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ANTHER CULTURE IN RICE: PROGRESS AND BREEDING PERSPECTIVE

Swapan K. Tripathy, Devraj Lenka, Arjun M. Prusti, Dayanidhi Mishra, Digbijaya Swain and Suraj K. Behera

Department of Agricultural Biotechnology, College of Agriculture, Orissa University of Agriculture & Technology, Bhubaneswar - 751 003, Odisa (India)
*e-mail: *swapankumartripathy@gmail.com*

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ABSTRACT

Anther culture is an important biotechnological tool for the rapid recovery of fixed breeding lines in rice. The method is largely species- and genotype-specific. Indica rice is known to have a recalcitrant genetic background. *Oryza glaberrima* responds better to the anther culture than *O. sativa* while japonica sub-group is more responsive to microspore embryogenesis than Indica types. Media recipes and culture techniques have been standardized for callus induction and green plantlet regeneration using Indica and Japonica varieties as well as their *inter se* hybrids. Additive effects seem more important than dominant effects of the genes concerned for callus induction while preponderance of dominant effects is reported for regeneration response. Sexual hybridization of rice sub-groups (Japonica x Indica) followed by the selection of favourable QTL for green plant regeneration may be adopted to improve plant regeneration from anther-derived callus in Indica rice. However, genetic transformation technology using individual genes related to different aspects of anther culture could be a more direct approach. This paper reviews the progress and breeding perspective of anther culture in rice for genetic improvement of agronomic- and stress-related traits using double haploid breeding.

Keywords: Anther culture, double haploids, genetic improvement, rice

1. INTRODUCTION

Rice (*Oryza sativa* L., 2n = 24, family Poaceae) is one of the world's most important staple cereal food crop. It feeds over half of the global population (Sasaki, 2005). Rice is highly self-pollinated so the development and selection of pure breeding lines with manifested superior phenotype is the ultimate objective for desired genetic improvement. It normally needs 6-9 cycles of selfing, followed by 3-5 years of field evaluation to develop a variety. In contrast, anther culture seems to be a suitable technique that significantly reduces breeding period due to early fixation of homozygosity (Otani *et al*., 2005). Genetic recombination occurs during micro-gametogenesis producing genetically unique male gametophyte. Anther culture induces haploid callus formation, which is converted to immortal true breeding double haploid (DH) embryos due to the spontaneous genome doubling. Consequently, each DH line obtained in this way would bypass the inbreeding process (Germana, 2011) and produce a new true breeding line with unique gene combination. This allows better discrimination between genotypes (Maria *et al.*, 2006) resulting in increased selection response. 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 gameto-genesis. However, these may be valuable to detect desirable recessive traits introduced through mutation (Chen *et al.*, 2001) or hybridization (He *et al*., 2006) and such plantlets may be converted to DHs by colchicine treatment.

Anther culture offers greater chance of recovery of otherwise desirable recessive genes as compared to the conventional breeding. Doubled haploid breeding involve only one cycle of

meiotic recombination. However, many agronomic traits are polygenically controlled, therefore one cycle of recombination is usually insufficient for the improvement of quantitative traits since crossing over between polygenes will not release all potential variations available in a cross. To overcome these disadvantages, Chinese developed a method of combining anther culture with sexual hybridization among anther-derived plants. The anthers of hybrid (F_1) progeny are excellent breeding material for raising pollen-derived homozygous plants (double haploids) in which complementary parental characteristics are combined in one generation.

Varieties developed through anther culture under moderate fertility conditions may yield as high as 10.3 t ha^{-1}. It can be utilized for developing direct one-step homozygous transgenic plants (Chen *et al*., 2006), increasingthe selection efficiency and genetic manipulation, widening the genetic variability through the production of gametoclonal variants and for producing double haploid populations and allow early expression of recessive genes. Since, the consumer's life style and demand for a suitable variety changes with time, therefore anther culture appears to be a suitable alternative for genetic improvement in rice.

2. ANTHER/POLLEN CULTURE IN RICE

The chance discovery of androgenic haploidy in *Datura* by Guha and Maheswari (1964) led several researchers to apply such novel technique in a wide range of crop plants. Haploid plant production was first reported in rice through anther culture (Niizeki and Oono, 1968). Anther culture is a two-step process (Fig. 1). The 1st step is initial callus development that leads to 2nd step of green plant regeneration from callus. The major constraints in double haploid breeding seem to be the limited morphogenetic potential of anther-derived calli and a higher percentage of regenerated albino plants (Talebi *et al.*, 2007). Maw and Kenji (2017) evaluated callus induction and plant regeneration in rice F_1 hybrids on different media. Gueye and Ndir (2010) reported a recovery of 93 regenerants out of which 79 were albinos. Many researchers reported genotypic specificity within Indica sub-species by using improved media (Talebi *et al.*, 2007). Green plant regeneration reportedly is enhanced by 6 days of cold pretreatment to the panicles (Sen *et al*., 2011). Nirouli and Bimb (2009) reported high callus induction frequency in N_6 medium with 2.5 mg L^{-1} 2,4-D (2,4-dichloro-phenoxyacetic acid) + 0.5 mg L^{-1} kinetin (Kn) than N_6 + 4 mg L^{-1} NAA (naphthalene acetic acid) + 0.5 mg L^{-1} Kn; but reverse was the case for green plant regeneration. Xa and Lang (2011) reported 5.1 to 9.3% callus induction and 6.2 to 14.0% regeneration from 4 crosses in MS medium supplemented with 1 mg L^{-1} BA (6 benzyl amino-purine) + 2 mg L^{-1} Kn + 3% sucrose. High frequency of callus induction was also obtained by incorporating 2,4-D, NAA and Kn to the anther culture media (Mukherjee *et al*., 2015). Profuse microtillering of androgenetic plantlets was found in elite Indica rice cv. 'IR 72' which can be further depressed by higher concentration of BAP (Roy and Mandal, 2011). F_1 hybrids are more responsive to anther culture than their parents. Herath *et al*. (2007) noticed high callus induction frequency (29.4%) in N_6 medium for F_1 hybrid 'Hu Lo Tao × BG 90-2' with green plant regeneration frequency of 41% in MS medium. However, positive relationship for both was noticed by Shahnewaz *et al.* (2004). Thuan *et al*. (2001) reported callus induction from anthers of F_1 plants-derived from 4 crosses of improved aromatic rice cultivars cultured in N_6 and MS media supplemented with 2,4-D (0.5 mg L^{-1}) + NAA (1.0 mg L^{-1}) + BAP (0.5 mg L^{-1}) showed better callus induction. Dash *et al.* (2014) reported a callus induction frequency of 37.83% from anther culture of a cross 'CRMS31B x CRMS24B' when incubated at 26±1°C for 24 h.

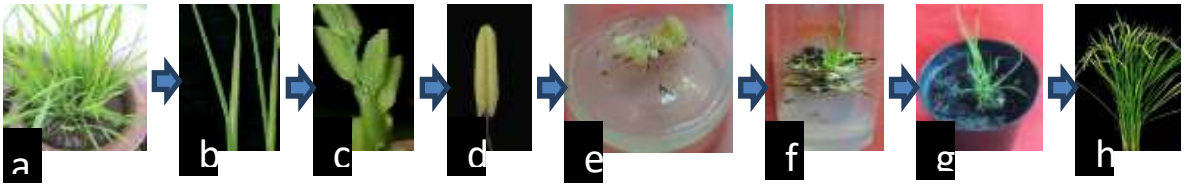

Fig. 1: Anther culture derived plantlet production in rice; a) Rice plant as source of explants, b) Boots, c) Spikelets, d) Anther, e) Callus induction from anthers, f) Regeneration of plantlet, g) Plant establishment in pot mixture, and h) Field grown plants at panicle stage

3. FACTORS AFFECTING ANTHER CULTURE

The success of anther culture depends upon exogenous and endogenous factors such as the maturity of donor plant, genotype, microspore developmental stages, panicle pretreatment, temperature and duration of pretreatment, culture medium used, growth conditions, etc.

i. Genotype: Response to anther culture varies within species, subspecies, or varieties. *O. glaberrima* is more potent for callusing and regeneration than *O. sativa* (Gueye and Ndir, 2010). Japonica types *in vogue* are more responsive to microspore embryogenesis than Indica rice types (López-Cristoffanini *et al.*, 2018; Sharma, 2018). Indica cultivars show poor callus growth, poor regeneration ability and low percentage of albino plants. Only 5 out of 18 Indica cultivars showed pollen callusing and 4 calli differentiated into plants (Guhaand Mukherjee, 1973); whereas, only one out of 35 Indica cultivars exhibited pollen callusing (Lentini *et al*., 1995). Tran and Vuong (2002) also recorded low response for callus induction (3.53%) and plantlet regeneration frequency (1.12%) in Indica rice. However, combining high yielding Indica rice with high anther culture responding Japonica genotype may improve anther culture response. Calli from a F_1 hybrid ('Bg 90-2': Indica rice × 'Hu Lo Tao': Japonica rice) showed higher frequency of green plantlet regeneration than their parents. High callus induction frequencies (30-34%) and low regeneration response in F_1s was obtained by Mishra *et al*. (2011). However, positive relationship for both was noticed by Javed *et al.* (2007).

ii. Physiological status of donor plants: Anthers collected at the beginning of flowering period respond better and it declines with the age of plants (Szarejko, 2003). Field grown plants show superiority over those grown in the glasshouse or pots (Veeraraghavan, 2007). Plants with excessive vegetative growth are not desirable and therefore, second application and/or foliar spray of nitrogen is avoided. Besides, a favourable day (34^{o}C) and night (25^{o}C) temperature at booting stage seems to be a determining factor for androgenic embryogenesis (Prem *et al.*, 2004). Anthers from the primary tillers are *in vogue* more responsive than secondary tillers (Asif, 2013). Besides, physiological status of anthers from middle portion of the panicles in boot stage seems to be favourable for callusing and regeneration (Jacquard *et al*., 2006).

iii. Developmental stage of pollen: The developmental stage of pollen can enhance anther culture efficiency. In rice, the most suitable stage is the early to mid-uninucleate pollen stage, while the older pollens at tetrad stage and after 1st pollen mitosis do not respond to culture due to starch deposition which leads to differentiation into male gametophyte (Silva, 2010). The above conditions correspond to the 3-4 cm distance between collar of flag leaf and ligule of penultimate leaf. Therefore, it is essential to optimize anther culture response in rice by judging optimum developmental stage of pollen grain in anther by acetocarmine staining.

iv. Pre-treatment: Anthers exposed to various kinds of stresses (like cold, heat, osmotic stress, sugar starvation, γ-irradiation and chemical treatment) prior to *in vitro* culture reportedly induce androgenesis (Shariatpanahi *et al*., 2006) and inhibit callus induction from somatic origin (anther wall and tapetum). However, the type and duration of pre-treatments varies with the species and variety of rice.

a) **<u>Cold pre-treatment</u>:** A low temperature stress induces and ensures continuance of sporophytic mode of development of microspores (Matsushima *et al*., 1988) instead of follow-up gamete formation. It triggers embryogenesis from microspores and increases the frequency of spontaneous development of DH plants. Pre-treatment of anthers at 10^{o}C for 11-12 days results in better callusing response but leads to albino plantlet regeneration (Gupta and Borthakur, 1987), while pretreatment at 12^{o}C for 5 days gives best regeneration response (Kaushal *et al.*, 2014a). Also, the cold pre-treatment eliminates weak or non-viable microspores in culture, delays anther wall senescence, increases symmetric division of pollen grains and improves release of necessary substances (cold shock proteins and amino acids) for androgenesis (Kiviharju and Pehu, 1998).

b) **Heat shock treatment:** Heat temperature pre-treatment synchronizes physiological states of microspores and stimulates the embryoid formation by disrupting cytoskeleton at initial stage of microspore development (Ferrie and Keller, 1995).

c) **Osmotic stress:** Osmotic stress e.g., mannitol can offer as a substitute or replace the pre-cold treatment as it enables physiological isolation microspores leading to embryoid formation. It improves sugar uptake so leads to increase in glucose level in anther tissue. Treatment of 0.4 M mannitol to anthers was useful to boost androgenesis in anther culture of Indica cultivar IR 43 from 3 to 33%. However, cold treatment combined with mannitol induced osmotic stresshad no added advantageor even detrimental (Pande, 1997).

d) **Sugar starvation:** Sugar starvation contributes to the promotion of high frequency embryogenesis and plantlet regeneration from microspores isolated from anthers of Indica and Japonica rice and also in isolated microspores (Raina and Irfan, 1998). Sugar starvation of anthers of Indica rice variety IR 43 for 2 days at the beginning of culture caused 12-fold increase in androgenic response (Ogawa *et al.*, 1995). However, cold treatment proved superior to sugar starvation.

e) **Gamma (γ)-irradiation:** Low irradiation pre-treatment promotes embryogenesis in anther culture. Chen *et al.* (2001) reported significant stimulatory effect of 20 kR γ-ray treatment on regeneration of green plantlets. Similarly, Mkuya *et al.* (2005) observed prolific doubled haploid production and green plant regeneration following γ-irradiation of Indica rice line TM7-5.

f) **Chemical treatment:** Ethrel and ethephon (ethylene releasers) have pronounced effect on haploid production in various plant species. Plants are sprayed with 4000 ppm ethrel just before meiosis in pollen mother culture which results in multinucleated (4-6) pollen with a few starch grains. Parthenogenic embryoids may be induced due to the additional mitosis from such pollen grains in culture (Chawla, 2010). Wang *et al*. (1979) reported enhancement in anther culture response with 4000 ppm ethrel (2-chloroethyl phosphonic acid) treatment for 48 h at 10^{o}C. Also, the androgenic response of rice anthers was enhanced when inflorescences were pretreated with ethrel.

v. Culture media: Haploid plants can be produced either through embryo formation or callusing (Niizeki and Oono, 1968) by varying medium composition. N_6, MS and SK1 solid media are *in vogue* used for anther culture with N_6 being more potent than other media (Siddique, 2015) and even in comparison to MO19, Blayde's, Heh 5, MSN1, SK 8 and R 2 media in callus induction and green plant regeneration (Mishra *et al*., 2011). Frequency of callus formation were better in N_6 medium as compared to MS medium (11.9 and 7.9%, respectively) (Thuan *et al.*, 2001). Lentini *et al*. (1995) favoured liquid media for callus induction as it provides greater access to nutrients and hormones with more chances of rapid dispersion of toxic substances. The choice of medium proved to be genotype-specific (Talebi*et al*., 2007). Khanna and Raina (1998) indicated significant genotype x culture media interaction for regeneration response in three Indica rice cultivars. N_6 medium responds better to anther culture in Japonica than Indica rice (Lentini*et al.*,1995). Kaushal *et al*. (2014b) reported highest callus induction, green plant regeneration and least albino plant development (40.6, 40.9 and 3.7%, respectively) in He-2 medium.

Higher doses of nitrogen, phosphorus and potassium in medium leads to better anther culture response in Indica rice (Silva, 2010). Generally, ammonium nitrogen (NH_4^+) is required in low amounts for anther culture in cereal. N_6 medium being high in NO_3^- and low in (NH_4^+) has proved very efficient for Japonica rice anther culture. Sucrose and iron are crucial for pollen embryo developmentand chelated form of iron (Fe-EDTA) is more effective than ferric citrates. Maltose showed better callusing and regeneration response than sucrose. Besides, higher ratio of green plantlets to albinos in both Japonica and Indica types was realized due to mannitol as carbon source (Javed *et al.*, 2007). Alternatively, ficoll (a synthetic polymer of sucrose) could be used to increase the surface density and improve the ratio of green to albino plantlets and to maintain optimum osmotic pressure. Gel rite, a gelling agent alternative to agar, seems to be the most effective one. Addition of organic additives (yeast extracts @100 mg L^{-1}, casein hydrolysate @ 500 mg L^{-1},

coconut water 5-10%) to N_6 further enhanced androgenic callus induction in Indica rice varieties (Roy and Mandal, 2005). Besides, the requirement of amino acids (e.g. glutamine and alanine) is realized in Indica rice for callus formation and green plant regeneration. $AgNO_3$ supplementation to medium induces callusing response and promotes regeneration of green plants (Sah, 2008).

vi. Hormonal requirement: 2,4-D and NAA alone or along with kinetin in culture medium seem to be the major determinants for embryogenic callusing from rice anthers (Mukherjee *et al.,* 2015). Neither 2,4-D nor NAA alone in media support regeneration, but the use of cytokinins like Kn and BAP with NAA are required for regeneration. A combination of 0.5 ppm IAA (indole acetic acid) and 2.0 ppm BAP facilitates germination of androgenic embryos. IAA and NAA may induce direct androgenesis, while 2,4-D promotes callus induction and rapid cell proliferation. Lower concentration of BAP enhances micro-tillering from androgenic plantlets (Roy and Mandal, 2011) and NAA promotes the formation of roots. Thuan *et al*. (2001) reported better callus induction from anthers of F_1 plants derived from four crosses of aromatic and improved rice cultivars cultured in N_6 and MS media supplemented with 2,4-D (0.5 mg L^{-1}) + NAA (1.0 mg L^{-1}) + BAP (0.5 mg L^{-1}). Nirouli and Bimb (2009) reported higher callus induction frequency in N_6 medium with 2,4-D (2.5 mg L^{-1}) + 0.5 mg L^{-1} Kn than N_6 + NAA (4 mg L^{-1}) + Kn (0.5 mg L^{-1}); but reverse was the case for green plant regeneration. Xa and Lang (2011) reported 5.1 to 9.3% callus induction and 6.0 to 14.0% regeneration from four crosses in MS medium with combination 1mg L^{-1} BA + 2 mg L^{-1} Kn + 3% sucrose. Besides, MS medium with 10% coconut milk and addition of Kn (0.5 mg L^{-1}), BAP (2 mg L^{-1}) and NAA (1 mg L^{-1}) gave consistently high frequency of plantlet regeneration from anther derived calli of a wide range of genotypes (Rout and Sharma, 2001).

Embryo formation (10.8%) markedly increased by ABA (abscisic acid) in callus induction media or its treatment during cold pre-treatment period (Sohn, 1996). Ethylene is produced in plant cell due to the presence of auxin, sucrose or calcium in callus induction medium (Hepler, 2005). Profuse callusing may be due to inhibitory effect of endogenously produced ethylene from excised anthers. $AgNO_3$ and polyamines also affect anther culture response in rice through inhibition of ethylene synthesis (Dewi and Purwoko, 2008). Putrescine application to callus medium also increase the frequency of green/albino regeneration frequency both in Japonica and Indica varieties.

vii. Culture conditions: Anther derived callus was observed in Azucena rice variety (6.66%) while no callusing was found in 'Budda' variety when the cultures were exposed to dark at 23±2°C (Lal *et al.,* 2014). Highest callus induction was observed from anther culture of rice variety 'Rajalaxmi and Ajay' by keeping the culture at 25±1°C for 3-4 weeks (Mishra *et al*., 2011) and from anthers of six F_1 hybrids within 60 days and highest regeneration frequency was obtained by keeping at 18 h photoperiod with a light intensity of 27 µmol m^{-2} s^{-1} at 25±1°C (Lee *et al.,* 2003a) which was also observed in Japonica variety (Mankeumbyeo, recurrent parent) and a recalcitrant Indica variety ('RantaEmas', donor parent) at the same culture conditions (Lee *et al.,* 2004).

High embryonic culture induction was obtained by culturing of anthers of rice cv. 'PT-1', 'KDML-105', and 'HJ' at 25±2°C in darkness for 6 weeks (Chaum *et al*., 2009). Highest callus induction frequency was obtained in anther culture of Japonica × Indica, Indica × Japonica hybrids (Trejo-Tapia *et al*., 2002) at 25±2°C for 8 weeks. Androgenic callus induction as well as green plantlet regeneration was observed by keeping anther culture of five Indica rice genotypes ('IR 72', 'Mansarovar', 'Taraori Basmati', 'Pusa Basmati' and 'Karnal local 95') (Roy and Mandal, 2005) and Indica rice genotypes ('GR 11' and 'Gurjari') in dark at 25±2°C (Patel *et al.,* 2014). Callus induction frequency and regeneration efficiency at 30/20°C day/night temperature in dark for 4 weeks was increased by 1.4 and >2 times when kept at 25°C (Okamoto *et al.,* 2001). Initial incubation in dark at 26±2°C for 6 weeks, followed by 12 h photoperiod for another 2 weeks resulted in highest callus induction and plant regeneration response in six Indica ('HKR120', 'HKR 86-3', 'HKR86-217', 'PR106', 'Gobind' and 'CH 2 double dwarf') and two Basmati rice ('Basmati 370', 'Taraori Basmati') varieties and 14 heterotic Indica Basmati F_1 hybrids. Though callus induction was observed by incubation at 27°C, but regeneration ability was lost after 11days (Chen

et al., 2001). In contrast, incubation at above temperature was satisfactory in an inter-specific hybrid (*O. sativa* x *O. rufipogon*) for callus induction in dark and for plantlet regeneration in 16 h photoperiod (2000 lux) (Mandal and Gupta, 1997). However, callus induction frequency (0.75 to 5.15%) was found from anthers of two Indica elite lines ('MRP5401' and 'BR29') and one Japonica variety ('Taipei 309') with exposure to white light for 16 h at 28±2^{o}C until shoots develop in about 3-6 weeks (Bhattacharya *et al*., 2014).

4. PLOIDY STATUS OF ANTHER DERIVED CALLI AND PLANTLETS

Various morphological attributes are used to verify the ploidy statusand DH's can be identified by comparing plant morphology such as plant height, leaf size, flower morphology, plant vigour and fertility. Haploid plants show reduced plant vigour with degenerated flowers or anthers and are mostly sterile or show greatly reduced fertility; whereas diploid individuals resemble the donor plant and are often characterized by normal flower and pollen development. However, this method is not 100% foolproof. Cytological analysis of anther/pollen culture can be used to unfold the factor(s) controlling pollen embryogenesis of higher plants. Study of meiotic behaviour of haploids provides the status of chromosome duplication. The principle of androgenesis is to arrest the development of male gametophytes (pollen grains) and to force them to follow sporopytic (somatic) pathway. Under certain culture conditions in anther culture, the pollen grain (haploid) induce, divide and double the chromosome number while reversing the process of gametophyte (early- to mid-uninucleate) to sporophytic stage. The ploidy status of regenerated plants can be confirmed by counting chromosome number in root tips and in pollen mother cells following usual acetocarmine staining technique. About 70% of regenerated plants are normally haploids; while rest account for *in vitro* euploids (including spontaneous diploids) and aneuploid. The ploidy level in plants can be also estimated by chloroplast count in guard cells or chromosome count of root tip cells (5-8 in haploids, 10-12 in diploids and 15-18 in triploids and still more in case of higher levels of ploidy). However, recently measuring the C-value (amount of DNA in unreplicated gametic nucleus) using flow cytometry seems to be a suitable approach (Ochatt *et al*., 2009).

5. PRODUCTION OF DOUBLE HAPLOIDS

Doubled haploid (DH) lines derived from anther culture of F_1 hybrids are promising tools to develop plant cultivars. Colchicine (a mitotic inhibitor) treatment is used to induce chromosome doubling by inhibiting anaphase movement in crop plants. Colchicine treatment of apical meristem of anther-derived haploid plants produce DH cells in small sectors which normally produce DH seeds. Microspore derived calli often show aneuploids, dihaploids and polyploids (Debata and Patnaik, 1989). Occurrence of mixoploids and polyploids resulting in chimeric plants and low seed set is prevalent in colchicine treatment, hence is normally avoided at whole plant level in rice. However, the application of colchicine during early stages of androgenesis could alleviate above problems (Castillo *et al*., 2009) and increase the frequency of DH development in the shortest time. Supplementation of 0.2-0.5 g colchicine L^{-1} for 24-48 h incubation results in viable double haploid embryo-like structure formation which on transfer to colchicine-free regeneration medium induces as high as 65-70% viable DH plantlets and it was twice in comparison to spontaneous doubling (Premvaranon *et al.*, 2011). Alternatively, the anther culture derived haploid plants may be immersed in 0.1% colchicine and 2% dimethyl suphoxide (DMSO) solution for 4-5 h (Zapata and Aryas, 2003) or colchicine (1.25 mM) and oryzalin (25 µM) solution for 12 h (Chen *et al.*, 2001) to obtain mirror image of haploid set of chromosomes in DH lines. In rice, haploid set of chromosomes in microspores also become spontaneously doubled under suitable culture conditions. Germana (2011) reported higher (50-60%) spontaneous chromosome doubling (endoreduplication) in culture. Lee *et al.* (2003b) recovered a maximum of 60% spontaneous DH green plantlets through mutagenesis in Japonica rice. Chen *et al*. (2001) reported that colchicine treatment (30 mg L^{-1}) has significant stimulating effect on regeneration of higher frequency of green DH mutant

plantlets from anther culture of rice varieties. Thus, colchicine treatment may be omitted for DH production in rice.

Jones *et al*. (1996) was successful to recover DH lines from hybrids *O. sativa* x *O. glaberrima.* Higher number of DH lines were recovered in Indica rice (var. 'Gobind', 'HKR120', 'Basmati 370' and 'Taraori Basmati') and an Indica-Basmati hybrid via colchicine treatment (0.1%, w/v). Rout *et al*. (2018) was able to produce DH lines from an elite long duration rice hybrid 'CRHR 32' through anther culture. Similarly, Naik *et al.* (1016) recovered a few promising uniform and stable DH lines from Indica rice hybrid using morphological and molecular markers. Sasmita (2009) observed that in> 90% cases of anther culture, homozygous and stable plants progeny were observed from generation to generation. However, Bhattacharya *et al*. (2014) advocated chromosome counting combined with segregation analysis to distinguish diploids, haploids and double haploids plants.

6. GENETIC BASIS OF ANTHER CULTURE RESPONSE

Genetic control of microspore response to *in vitro* culture in rice has been studied by many workers and it is largely considered specie- and genotype-specific (Dash *et al.*, 2014). *O. glaberrima* responds more to callus induction and plantlet regeneration than *O. sativa* genotypes. All *O. glaberrima* genotype regenerated plants and a 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 are >20-fold responsive (28.1%) to microspore embryo-genesis (Grewal *et al.*,2011). Diverse genotype-specificity of anther culture exists within Indica subspecies (Talebi*et al.,* 2007). Temperate rice (hill rice) cultivars are more responsive to anther culture than tropical rice (Niroula and Bimb, 2009). Anther culture ability is decreased in the order of Japonica/Japonica F_1> Japonica> Indica/Japonica F_1> Indica/IndicaF_1> Indica (Yan *et al.*, 1996). 'IR 43' produced maximum embryiods (16.1%) and green plantlets (11.9%) followed by 'BRRI Dhan 33', 'IR 54', 'Jaya' and 'BR3' (Khatun *et al*., 2012). Indica x Indica hybrids produce more albino plantlets as compared to Indica x Japonica hybrids (Rangasamy *et al.,* 1992). However, 'CRMS31B x CRMS24B' among 4 inter-varietal crosses was highly responsive for callus induction (37.8%) and shoot regeneration (41.1%) and green plant regeneration (15.0%). Genes encoding certain enzymes are essential for metabolism of differentiating cells, tissues and organs. Calli with higher levels of peroxidase enzyme reportedly display greater regeneration potential than those having low levels of enzyme producing predominantly albinos. Induction of sporophytic haploidy in rice anther culture is controlled by haploid (gametophytic) inhibitor gene '*hap'* (Kasha and Sequin-Swartz, 1983) which is activated by cold pre-treatment. This reverses the gametophytic development of pollen grain to sporophytic status. Kiruchi *et al.* (2003) have reported that androgenesis in rice may be due to the activation of a new class of Miniature Inverted-repeat Transposable Elements (MITE: mapping elements) in anther derived calli. Among 13 genotypes evaluated, a rice genotype 'IR58025B' with *eui* (25eB) gene was reported to be highly responsive for both callus induction and green plantlet regeneration (Kaushal *et al*., 2014b). Japonica/Indica crosses usually result in partial sterility due to the interaction between Indica allele S-5i and Japonica allele S-5j in chromosome 6. This causes partial abortion of female gamete containing S-5j. A recent report has shown that "Indica T 23" rice genetic stock with wide compatible allele S-5n (in chromosome 6) responds well to anther culture (Nguyen *et al*., 2016). Dular – an Indica variety has also the allele S-5n at S-5 locus which is widely compatible with both S-5i and S-5j. However, many often segregation distortion (SD) at S-5 locus is reported which may be due to the preferential selection of gametes with S-5i Indica allele for androgenesis (Yang *et al*., 2012). 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 induced callus in rice are the two independent traits displaying quantitative inheritance (Bagheri and Jelodar, 2008). Genetic analysis

could be performed on haploid population to establish inheritance patterns. Additive effects seem more important than dominant effects of the genes concerned for callus induction while preponderance of dominant effects is reported for regeneration response (He *et al.*, 2006). Quimo and Zapata (1990) has suggested the predominance of additive effects controlling both characters with Japonica cultivars having higher general combining ability for green plant regeneration. However, callus induction is mainly controlled by gametic additive effects than maternal effects, while reverse is the case for green plantlet regeneration in anther culture (Yan *et al.,* 1996). 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 reportedly follows recessive inheritance (Miah *e t al.*, 1985) conditioned by block of genes (QTLs) on two specific chromosomal regions and Japonica appears to be a good combiner for callus induction. A QTL on chromosome 1 controls callus formation and another on chromosome 10 determines the balance between albino/green plant regeneration ability (Yamagishi *et al.*, 1998). He *et al*. (1998), however, 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*. (2002) 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, AFLP and SSR have proved powerful tools for the characterization of DH lines (Lee *et al*., 2014). Grewal *et al*. (2011) used a set 124 DH lines for SSR marker analysis generated from 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 partially dominant. Hemaprabha *et al.* (2013) 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 (Bolibok and Rakcoczy-Trojanowska, 2006). Silva (2010) has identified several genes that are specifically expressed in calli with good regeneration ability and some of these have been cloned. Transformation of a genotype, showing poor response in tissue culture with a gene that putatively encodes glucose dehydrogenase, has greatly enhanced the differentiation rate of calli (Ozawa *et al.*, 2003).

7. DOUBLE HAPLOID BREEDING

Anther culture is a useful biotechnological tool applied in plant breeding (Tripathy, 2018; Ruwani *et al*., 2018). Creation of genetic variability is an essential step in any crop improvement programme. Breeders often resort to hybridization or mutation breeding and even genetic transformation for genetic improvement of specific trait(s). Hybrids possess good broad genetic backgrounds than their parents. Anther culture in rice has potential to proportionally rescue microspores of hybrids according to gametophytic gene effect and enhance genetic study of hybrid sterility (Kanaoka *et al*., 2018). So, breeding them through double haploid production is an important method. DH breeding provides fertile segregants with fixed derived character combination (Fig. 2) that might otherwise disappear in the course of an extended series of segregating generations in conventional breeding methods. Variation in DH lines from F_1 hybrids may be due to the recovery of rare gene combinations (following meiosis) even in a small population size of 200 DH plants. However, the plants derived from multiple shoots of a single callus show high phenotypic similarity than those derived from different calli. The regenerants, in addition to biochemical and chromosomal changes, may show heterozygosity due to anther wall somatic tissues and a wide range of gametoclonal variation (Narasimman and Rangasamy, 1993). Such genetic variation among DH lines can be harnessed for improvement of agronomic characters and the production of new cultivars and breeding lines.

Sterility problem in interspecific cross (*O. sativa* x *O. glaberrima*) was overcome by double haploid approach (Enriquez *et al.*, 2000). A few DH lines derived from anther culture of hybrids of

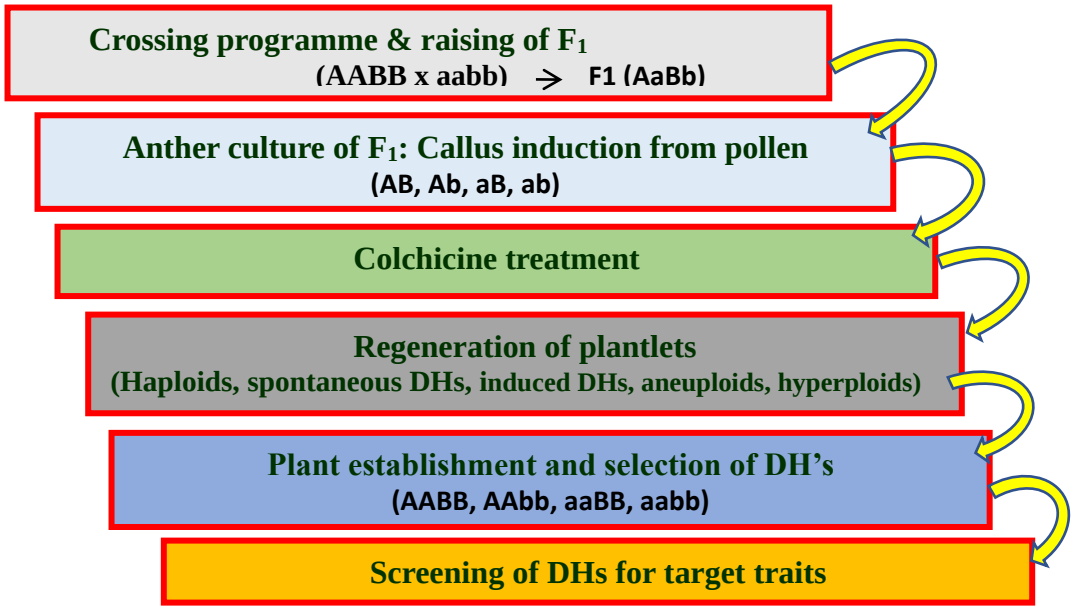

Fig. 2: Steps in double haploid breeding for genetic improvement of rice

genetically diverse parents could reach the heterotic level of hybrid (Ba Bong and Swaminathan, 1995) which pinpointed the prospect of anther culture technique in rice. DH lines with earliness, enhanced biomass production, high panicle density and increased yield potential with resistance top stand grain quality could be produced from anther culture of different crosses. DH lines from cv. 'Calrose 76' showed variation with improved agronomic characters e.g. seed number size, panicle size, plant height and tiller number and improved protein content and higher yields. Roy and Mandal (2005) reported high genetic variability for filled grains panicle^{-1}, grains yield plant^{-1} and number of panicles plant^{-1} among androgenic plantlets derived from scented Indica rice cv. 'Karnal local 95', suggesting the scope of haploid breeding for higher seed yield. Xa and Lang (2011) developed 22 outstanding DHLs for high grain quality through anther culture. Bhattacharya *et al.* (2014) confirmed 98 lines to be DHs out of 232 lines recovered from 'Mahyco Hybrid MRP5401' which had dwarf height, early to late flowering and high yield characters. Development of improved DHs for salt tolerance (Lee *et al.*, 2003a), submergence tolerance (Mandal and Gupta, 1997) and bacterial blight resistance (Sengsai *et al.*, 2007) have been reported. Mohiuddin *et al.* (2014) recovered genetically uniform dwarf DHs from anther culture of an advance breeding line 'BR802-78-2-1-1'. A few of these DHs showed high fertility status of spikelet with long-bold and long-slender grain. Fertile DH plantlet regeneration was also reported in a salt susceptible × salt tolerant rice hybrid (Chowdhury and Mandal, 2001). DH lines produced from anther culture have many advantages like improved grain quality, resistance to diseases, tolerance to biotic and abiotic stress, superior performance for some agronomic traits over parents and/or lines used as checks. Mishra *et al.* (2015) studied physicochemical characteristics and cooking quality in 20 recombinant DH lines derived from rice hybrid 'Ajay and Rajlaxmi'. Eight derivatives from each hybrid possessed grain quality characteristics at desired levels and some were better than their respective parents in terms of both quality and uniformity. Xa and Lang (2011) recovered 133 DH lines out of which 22 outstanding DH lines were selected for yield and grain quality. Purwoko *et al.* (2010) recovered 92 DH lines from 13 crosses of which 24 lines had seed yield more than 26 g hill^{-1} (transplanted single seedling hill^{-1}) with tolerance to biotic and abiotic stresses.

More than 200 crop varieties have been developed worldwide by DH approach (Thomas *et al.*, 2003) amongst which 40 are rice varieties (Table 1). Senadhira *et al.* (2002) developed first salt tolerant rice cultivar through Indica/Indica anther culture. 'Dama' was the first Hungarian rice variety developed by haploid somaclone breeding in 1992 (Heszky and Simon-Kissn, 1992). In

Table 1: List of rice varieties released using double haploid breeding

Country	Rice variety	Trait(s)	Reference(s)
China	Guan 18, Gan Xhao Xian No. 11	Early maturity; good quality and disease resistance	Zhu and Pan (1990)
South Korea	Hwacheongbyeo, Joryeongbyeo, Hwajinbyeo	Brown plant hopper, stripe virus, blast and bacterial blight resistance	Lee *et al.* (1989)
India	CR Dhan 801, Phalguni	Leaf blast, gall midge resistance	NRRI (2010)
India	CR Dhan 10, Satyakrishna	Resistant to neck blast, sheath rot	NRRI (2008)
China	Huayu 15	Lodging and diseases resistance; good grain quality	Shouyi and Shouyin (1991)
Republic of Korea	PSBRc 50 Bycol (IR 51500-AC-11-1)	Salt tolerance	Senadhira *et al.* (1996)
Philippines	PSBRc 50 Bycol (IR51500AC-11-1)	Salt tolerance	Senadhira *et al.* (2002)
India	Janka	Drought tolerance; good grain quality	Pauk *et al.* (2009)
India	Abel	Cold tolerance at early stage	Pauk *et al.* (2009)
Japan	Joiku N 394, Hirohikari, Hirohonami, AC. No.1, Kibinohana	Cold tolerance and good taste	Singh (1998)
China	Huayu I, Huayu II, Xin Xiu, Late Keng 959, Tunghua 1, Tunghua 2, Tunghua 3, Zhonghua 8, Zhonghua 9, Hua 03, Huahanzao, Huajian 7902, Tanghuo 2, Shanhua 7706, Huahanzao 77001, Noll	High yield, superior grain quality; blast and bacterial blight resistance	Yang and Fu (1989)
Hungary	Dama	High yield, resistant to *pyricularia*, good cooking quality	Heszky and Simon-Kiss (1992)
China	Milyang 90	Good grain quality; brown plant hopper and stripe virus resistance	Chung (1987)
India	Parag 401	Superior grain quality and resistant to iron chorosis	Patil *et al.* (1997)
China	Hua 03	High protein content (13.7%)	Yang and Fu (1989)
India	Risabell	High milling, good cooking quality and resistant to blast	Pauk *et al.* (2009)

India, ‘Satyakrishna’ (CR Dhan 10) and ‘Phalguni’ (CR Dhan 801) are the first released varieties from DH lines. ‘Guan 18’ and ‘Gan Xhao Xian No. 11’ are the outcome of anther culture in China which mature within 105 days and possess high yield potential (5.6-6.0 t ha^{-1}). Besides, several rice varieties have been developed by anther culture in China (‘Tanfeng 1’, ‘Bua Yu 2’, ‘Hua Yu 1’, ‘Hua 03’, ‘Xin Xiu’, ‘Xhongua 8’, ‘Ta Be 78’) and Argentina (‘Patei’ and ‘Moccoi’).

Anther culture derived DH populations provide a link between conventional plant breeding and genomics. DH populations are quite useful for chromosome mapping (Forster and Thomas, 2005) and genetic analysis of QTLs (Lu *et al.*, 1996). DH lines are permanent fixed genotypes (Semagn *et al*., 2006) which 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 and BC_3 populations and is often preferred over near isogenic lines (NILs), backcross 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 (Singh and Singh, 2015). In DH populations, the identification of DNA markers seems much reliable (Sammour *et al.*, 2015) as the gene segregates in 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 (Guo *et al.*, 2004), grain quality (Ren *et al.*, 2016), cooking quality (Tian *et al.*, 2005), seed yield (Park *et al.*, 2014) and resistance to brown plant hopper (Hu *et al.*, 2016), leaf folder (Rao *et al.*, 2010), blast (Jichen *et al.*, 2004), drought (Xu *et al.*, 2001) and salt stress (Fan *et al.*, 2015). Besides, validation of quantitative trait loci associated with reproductive-growth traits and grain yield under drought stress in a DH population of rice (*O. sativa*) was studied by Sellamuthu *et al.* (2011). DH line population derived from cross 'CT9993-5-10-1-M/IR62266-42-6-2' is tested for 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 (Kapusi *et al.*, 2013). Jiang *et al*. (2004) elucidated the genetic basis of stay-green 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 (Chaleff and Stolarz, 1982).

7. LIMITATIONS

Early anther necrosis, poor callus proliferation and albino-plant regeneration are currently recognized as the major problems in Indica rice varieties (Khatun *et al*., 2012). In Indica rice, callusing and follow-up regeneration response from anthers is very meager. At least 15,000 anthers need to be inoculated for *in vitro* culture to obtain regeneration of 150 plantlets (Huan, 1995). A higher percent-age of regenerated plants in anther culture are albinos (Talebi*et al.*, 2007). Gueye and Ndir (2010) could recover 93 regenerants out of which 79 were albinos. The basic cause for albino plant production is breakage of DNA in plastids and nuclei (Kumari *et al.*, 2009). This problem is more serious in Indica rice than Japonica rice. As per Asaduzzaman *et al*. (2003), albinism can be reduced by shortening the culture period. Frequency of albinism among regenerated is controlled by QTL on chromosome 9 and 10 (Yamagishi *et al.*, 1998). This problem can be minimizedby early transfer of anther culture-induced calli into regeneration media, a low temperature incubation($<26^{o}C$) or medium modification for callus induction and plant regeneration.

8. EPILOGUE

In rice, maximum number of crop varieties has been developed through recombination breeding (hybridization). Several breeding methods are now available to broaden genetic variation and recover desirable gene combinations. In each case, the breeding population is derived from a single zygote. In contrast, each anther of an inter-varietal/interspecific hybrid carries thousands of pollen grains with different genotype and these can be induced to form stable DH plantlets within a short time. Hence, a highly reproducible anther culture technique can be an alternative to recover homozygous plants with rare gene combinations and accelerate the breeding process in order to respond to climate change. The anther culture derived DH populations may be amenable for molecular mapping of valuable genes and isolation of plant types with high yield, disease resistance and improved quality traits. Besides, a significant proportion of haploids regenerated through anther culture of a plant variety may be valuable to detect and fix (by colchicine) desirable recessive traits induced through mutation or spontaneous gameto-clonal variation.

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REFERENCES

Asaduzzaman, M., Bari, M.A., Rahman, M.H., Khatun, N., Islam, M.A. and Rahman, M. 2003. *In vitro* plant regeneration through anther culture of five rice varieties. *Journal of Biological Sciences*, **3**: 167-171.

Asif, M. 2013. History, production methods and types of haploids. pp. 10-11. **In**: *Progress and Opportunities of Double Haploid Production* (Ed. Mahammad Asif). University of Alberta, Alberta, Canada.

Ba, Bong and Swaminathan, M.S. 1995. Magnitude of hybrid vigour retained in double haploid lines of some heterotic rice hybrids. *Theoretical & Applied Genetics*, **90**: 253-257.

Bagheri, N. and Jelodar, N.B. 2008. Combining ability and heritability of callus induction and green plant regeneration in rice anther culture. *Biotechnology*, **7**(2): 287-292.

Bhattacharya, A., Mikkilineni, V., Verma, L., Palan, B., Mali. K. and Char, B. 2014. Evaluation of doubled haploid culture conditions and regeneration of an Indica rice hybrid. *Indian Journal of Genetics*, **74**: 384-386.

Bolibok, H. and Rakcoczy-Trojanowska, M. 2006. Genetic mapping of QTL for tissue culture response in plants. *Euphytica*, **149**(1-2): 73-83.

Castillo, A.M., Cistué, L., Vallés, M.P. and Soriano, M. 2009. Chromosome doubling in monocots. pp. 329-338. **In**: *Advances in Haploid Production in Higher Plants* (eds. A. Touraev, B.P. Forster and S.M. Jain), Springer, Dordrecht, Netherlands.

Chaleff, R.S. and Stolarz A. 1982. The development of anther culture as a system for *in vitro* mutant selection. pp. 63-74. **In**: *Rice Tissue Culture Planning Conference.* International Rice Research Institute. Los Banos, Laguna, Manila, Philippines.

Chaum, S., Srianan, B., Pichakum, A. and Kirdmanee, C. 2009. An efficient procedure for embryogenic callus induction and double haploid plant regeneration through anther culture of Thai aromatic rice (*Oryza sativa* L. subsp. Indica). *In vitro Cellular & Development Biology - Plant*, **45**: 171-179.

Chawla, H.S. 2010. *In vitro* production of haploids. pp. 94-95. **In**: *Introduction to Plant Biotechnology* (3rd edn.). Oxford & IBH Pub., New Delhi, India.

Chen, C., Xiao, H., Zhang, W., Wang, A., Xia, Z., Li, X., Zhai, W., Cheng, Z. and Zhu, L. 2006. Adapting rice anther culture to gene transformation and RNA interference. *Science China C: Life Science*, **49**: 414-428.

Chen, Q.F., Wang, C.L., Lu, Y.M., Shen, M., Afza, R., Duren, M.V. and Brunner, H. 2001. Anther culture in connection with induced mutations for rice improvement. *Euphytica*, **120**: 401-408.

Chowdhury, B. and Mandal, A.B. 2001. Microspore embryogenesis and fertile plantlet regeneration in a salt susceptible × salt tolerant rice hybrid. *Plant Cell, Tissue Organ Culture*, **65**: 141-147.

Chung, G.S. 1987. Application of anther culture technique for rice (*Oryza sativa* L.) improvement. pp. 36-56. **In**: *Korea-China Plant Tissue Culture Symposium.* Academia Sinica Publishers, Beijing, China.

Dash, A.K., Rao, J.G.N. and Rao, R.N. 2014. Effect of genotype on anther culture response in Indica rice hybrids of maintainer lines. *Oryza*, **51**: 165-167.

Debata, B.K. and Patnaik, S.N. 1989. *In vitro* anther and pollen culture for induction of haploids. pp. 885-901. **In**: *Plant Science Research in India* (eds. M.L. Trivedi, B.S. Gill and S.S. Saini), Today and Tomorrow's Printers and Publishers, New Delhi, India.

Dewi, I.S. and Purwoko, B.S. 2008. Role of polyamines in inhibition of ethylene biosynthesis and their effects on rice anther culture development. *Indonesian Journal of Agricultural Science*, **9**(2) :60-67.

Enriquez, E., Brar, D.S., Rosario, T.L., Jones, M. and Khush, G.S. 2000. Production and characterization of doubled haploids from anther culture of the F_1s of *Oryza sativa* L. x *O. glaberrima* Steud. *Rice Genetics Newsletter*, **17**: 67-69.

Fan, Y., Shabala, S., Ma, Y., Xu, R. and Zhou, M. 2015. Using QTL mapping to investigate the relationships between abiotic stress tolerance (drought and salinity) and agronomic and physiological traits. *BMC Genomics*, **16**: 43.

Ferrie, A.M.R. and Keller, W.A.1995. Development of methodology and applications of doubled haploids in *Brassica rapa*. pp. 807-809. **In**: *Proceedings of the 9th International Rapeseed Congress.* Cambridge, UK.

Forster, B.P. and Thomas, W.T.B. 2005. Doubled haploids in genetics and plant breeding. *Plant Breeding Reviews*, **25**: 57-88.

Germana, M.A. 2011. Anther culture for haploid and doubled haploid production. *Plant Cell Tissue and Organ Culture*, **104**: 283-300.

Grewal, D., Manito, C. and Bartolome, V. 2011. Doubled haploids generated through anther culture from crosses of elite Indica and Japonica cultivars and/or lines of rice: Large-scale production, agronomic performance, and molecular characterization. *Crop Science Society of America*, **51**: 2544-2553.

Gueye, T. and Ndir, K.N. 2010. *In vitro* production of double haploid plants from two rice species (*Oryza sativa* L. and *Oryza glaberrima* Steudt.) for the rapid development of new breeding material. *Scientific Research and Essays*, **5**(7): 709-713.

Guha, S and Mukherjee, S. 1973. Genotypic differences in the *in vitro* formation of embryoids from rice pollen. *Journal of Experimental Botany*, **24**: 139-144.

Guha, S. and Maheswari, S.C. 1964. *In vitro* production of embryos from anthers of *Datura*. *Nature*, **204**: 497.

Guo, L., Zhu, L., Xu, Y., Zeng, D., Wu, P., and Qian, Q. 2004. QTL analysis of seed dormancy in rice (*Oryza sativa* L.). *Euphytica*, **140**(3): 155-162.

Gupta, H.S. and Borthakur, D.N. 1987. Improved rate of callus induction from rice anther culture following microscopic staging of microspores in iron alum-haemotoxylin. *Theoretical & Applied Genetics*, **74**: 95-99.

He, P., Shen, L.S., Lu, C.F., Chen, Y. and Zhu, L.H. 1998. Analysis of quantitative trail loci which contribute to anther culturability in rice (*Oryza sativa* L.). *Molecular Breeding*, **4**: 165-172.

He, T., Yang, Y., Tu, S.B., Yu, M.Q. and Li, X.F. 2006. Selection of interspecific hybrids for anther culture of Indica rice. *Plant Cell Tissue and Organ Culture*, **86**: 271-277.

Hemaprabha, K., Hemalatha, T., UmaMaheswari. T., Anbukkarasi. K. and Shanmugasundaram. 2013. Genetic diversity analysis of anther culture derived rice plants using molecular markers. *International Journal of Advanced Research*, **1**(5): 103-112.

Hepler, P.K. 2005. Calcium: A central regulator of plant growth and development. *The Plant Cell*, **17**: 2142-2155.

Herath, H.M.I., Bandara, D.C. and Samarajeewa, P.K. 2007. Effect of culture media for anther culture of Indica rice varieties and hybrids of Indica and Japonica. *Tropical Agricultural Research & Extension*, **10**: 17-22.

Heszky, L.E. and Simon-Kiss, I. 1992. The first plant variety of biotechnology origin in Hungary, registered in 1992. *Hungarian Agricultural Research*, **1**: 30-32.

Hu, J., Xiao, C. and He, Y. 2016. Recent progress on the genetics and molecular breeding of brown planthopper resistance in rice. *Rice*, **9**: 30.

Huan, N.T. 1995. Efficiency of different media used in anther culture of hybrid rice. *International Rice Research Newsletter*, **20**(2): 7.

Jacquard, C., Asakaviciute, R., Hamalian, A.M., Sangwan, R.S., Devaux, P. and Clement, C. 2006. Barley anther culture: Effects of annual cycle and spike position on microspore embryogenesis and albinism. *Plant Cell Reports*, **25**: 375-381.

Javed, M.A., Ishi, T., Kamijima, O. and Misoo, S. 2007. The role of alternating culture temperatures and maltose in enhancing anther culture efficiency of salt tolerant Indica rice (*Oryza sativa* L.) cultivars, Pokkali and Nona Bokra. *Plant Biotechnology*, **24**: 283-287.

Jiang, G.H., He, Y.Q., Xu, C.G., Li, X.H. and Zhang, Q. 2004. The genetic basis of stay-green in rice analyzed in a population of doubled haploid lines derived from an Indica by Japonica cross. *Theoretical & Applied Genetics*, **108**: 688-698.

Jichen, X., Jiulin, W., Zhongzhuan, L. and Lihuang, Z. 2004. Analysis of rice blast resistance genes by QTL mapping. *Chinese Science Bulletin*, **49**(4): 337-342.

Jones, M.P., Dingkuhn, M., Aluko, G.K. and Semon, M. 1996. Using back-crossing and doubled haploid breeding to generate weed competitive rices from *O. sativa* L. x *O. glaberrima* Steud.

pp.16-18. **In:** *Africa/Asia Joint Research on Interspecific Hybridization Between the African and Asian Rice Species (O. glaberrima & O. sativa).* WARDA, Mbe, Ivory Coast, West Africa.

Kanaoka, Y., Kuniyoshi, D., Inada, E., Koide, Y., Okamoto, Y., Yasui, H. and Kishima, Y. 2018. Anther culture in rice proportionally rescues microspores according to gametophytic gene effect and enhances genetic study of hybrid sterility. *Plant Methods*, **14**: 102.

Kapusi, E., Hensel, G., Coronado, M.J., Broeders, S., Marthe, C. and Otto, I. 2013. The elimination of a selectable marker gene in the doubled haploid progeny of co-transformed barley plants. *Plant Molecular Biology*, **81**: 149-160.

Kasha, K.J. and Sequin-Swartz, G. 1983. Haploidy in crop improvement. pp. 19-68. **In**: *Cytogenetics of Crop Plants* (eds. M.S. Swaminanthan, P.K. Gupta and U. Sinha). MacMillan, New Delhi, India.

Kaushal, L., Balachandran, S.M., Ulaganathan, K. and Shenoy, V. 2014a. Effect of culture media on improving anther culture response of rice (*Oryza sativa* L.). *International Journal of Agricultural Innovation & Research*, **3**(1): 218-224.

Kaushal, L., Sharma, R., Balachandran, S.M., Ulaganathan, K. and Shenoy, V. 2014b. Effect of cold pretreatment on improving anther culture response of rice (*Oryza sativa* L.). *Journal of Experimental Biology and Agricultural Science*, **2**(2S): 234-242.

Khanna, H.K. and Raina, S.K. 1998. Genotype x culture media interaction effects on regeneration response of three Indica rice cultivars. *Plant Cell Tissue & Organ Culture*, **52**: 145-153.

Khatun, R., Shahinul Islam, S.M., Ara, I., Tuteja, T. and Bari, M.A. 2012. Effect of cold treatment and different media in improving anther culture response in rice (*Oryza sativa* L.) in Bangladesh. *Indian Journal of Biotechnology*, **11**: 458-463.

Kiruchi, K., Terauchi, K., Wada, M. and Hirano, H.Y. 2003. The plant MITE mping is mobilized in anther culture. *Nature*, **421**(6919): 167-170.

Kiviharju, E. and Pehu, E. 1998. The effect cold and heat pretreatments on anther culture response of *Avena sativa* and *A. sterilis*. *Plant Cell Tissue & Organ Culture*, **54**: 97-104.

Kumari, M. and Clarke, H.J., Small, I., Siddique, K.H.M. 2009. Albinism in plants: A major bottleneck in wide hybridization, androgenesis and doubled haploid culture. *Critical Reviews in Plant Science*, **28**(6): 393-409.

Kwon, Y.S., Kim, K.M., Eun, M.Y. and Sohn. J.K. 2002. QTL mapping and associated marker selection for the efficacy of green plant regeneration in anther culture of rice. *Plant Breeding*, **121**: 10-16.

Lal, D., Shashidhar, H.E., Ramanjini, Godwa, P.H. and Ashok, T.H. 2014. Callus induction and regeneration from *in vitro* anther culture of rice (*Oryza sativa* L.). *International Journal of Agriculture, Environment & Biotechnology*, **7**(2): 213-218.

Lee, S.Y., Cheong, J.I. and Kim, T.S. 2003b. Production of doubled haploids through anther culture of M_1 rice plants derived from mutagenized fertilized egg cells. *Plant Cell Reports*, **22**: 218-223.

Lee, S.Y., Lee, J.H. and Kwon, T.O. 2003a. Selection of salt-tolerant doubled haploids in rice anther culture, *Plant Cell, Tissue and Organ Culture*, **74**: 143-149.

Lee, S.Y., Lee, J.H. and Kwon, T.O. 2004. Variation in anther culture response and fertility of backcrossed hybrids between Indica and Japonica rice (*Oryza sativa*). *Plant Cell, Tissue and Organ Culture*, **79**: 25-30.

Lee, Y.T., Lim, M.S., Kim, H.S., Shin, H.T., Kim, C.H., Bae, S.H. and Cho, C.I. 1989. An anther derived new high quality variety with disease and insect resistance 'Hwacheongbyeo'. *Research Report - Rural Development Administration Rice*, **31**(2): 27-34.

Lee. G.H., Yun, B.W. and Kim, K.M. 2014. Analysis of QTLs associated with the rice quality related gene by double haploid populations. *International Journal Genomics*, **2014**: 1-6.

Lentini, Z., Reyes, P., Martinez, C.P. and Roca, W.M. 1995. Androgenesis in highly recalcitrant rice genotypes with maltose and silver nitrate. *Plant Science*, **110**: 127-138.

López-Cristoffanini, C., Serrat, X., Ramos-Fuentes, E., Hooghvorst, I., Llaó, R., López-Carbonell, M., Nogués, S. 2018. An improved anther culture procedure for obtaining new commercial

Mediterranean temperate japonica rice (*Oryza sativa*) genotypes. *Plant Biotechnology (online)* [doi: *10.5511/plantbiotechnology.18.0409a*].

Lu, C., Shen, L., Tan, Z., Xu, Y., He, P., Chen, Y. and Zhu, L. 1996. Comparative mapping of QTLs for agronomic traits of rice across environments using a doubled haploid population. *Theoretical & Applied Genetics*, **93**: 1211-1217.

Mandal, N. and Gupta, S. 1997. Anther culture of an interspecific rice hybrid and selection of fine grain type with submergence tolerance. *Plant Cell, Tissue and Organ Culture*, **51**: 79-82.

Maria, A.M., Adriana, S. and Ana, M.G. 2006. Plant regeneration from rice anthers cryopreserved by an encapsulation/dehydration technique. *In vitro Cell Development & Biology - Plant*, **42**: 31-36.

Matsushima, T., Kikuchi, S., Takaiwa, F. and Oono, K. 1988. Regeneration of plants by pollen culture in rice (*Oryza sativa* L.). *Plant Tissue Culture Letters*, **5**: 78-81.

Maw, Z.W. and Kenji, I. 2017. Evaluation of callus induction and plant regeneration on different media in rice F_1 hybrids using anther culture. *International Journal of Scientific & Engineering Research*, **8**(10): 363-369.

Miah, M.A.A., Earle, E.D. and Khush, G.S. 1985. Inheritance of callus formation ability in anther cultures of rice, *Oryza sativa* L. *Theoretical & Applied Genetics*, **70**: 113-116.

Mishra, R., Rao, G.J.N., Rao, R.N. and Sharma, S.G. 2015. Physicochemical and cooking characteristics of anther derived doubled haploid lines of two elite Indica hybrid rice varieties - Ajay and Rajalaxmi. *Oryza*, **52**: 19-26.

Mishra, R., Rao, R.N. and Rao, G.J.N. 2011. Anther culture response of Indica rice hybrids. *Oryza*, **48**: 375-377.

Mkuya, M.S., Si, H.M., Liu, W.Z. and Sun, Z.X. 2005. Effect of 137Cs gamma rays to panicles on rice anther culture. *Rice Science*, **12**(4): 299-302.

Mohiuddin, A.K.M., Karim, N.H. and Sultana, S. 2014. Development of improved doubled-haploids through anther culture of Indica rice (*Oryza sativa* L.). *Annals of Biological Research*, **5**(10): 6-13.

Mukherjee, A., Islam, M.R., Nasiruddin. K.M. and Banerjee, P. 2015. Study on callus initiation and plantlet regeneration ability of some rice genotypes. *International Journal of Science & Technology Research*, **4**(10): 354-361.

Naik, N., Rout, P., Verma, R.L., Ngangkham, U., Katara, J.L., Sahoo, K., Singh, O.N. and Samantaray, S. 2016. Development of promising doubled haploids from Indica rice hybrid: Evaluation of uniformity and stability through morphological and molecular markers. *International Journal of Agriculture Sciences*, **8**(47): 1987-1992.

Narasimman, R. and Rangaswamy, S.R. 1993. Correlations and heritability for callus induction and regeneration *in vitro* anther culture of Indica Japonica rice hybrids. *Journal of Genetics & Breeding*, **47**(3): 187-189.

Nguyen, H., Chen, X.Y., Jiang, M., Wang, Q., Deng, L., Zhang, W.Z. and Shu, Q.Y. 2016. Development and molecular characterization of a doubled haploid population derived from a hybrid between Japonica rice and wide compatible Indica rice. *Breeding Science*, **66**: 552-559.

Niizeki, H. and Oono, K. 1968. Induction of haploid rice plant from anther culture. *Proceedings of Japan Academy*, **44**: 554-557.

Niroula, R.K. and Bimb, H.P. 2009. Effect of genotype and callus induction medium on green plant regeneration from anthers of Nepalese rice cultivars. *Asian Journal of Plant Sciences*, **8**: 368-374.

NRRI. 2008. *Annual Report 2007–2008: 34*, National Rice Research Institute, Cuttack, India.

NRRI. 2010. *Annual Report 2009-2010: 35*, National Rice Research Institute, Cuttack, India.

Ochatt, S., Pech, C., Grewal, R., Conreux, C., Lulsdorf, M. and Jacas, L. 2009. Abiotic stress enhances androgenesis from isolated microspores of some legume species (Fabaceae). *Journal of Plant Physiology*, **166**: 1314-1328.

Ogawa, T., Fukuwa, H. and Ohkawa, Y. 1995. Plant regeneration through direct culture of isolated pollen grains in rice. *Breeding Science*, **45**: 301-307.

Okamoto, Y., Kinoshita, A. and Satake, T. 2001. Enhancement of the frequency of callus formation and plant regeneration in rice anther culture by alternating temperature. *Breeding Research*, **3**: 87-94.

Otani, M., Wakita, Y. and Shimada, T. 2005. Doubled haploid plant production of transgenic rice (*Oryza sativa* L.) using anther culture. *Plant Biotechnology*, **22**: 141-143.

Ozawa, K., Kawahigashi, H., Kayano, T. and Ohkawa, Y, 2003. Enhancement of regeneration of rice (*Oryza sativa* L.) calli by the integration of the gene involved in regeneration ability of the callus. *Plant Science*, **165**: 395-402.

Pande, H. 1997. *Androgenesis in Anther Cultures of an Indica Cultivar of Oryza sativa L.* Ph.D. thesis, University of Delhi. Delhi, India.

Park, G.H., Kim, J.H. and Kim, K.M. 2014. QTL Analysis of yield components in rice using a Cheongcheong/Nagdong doubled haploid genetic map. *American Journal of Plant Science*, **5**: 1174-1180.

Patel, J.K., Subhash, N. and Fougat, R.S. 2014. *In vitro* callus induction and plantlet regeneration studies through anther culture in two Indica rice (*Oryza sativa* L.) varieties. *Current Trends in Biotechnology & Pharmacy*, **8**: 152-159.

Patil, V.D., Nerkar, Y.S., Misal, M.B. and Harkal, S.R. 1997. Parag 401, a semidwarf rice variety developed through anther culture. *International Rice Research Newsletter*, **22**(2): 19.

Pauk, J., Janeso, M. and Simon-Kiss, I. 2009. Rice doubled haploids and breeding. pp.189-197. **In**: *Advances in Haploid Production in Higher Plants* (eds. A. Touraev, B.P. Forster, S.M. Jain), Springer, Amsterdam, Netherlands.

Prem, D., Gupta, K. and Agnihotri, A. 2004. Doubled haploids: A powerful biotechnological tool for genetic enhancement in oilseed *Brassicas*. pp. 18-30. **In**: *Plant Biotechnology and Molecular Markers* (eds. P.S. Srivastava, A. Narula and S. Srivastava). Anamaya Publishers, New Delhi, India.

Premvaranon, P., Vearasilp, S., Thanapornpoonpong, S., Karladee, 1.D. and Gorinstein, S. 2011. *In vitro* studies to produce double haploid in Indica hybrid rice, *Biologia*, **66**(6): 1074-1081.

Purwoko, B.S., Dewi, I.S. and Khumaida, K. 2010. Rice anther culture to obtain doubled-haploids with multiple tolerances. *AsPac Journal of Molecular Biology & Biotechnology*, **18**: 55-57.

Raina, S.K. and Ifran, S.T. 1998. High-frequency embryogenesis and plantlet regeneration from isolated microspores of Indica rice. *Plant Cell Reports*, **17**: 957-962.

Rangasamy, S.R., Sukumar, S. and Manonmoni, S. 1992. *Rice Improvement through Anther Culture.*DRR Report, National Rice Biotechnology Network, India.

Rao, Y., Dong, G., Zeng, D., Hu, J., Zeng, L., Gao, Z., Zhang, G., Guo, L. and Qian, Q. 2010. Genetic analysis of leaf folder resistance in rice. *Journal of Genetics & Genomics*, **37**: 325-331.

Ren, D., Rao, Y., Huang, L., Leng, Y., Hu, J., Lu, M., Zhang, G., Zhu, L., Gao, Z., Dong, G., Guo, L., Qian, Q. and Zeng. Fine mapping identifies a new QTL for brown rice rate in rice (*Oryza sativa* L.). *Rice*, **9**: 4.

Rout, J.R. and Sharma, N.P. 2001. High frequency plantlet regeneration in rice anther callus cultures. *Rice Genetics Newsletter*, **29**: 175-182.

Rout, P., Naik, N., Ngangkham, U., Verma, R.L., Katara, J.L., Singh, O.N., Samantaray, S. 2018. Doubled haploids generated through anther culture from an elite long duration rice hybrid, CRHR32: Method optimization and molecular characterization. *Plant Biotechnology (online).* [doi:*10.5511/plantbiotechnology.16.0719a]*.

Roy, B. and Mandal, A.B. 2005. Anther culture response in Indica rice and variations in major agronomic characters among androclones of a scented cultivar, Karna Local. *African Journal of Biotechnology*, **4**(3): 235-240.

Roy, B. and Mandal, A.B. 2011. Profuse microtillering of androgenic plantlets of elite Indica rice variety IR 72. *Asian Journal of Biotechnology*, **3**: 165-176.

Ruwani, D.M., Mayakudua, G. and Silva, T.D. 2018. Anther culture as a supplementary tool for rice breeding. pp. 1-15. **In***: Rice Crop - Current Developments.* [doi: *10.5772/intechopen.76157*].

Sah, B.P. 2008. Response of genotypes to culture media for callus induction and regeneration of plants from rice anthers. *Scientific World*, **6**(6): 37-43.

Sammour, R.H., El-Salam, A., Draz, E, Mohammad Nofal, R.S. 2015. Improvement in *Oryza sativa* L. production using anther culture and molecular markers. *Biosciences Review*, **10**(6): 219-230.

Sasaki, T. 2005. The maped base sequence of the rice genome. *Nature*, **436**: 793-800.

Sasmita, P. 2009. Evaluation of uniformity, variability and stability of agronomic traits of doubled haploid rice lines resulting from anther culture. *Nusantara Bioscience*, **2**(2): 67-72.

Sellamuthu, R., Liu, G.F., Ranganathan, C.B. and Serraj, R. 2011. Genetic analysis and validation of quantitative trait loci associated with reproductive-growth traits and grain yield under drought stress in a doubled haploid line population of rice (*Oryza sativa* L.), *Field Crops Research*, **124**: 46-58.

Semagn, K., Bjørnstad. A. and Ndjiondjop, M.N. 2006. An overview of molecular marker methods for plants. *African Journal of Biotechnology*, **5**(25): 2540-2568.

Sen, C., Singh, R.P., Singh, M.K. and Singh, H.B. 2011. Effect of cold pretreatment on anther culture of boro rice hybrids. *International Journal of Plant Reproductive Biology*, **3**: 69-73.

Senadhira, D., Gregorio, G.B., Mendoza, R.D., Roxas, J.P., Alejar, M.S, Reyes, R.Y., Patena, C.C., DelaCruz H.C., Padolina, T.F., Galvez, A.M. and Gamiao, B.P. 1996. *New Rice Varieties that Defy Soil Salinity.* University of the Philippines, Los Baños, Philippines.

Senadhira, D., Zapata-Arias, F.J., Gregorio, G.B., Alejar, M.S., De La Cruz, H.C., Padolina, T.F. and Galvez, A.M. 2002. Development of the first salt tolerant rice cultivar through Indica/ Indica anther culture. *Field Crops Research*, **76**: 103-110.

Sengsai. S., Peyachoknagul, S., Sripichitt, P., Thongpan, A. and Pongtongkam, P. 2007. Anther culture of BC_1F_1 (KDML105/IRBB5/KDML105) hybrid to produce bacterial blight resistance double haploid rice. *Kasetsart Journal*, **41**: 251-261.

Shahnewaz, S., Bari M.A., Siddique, N.A. and Rahman, M.H. 2004. Effects of genotype on induction of callus and plant regeneration potential *in vitro* anther culture of rice (*Oryza sativa* L.). *Pakistan Journal of Biological Science*, **7**: 235-237.

Shariatpanahi, M.E., Bal, U., Heberle-Bors, E. and Touraev, A. 2006. Stresses applied for the re-programming of plant microspores towards *in vitro* embryogenesis. *Physiologia Plantarum*, **127:** 519-534.

Sharma, A. 2018. Morphology response of growth regulators on callus of Japonica rice through anther culture. *Clinical Biotechnology and Microbiology*, **2**: 424-442.

Shouyi, L. and Shouyin, H. 1991. Huayu 15, a high yielding rice variety bred by anther culture. pp. 230-247. **In**: *Rice. Part III - Biotechnology in Agriculture and Forestry, Volume 14.* (ed. Y.P.S. Bajaj), Springer, New York, USA.

Siddique, R. 2015. Impact of different media and genotypes in improving anther culture response in rice (*Oryza sativa*) in Bangladesh. *European Scientific Journal*, **11**(6): 164-169.

Silva T.D. 2010. Indica rice anther culture: Can the impasse be surpassed? *Plant Cell, Tissue & Organ Culture*, **100**(1): 1-11.

Singh, B.D. and Singh, A.K. 2015. Mapping populations. pp. 125-150. **In**: *Marker-Assisted Plant Breeding: Principles and Practices, Part III.* Springer, New York, USA.

Singh, R.B.1998. Agricultural biotechnology in Asia-Pacific region. pp. 53-55. **In**: *Agricultural Biotechnology in the Developing World.* FAO Research & Technology Development Division, Daya Publishing House, New Delhi, India.

Sohn, J.K. 1996. Factors affecting pollen embryogenesis of rice anther culture. *International Rice Research Newsletter*, **21**(2-3): 41-42.

Szarejko, I. 2003. Anther culture for double haploid production in barley (*Hordeum valgare* L.). pp. 35-42. **In**: *Double Haploid Production in Crop Plants: AManual* (eds. M. Maluszynski, K. Kasha, B.P. Forster and I. Szarejko). Kluwer Academic Publishers, Dordrecht, Netherlands.

Talebi, R., Rahemi, M.R., Arefi, H., Nourozi, M. and Bagheri, N. 2007. *In vitro* plant regeneration through anther culture of some Iranian local rice (*Oryza sativa* L.) cultivars. *Pakistan Journal of Biological Sciences*, **10**: 2056-2060.

Thomas, W.T.B., Foster, B.P. and Gertsson, B. 2003. Double haploids in breeding. pp. 337-350. **In**: *Doubled Haploid Production in Crop Plants* (eds. M. Maluszynski, K. Kasha, B.P. Foster and I. Szarejko), Kluwer Academic Publishers, Dordrecht, Netherlands.

Thuan, O.T., Tuan, V.D. and Ba Bong, B. 2001. Study on anther culture of F_1 plants from crosses between aromatic and improved rice cultivars. *Omonrice*, **9**: 41-45.

Tian, R., Jiang, G.H., Shen, L.H., Wang, L.Q. and He, Y.Q. 2005. Mapping quantitative loci underlying the cooking and eating quality of rice using a DH population. *Molecular Breeding*, **15**: 117-124.

Tran, D.G. and Vuong, D.T. 2002. Effect of different media and genotypes on anther culture efficiency of F_1 plants derived from crosses between IR64 and new plant type rice cultivars. *Omonrice*, **10**: 107-109.

Trejo-Tapia, G., Amaya, U.M., Morales, G.S., Sanchez, A.D.J., Bonfil, B.M., Rodriguez-Monroy, M. and Jimenez-Aparicio, A. 2002. The effects of cold-pretreatment, auxins and carbon source on anther culture of rice. *Plant Cell, Tissue and Organ Culture*, **71**: 41-46.

Tripathy S.K. 2018. Anther culture for double haploid breeding in rice - A way forward. *Rice Genomics & Genetics*, **9**(1): 1-6.

Veeraraghavan, R. 2007. *A Study on the Comparison of Anther Culture Response in Different Varieties of Rice, Oryza sativa L. subspecies Indica*. University of Colombo, Colombo, Sri-Lanka.

Wang, R.F., Zuo, Q.Z., Zheng, S.W. and Tiang, W.Z. 1979. Induction of plantlets from isolated pollen culture in rice (*Oryza sativa* L.). *Acta GeneticaSinica*, **6**: 7.

Xa, T.T. and Lang, N.T. 2011. Rice breeding for high grain quality through anther culture. *Omonrice*, **18**: 68-72.

Xu, J., Li, J., Zheng, X., Zou, L. and Zhu, L. 2001. QTL mapping of the root traits in rice. *Acta Genetica Sinica*, **28**: 433-438.

Yamagishi, M., Otani, M., Higashi, M., Fukuta, Y., Fukui, K., Yano, M. and Shimada, T. 1998. Chromosomal regions controlling anther culturability in rice (*Oryza sativa* L.). *Euphytica*, **103**: 227-234.

Yan, J, Xue, Q. and Zhu, J. 1996. Genetic studies of anther culture ability in rice (*Oryza sativa*). *Plant Cell, Tissue and Organ Culture*, **45**: 253-258.

Yang, J.Y., Zhao, X.B., Cheng, K., Du H.Y., Ouyang, Y.D., Chen, J.J., Qiu, S.Q., Huang, J.Y., Jiang, Y.H., Jiang, L.W., Ding, J.H., Wang, J., Xu, C.G., Li, X.H. and Zhang, Q.F. 2012. A killer-protector system regulates both hybrid sterility and segregation distortion in rice. *Science*, **337**: 1336-1340.

Yang, X.R., Fu, H.H. 1989. A high protein Indica rice. *International Rice Research Newsletter*, **14**(3): 14-15.

Zapata-Arias, F.J. 2003. Laboratory protocol for anther culture technique in rice. pp. 109-116. **In**: *Doubled Haploid Production in Crop Plants, A Manual* (eds. M. Maluszynski, K.J. Kasha, B.P. Forster and I. Szarejk). Kluwer Academic Publishers, Dordrecht, Netherland.

Zhou, H., Zheng, Y. and Konzak, C.F. 1991. Osmotic potential of media effecting green plant percentage in wheat anther culture. *Plant Cell Reports*, **10**: 63-66.

Zhu, D.Y., Pan, X.G. 1990. Rice (*Oryza sativa* L.): Guan 18: An improved variety through anther culture. pp. 204-211. **In**: *Biotechnology in Agriculture and Forestry Volume 12*: *Rice* (ed. Y.P.S. Bajaj). Springer, Berlin-Heidelberg, Germany.