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Development of Double Haploid Lines from F1 Cross of Yar-8 x Thee Htat Yin Genotypes through Anther Culture

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Abstract

Anther culture has become a powerful technique for the rapid production of double haploid lines in crop breeding program. The objectives of this experiment were to examine the callusing and green plant regeneration ability of parents (Yar -8 and Thee Htat Yin) and their F_1, and to develop the double haploid lines from the cross of Yar-8 and Thee Htat Yin genotypes. Nitsch and Nitsch (N6) medium with 2 mg.L^{-1}2, 4-Dichlorophenoxyacetic acid and 0.5 mg.L^{-1} kinetin was used for callus induction and Murashige and Skoog (MS) medium with 1 mg.L^{-1} Naphthalene acetic acid, 1 mg.L^{-1} Indole 3-acetic acid, 1 mg.L^{-1} Indole 3-butric acid, and 2 mg.L^{-1} kinetin was used for green plant regeneration. Callus induction was successfully observed in both parents and their F_1. Plant regeneration from regenerated callus was dependent on the genotypes. Only Yar-8 and F_1 progenies produced green plants as well as albino plants. Out of total 14 plants, 10 plants were double haploid (DH) plants. These double haploid lines (DH) could be done further evaluation to develop improved rice lines in Myanmar.

Key words: anther culture, double haploid and 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 (Sasaki, 2005). In particularly, nearly 90 % of the world's rice is produced in Asia. Some advances have been performed in rice improvement program; production of high-yielding varieties with good grain quality and resistance to biotic and abiotic stresses. In Myanmar, rice is the most important dominating crop. To hasten the development of elite rice breeding lines with good yield and quality, a breeding program based on anther culture derived plants (double haploid lines) was introduced.

Anther culture is an effective and time saving technology for obtaining homozygous lines in varietal improvement (Chu, 2002). Doubled haploids lines derived from the anther culture are a vital source of permanent mapping populations. The production of homozygous lines from heterozygous parents is feasible and shortens the time required to obtain them (Germana, 2011).

Mostly cultivated rice varieties with high yielding and desirable characteristics in Myanmar are indica type which is recalcitrant to anther culture ability (callus induction and green plant regeneration) which limits its practical application in rice breeding. The genotype barrier can be overcome by crossing highly responsive to non-responsive genotypes in double haploid breeding. A few studies have been conducted on anther culture of F_1 hybrids from crosses of indica x indica. Therefore, it is important to investigate whether good combinations with high callus induction and green plant regeneration frequency could be selected by crossing of suitable parents. The objectives of this experiment were to examine the callusing and green plant regeneration ability of parents (Yar -8 and Thee Htat Yin) and their F_1, and to develop the double haploid lines from the cross of Yar-8 and Thee Htat Yin genotypes.

Materials and Methods

Plant materials: Two indica rice genotypes, Yar-8 (drought tolerance) and Thee Htat Yin

(quality rice) were selected as parental lines for hybridization. The parental lines and their F_1 seeds were grown in the field of Department of Horticulture, and the anther culture process was conducted at plant tissue culture laboratory, Department of Horticulture, Yezin Agricultural University, Myanmar from June 2015 to February 2017.

Pre-culture treatment: Healthy panicles were collected during 8:00-10:00 a.m when the distance between flag leaf and penultimate leaf was 7-12 cm depending on genotypes. The collected panicles were sterilized with 70% ethanol and kept in low temperature incubator at 10ºC for 7-10 days for cold treatment. The cold treated panicles were sterilized in Sodium Hypochloride 2% (Clorox) for 25 minutes, followed by rinsing three times with double distilled water.

Anther culture: The spikelets in which anthers developed at early uni-nucleate to early bi-nucleate stage were cultured on N6 medium with 2 $mg.L^{-1}$2, 4-Dichlorophenoxy acetic acid and 0.5 $mg.L^{-1}$ kinetin. Callus which has 2-5mm size were transferred into Liquid MS medium for 2 weeks and then transferred onto MS medium with 1$mg.L^{-1}$ Naphthalene acetic acid, 1$mg.L^{-1}$Indole 3-acetic acid, 1$mg.L^{-1}$ Indole 3-butric acid, and 2$mg.L^{-1}$ kinetin for plant regeneration. The cultured tubes were incubated in completely dark condition at 25 ± 2ºC till callus induction and under 16/8 hours light/ dark at 25 ± 2ºC till green plant formation.

Acclimatization: The completely regenerated green plants were transferred into Yoshida solution for stronger root formation. After 2 weeks, well rooted plants were transferred to sterile paddy soil before growing in the field condition. The anther-derived rice plants were grown individually in each pot under natural environment.

Data Analysis

The experiment was conducted at Plant Tissue Culture Laboratory under uniform condition of light, temperature and humidity. The completely randomized design (CRD) was used. The frequencies of callus induction and green plant regeneration were estimated as follows:

$$Callus\ induction\ frequency = \frac{\text{Number of anther producing calli}}{\text{Number of anther plated}} \times 100$$

$$Green\ plant\ regeneration\ frequency = \frac{\text{Number of green regenerating calli}}{\text{Number of calli transfered}} \times 100$$

Results and Discussions

Callus induction: Anthers of all genotypes changed color from yellow to brown and then into dark brown within 1-4 weeks after inoculation. The responsive anthers showed slight swelling around it and subsequently induced callus. Anthers of F_1 plants started callus initiation within 4 weeks, followed by the parental genotypes within 6 weeks after inoculation. It was observed that callus initiation was earlier in F_1 plants than their parents. This finding was in accordance with the report of Herath et al., (2007), which documented that the time requirement for callus initiation was genotype dependent. The highest callus induction frequency was occurred in Yar-8 genotype (2.6%), followed by F_1 (1.8%) and Thee Htat Yin (0.5%) as shown in Table 1. It was found that F_1 showed the best callus induction by crossing high callusing genotype (Yar-8) with low callusing genotype (Thee Htat Yin). Therefore, the genotype selection is an important factor for callus induction response in rice anther culture. Narasimman and Rangasamy (1993) stated that both callus induction and green plant regeneration have varied depending on the specific genotypes to construct the hybrids.

Plant regeneration: Calli which have 2-5 mm size were transferred to Liquid MS medium for 2 weeks and then transferred onto MS medium for plant generation. During this period, some calli responded to brown, white and light yellow color. It was found that only the light yellow calli turned to green color which can regenerate green shoot or albino shoots. It was also noted that all green region of calli could not produce green shoots. The green plant formation was found in F_1 plants (9.4%) and Yar-8 (37%), however did not found in Thee Htat Yin (Table 1). In this case, it was found that some callusing

responsive genotypes have not ability to produce plant regeneration. There was a strong genotypic effect on green plant regeneration ability in anther culture of rice. This finding was agreed with the report of Sree et al., (1992), the green plant formation of anther culture varied greatly with genotypes.

Table 1. Callus induction and green plant regeneration ability of parents and their F_1

Genotypes	Anther No.	Callus No.	Callus induction %	Green calli No.	Green plant regeneration %
Yar-8	120	46	2.6	17	37
Thee Htat Yin	120	8	0.5	0	0
Yar-8 x Thee Htat Yin	120	32	1.8	3	9.4

Survival rate: Anther-derived plantlets were individually transferred into the plastic pots. All transplanted anther-derived plants had 100 % survival rate (Table 2). This finding was a little variation with other research findings. A survival rate of anther derived plant was reported 50-75 % and 87 % (Herath *et al.,* 2007 and Wang *et al.,* 2011). In plant tissue culture, adaptation processes is very important for *in vitro* derived plants to survive well before transferring to natural environment. Therefore, it can be assumed that this variation was due to the adaptation process. In this study, the adaptation process was done well.

Table 2. Survival rate of the transplanted anther derived plants

Genotypes	Plant transferred	Plant survived	Survival %
Thee Htat Yin	40	40	100
Yar-8 x Thee Htat Yin	14	14	100

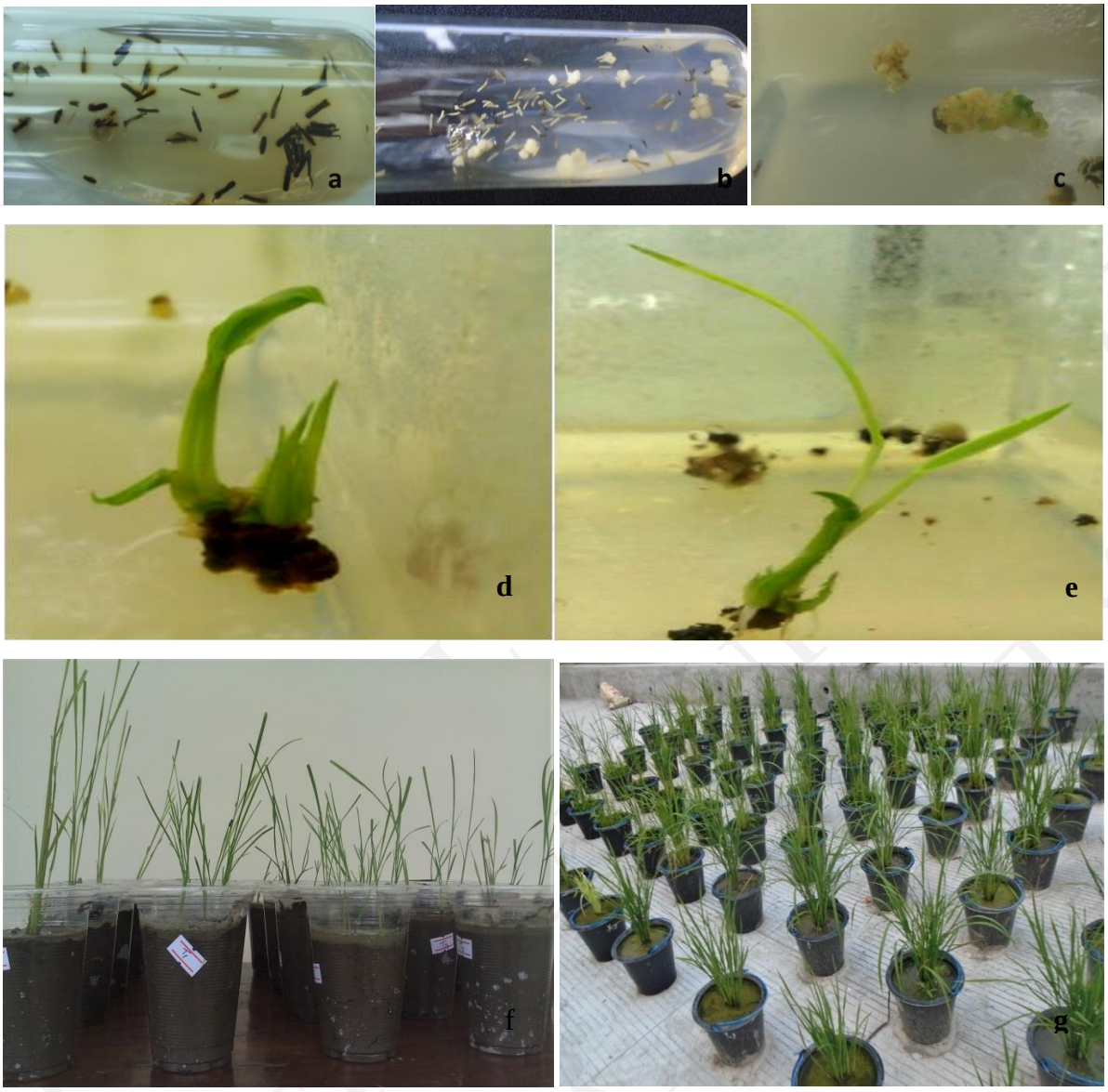

Figure1.Production of double haploid lines through anther culture (a) anther browning, (b) callus induction, (c) green spot formation from callus, (d) green shoot formation, (e) green plant, (f) acclimatization of anther derived plants and (g) anther derived plants under natural environment

Ploidy level of the anther derived plants: Anther derived green plants could be characterized as haploid, diploid, double haploid, etc. based on morphological characters (Figure 2). According to Mishra (2015), ploidy level based on the morphological characteristics of the anther derived plants, revealed that the haploid plant panicles were fully sterile and the double haploid plant panicles were fully fertile. In this experiment,

all panicles of Yar-8 anther derived plants were fully sterile and some of the plants were abnormal plant type. It can be assumed that they were probably haploid plants. Some panicles of F_1 plants were fully fertile and all plants were normal plant type. Out of 14 F_1 anther derived plants, 10 plants with fertile panicles could be assumed as double haploid plants. This finding was in line with the report of Germana (2011), 40-60% of the anther derived plants undergo spontaneous chromosome (endoreduplication) in rice.

Table 3. Characterization of ploidy level of the anther derived plants

Genotypes	No. anther derived plants	Haploid Plants		Double Haploid Plants	
		No.	%	No.	%
Thee Htat Yin	40	40	100	0	0
Yar-8 x Thee Htat Yin	14	4	28.57	10	71.43

Figure2. Ploidy level characterization by morphological characters (a) Normal plant type of Yar-8, (b) Abnormal plant type of Yar-8, (c) Normal plant type of F_1, (d) Sterile panicle and (e) Fertile panicle

Conclusion

The anther culture ability (callus induction and green plant regeneration) was dependent on genotypes. Although parents and their F_1 plants were responsive to callus induction, one parent (Thee Htat Yin) did not produce green plant regeneration. Therefore, the anther culture ability could be improved by crossing with high responsive genotypes. The ploidy level characterization of the anther derived plants is needed to address the confirmation of the double haploid of the anther derived plants by cytological determination or molecular marker characterization.

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