Androgenesis of Selected Indica Rice (*Oryza sativa* L.) Genotypes and F_1 Hybrids

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Abstract

This experiment was carried out to evaluate the callusing ability and green plant regeneration of selected indica rice genotypes and F_1 progenies after crossing . Shwe Man-1, Sin Akari-3, Sin Thwe Latt, Thee Htat Yin and Yar-8 genotypes were selected as parental lines based on callusing ability. Five crosses were obtained by crossing the selected genotypes. Anthers of selected indica rice genotypes and F_1 hybrids were cultured in N_6 medium supplemented with 2.0 mg·L^{-1} 2,4-Dichloro phenoxyacetic acid (2,4-D) and 0.5 mg·L^{-1} kinetin for callus induction. Calli were transferred onto MS medium supplemented with 1 mg.L^{-1} Naphthalene acetic acid (NAA), 1 mg.L^{-1} Indole 3-acetic acid (IAA), 1 mg.L^{-1} 6-Benzylaminopurine (BAP) and 2.0 mg·L^{-1} kinetin. All tested genotypes showed callus formation except one cross (Sin Thwe Latt x Sin Akari-3) that showed no response on anther culture. The callus induction percentage of response genotypes was 0.1-2.6 %. Green plant formation was 6.3-37.0 %. Among parents, Yar-8 and Shwe Man-1 gave the highest response on callus induction and green plant formation. Cross of Yar-8 x Thee Htat Yin increased callusing and green plant formation. The anther-derived plants of these three genotypes were haploid and double haploids. Therefore, these genotypes have the potential in double haploid production through anther culture in rice breeding program.

Key words: callus, green plant formation, double haploid

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops cultivated in the world. It provides food for more than half of the world population. In Myanmar, rice is the most important dominating crop and is extensively grown throughout the country. Therefore, increasing rice pro-duction to support food security has been given a first priority in agricultural development in Myanmar. Indica rice varieties are mainly grown throughout the country but some japonica rice varieties are rarely cultivated. Plant breeders used conventional methods such as hybridization, selection, mutation, etc., to produce new rice varieties. The conventional plant breeding methods can be achieved by combining the desired traits through crossing with another desired characters. However, rice yields are affected by biotic and abiotic stress. They attempt to improve rice by using biotechnology. Therefore, plant tissue culture has become an important tool for breeding improvement in rice. Among plant tissue culture techniques, anther culture is the simplest and more efficient method (Niizeki and Oono 1968).

Anther culture technique has been widely used in breeding programs of many crops. It can be used as an effective and time saving method for obtaining homozygous lines in varietal improvement (Chu *et al*, 2002). Production of double haploids through anther culture is a rapid approach to homozygosity that shortens the time required for the development of new rice cultivars as compared to conventional methods, which require at least 6-7 generations. Anther culture involves two steps; callus induction from microspores and regeneration of green plants from calli.

Callus induction and green plant regeneration is a pre-requisite for utilization of anther culture in

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breeding programs. The callus induction percent of indica rice genotypes was approximately 1-15%. Unfortunately, low percentages of both callus induction and plant regeneration are the principal constraints in successful anther culture in some rice varieties especially in indica rice since these critical culturing responses are genotype dependent (Roy and Mandal 2005). Breeders can overcome the barrier due to genotypes by crossing highly responsive to non responsive genotypes in rice breeding. To obtain good combinations with high callus induction and green plant regeneration, the suitable parents could be selected for hybridization (He *et al.,* 2006). Based on the above information, this experiment was conducted with the following objectives;

1. To evaluate the callusing ability of selected indica rice genotypes and F_1 progenies after crossing selected genotypes
2. To examine the green plant regeneration of selected indica rice genotypes and their F_1 hybrid

Materials and Methods

The experiment was carried out at Field and Plant Tissue Culture Laboratory, Department of Horticulture and Agricultural Biotechnology, Yezin Agricultural University, from January 2015 to October 2016. Five indica rice genotypes were selected as parental lines for hybridization based on callusing ability of anther culture (Table 1).

Table 1. Selected indica rice genotypes

No.	Genotypes	Callusing ability
1	Shwe Man -1	High
2	Sin Thwe Latt	Medium
3	Sin Akari-3	No response
4	Thee Htat Yin	High
5	Yar-8	Medium

They were grown and crossings of indica x indica were conducted to obtain F1 hybrids. Five crossings were produced after crossing.

Anther pre-treatment: Healthy panicles were collected from the plants of the parents and F_1 hybrids during 8:00-10:00 a.m. when the distance between flag leaf and penultimate leaf was 5-12 cm depending on genotypes. The selected panicles were washed thoroughly with water and then sterilized with 95% ethanol for 30 seconds. They were wrapped with black plastic sheet and kept in low temperature incubator at 10ºC for 8-10 days for cold treatment. Before inoculating the anthers, the cold treated panicles were sterilized in Clorox (20%) for 25 minutes, followed by rinsing three times thoroughly with double distilled water.

Callus induction: Anthers at early uninucleate to early binucleate stage were cultured on N_6 (Nitchs') medium supplemented with 2.0 $mg.L^{-1}$ 2,4-D and 0.5 $mg.L^{-1}$ Kinetin for callus induction. Cultured anthers were incubated in dark condition at 25 ± 2ºC till callus induction. The anther culture response was observed for callus induction for 3 months.

Green plant formation: Calli (1-3 mm) were precultured on 'M' shaped-paper bridges with MS liquid medium (Murashige and Skoog) with 2 $mg.L^{-1}$ 2,4-D and 0.5 $mg.L^{-1}$ Kinetin. After 2 weeks, the calli were transferred onto the MS medium supplemented with 1 $mg.L^{-1}$ NAA, 1 $mg.L^{-1}$ IAA, 1 $mg.L^{-1}$ BAP and 2 $mg.L^{-1}$ kinetin. The regenerated calli were observed till green plant formation under 16/8 light/ dark hours at 25 ± 2ºC.The completely regenerated plants were transferred into Yoshida solution for stronger root formation for 2 weeks. After 2 weeks, well rooted plants were accalimatized and grown to maturity in sterile paddy soil under open condition. The process for androgenesis of rice anther culture was shown in Figure 2.

Data analysis: The experiment was conducted to analyze callus induction and green plant formation percentage in Completely Randomized Design (CRD).

Results and Discussion

Callus induction

Callus induction started within 2 months of

Table 1. Callus induction (%) of selected indica and F_1 hybrids rice genotypes

No.	Genotypes	Number of Callus	Callus Induction %
1	Shwe Man-1	26	1.5
2	Sin Akari-3	9	0.8
3	Sin Thwe Latt	14	0.5
4	Thee Htat Yin	8	0.5
5	Yar-8	46	2.6
6	Shwe Man-1× Thee Htat Yin	16	0.9
7	Thee Htat Yin × Sin Thwe Latt	2	0.1
8	Sin Thwe Latt × Sin Akari-3	0	0.0
9	Yar-8 × Thee Htat Yin	32	1.8
10	Sin Thwe Latt × Yar-8	13	0.7

culture in both parents and F_1 hybrids. The callus induction percent of five selected indica rice and F_1 hybrids is showed in Table 1. The callus induction frequency ranges from 0.1 to 2.6% among the genotypes. Among parents, Yar-8 produced the maximum callus induction (2.6%) and the minimum callus induction was found in Sin Thwe Latt and Thee Htat Yin (0.5%). It was found that callus induction varied with the different genotypes. This finding agrees with those of Medhabati (2014), who observed that the callus induction varied with the different genotypes on the same medium.

Among crosses, Yar-8 x Thee Htat Yin gave the highest callus induction (1.8%), the lowest callus induction was found in Thee Htat Yin x Sin Thwe Latt (0.1%). Crossing of Yar-8 x Thee Htat Yin showed the best callus induction among crosses. In this case, the genotypes as female parents (Yar-8 and Shwe Man-1) showed the highest response on callus induction, but Thee Htat Yin showed the lowest callus induction among parents. In this experiment, it was observed among indica types that the less response genotypes can increase in callusing ability of anther culture by crossing with the high responsive one. Narasimman and Rangasamy (1993) stated that both callus induction and green plant formation varied depending on the specific genotypes used to construct the hybrids. Moreover, Imuta et al., (1991) also reported that callus induction of anther varied with different genotypes. In this study, similar results were observed that both selected indica rice and F1 hybrids showed differently to produce callus on the same medium.

Green plant formation

Green plant formation from regenerated calli of callusing response rice genotypes was shown in Table 2. Out of five parents, only two genotypes gave the green plant formation. Yar-8 and Shwe Man-1 gave the green plant formation 37% and 19.2% respectively. The remaining three genotypes did not produce green plants. Among crosses, only

two crosses (Yar-8 x Thee Htat Yin and Shwe Man-1 x Thee Htat Yin) exhibited 9.4 % and 6.3% green plant formation. Although almost all tested genotypes formed callus induction, only four genotypes produced green plants. Therefore, it was found that green plant formation depended on genotypes.

Although Thee Htat Yin did not induce green plant regeneration, the crosses of Shwe Man-1 x Thee Htat Yin and Yar-8 x Thee Htat Yin could produce green plants. It means that the green plant regeneration ability was dependent on their corresponding parental genotypes. Moreover, Sree *et al.,* (1992) stated that green plant regeneration of anther culture varied greatly with genotypes.

Characterization of anther-derived plants by panicle status

Several methods are available for determining the ploidy level of regenerated anther-derived plants. According to the Mishra *et al.,* (2013), ploidy levels based on morphological characteristics of the anther-derived plants, revealed that the haploid plants were fully sterile and double haploid plants were fully fertile. In this observation, the anther-derived plants may be haploid or double haploid plants. All panicles of Yar-8 anther derived plants were sterile (Figure 1.c). Although some panicles of Shwe Man-1 and cross of Yar-8 x Thee Htat Yin were fertile (Figure 1.a and b), some panicles were sterile.

It can be clearly seen in Table 3 that Yar-8 may be assumed as haploid due to fully sterile panicles and some of Shwe Man-1 and Yar-8 x Thee Htat Yin genotypes were probably double haploid

Table 3. Characterization of ploidy levels for anther-derived plants by panicle status

Genotypes	No. of Anther-derived Plants	Ploidy Level of Anther-derived Plants	
		Haploid	Double Haploid
Shwe Man-1	6	2	4
Yar-8	40	40	0
Yar-8 × Thee Htat Yin	14	4	10

Figure 1. Panicle status of anther-derived plants (a) fertile panicle of Shwe Man-1 (b) fertile panicle of Yar-8 x Thee Htat Yin (c) sterile panicle of Yar-8

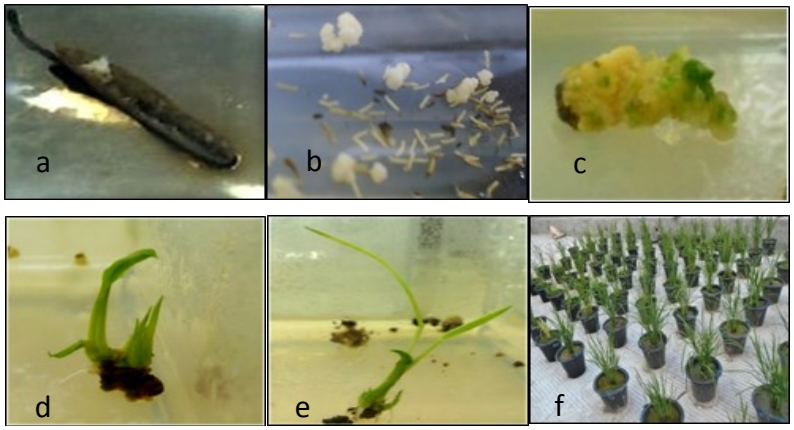

Figure 2. Callus induction and green plant production of rice anther culture (a) anther browning, (b) callus induction from responsive anther (c) green spot formation from regenerated calli (d) green shoot

plants. Hence, identification of haploid plants is needed for double haploid production. The most frequently used application is treating with colchicine (Jake *et al*., 2003). The haploid plants can be treated with colchicines solution for doubling chromosome to get double haploids. The finding of this experiment seems to the report of Mishra *et al.,* (2013), who reported that rice is a unique material in which around 30-40% of the anther-derived plants are double haploids due to the spontaneous doubling of the haploids. Therefore, further experiment is needed to address the confirmation of double haploid lines of the anther-derived plants with fertile panicle by cytological examination and molecular marker technology. Haploid plants of Yar -8 genotype have the potential to produce double haploid lines by using colchicines solution.

Conclusion

There was strong genotypic effect on callus induction and plant regeneration in rice anther culture. Among tested genotypes, Yar-8 and Shwe Man -1and Yar-8 x Thee Htat Yin produced the highest callus induction and green plant formation. The anther-derived plants of these three genotypes were haploid and double haploid plants by panicle performance. Haploid plants can be treated with colchicine solution to obtain homozygous double haploid lines. Therefore, Yar-8 genotype have the potential to produce double haploid lines and it should be selected a suitable genotype for double haploid production of rice breeding program in Myanmar.

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