_1 through recombination breeding (hybridization). DHs produced through anther culture of a cross involving diverse parents are stable pure breeding lines and each of these carry different gene combination. This paves the way for increased selection response and allows identification of desirable genotypes. But, the success of the technique is limited due to recalcitrance of indica rice to anther culture and recovery of high frequency of albino plants in regeneration medium. This poses a serious problem for practical use of this technique in rice breeding. Until now, a number of research papers have been piled up for elucidation of the genetic basis of anther culture response, but it still remained inconclusive. Additive

effects seems to be more important than dominant effects of the genes controlling callus induction while dominant effects largely contributes to regeneration response. A few genomic regions (QTLs) have been identified for anther culture response and recovery of albino plants in culture. Hybridization of rice sub-groups "japonica x indica" followed by selection of favorable QTL for green plant regeneration could be a better option to improve plant regeneration from anther-derived callus in indica rice.

6. Acknowledgements
We sincerely acknowledge and thank all researchers for their valuable contributions included in this pursuit.

7. Conflict of interest
It is declared that there is no conflict of interest.

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