In Vitro Regeneration of Selected Rice Genotypes (Oryza sativa L.) through Anther Culture

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Abstract

Rice is one of the most important cereal crops in the world. Anther culture, one of the double haploid techniques, is simple and efficient for rice breeding. The present study was carried out to evaluate callus induction ability of the selected rice genotypes on the media supplemented with two different carbon sources and to investigate suitable Benzylaminopurine (BAP) concentrations on plant regeneration of anther-derived calli. The selected 19 rice genotypes (14 indica and 5 tropical japonica) were used in the experiment. Anthers of each genotype were cultured on Chu (N6) medium supplemented with two types of carbon sources (4% maltose and 4% sucrose), 2mg.L-1 2,4-Dichlorophenoxyacetic acid (2,4-D) and 0.5 mg.L-1 kinetin for callus induction. Anther-derived calli were transferred to Murashige and Skoog (MS) medium supplemented with (0, 0.5, 1) mg.L-1 BAP, 1 mg.L-1 1-Naphthaleneacetic acid (NAA) and 1 mg.L-1 l kinetin for regeneration. The seventeen out of 19 rice genotypes produced callus on media supplemented with maltose while only 13 genotypes produced callus on media supplemented with sucrose due to genotype dependency. Tropical japonica had more callus induction from anther than indica among the tested genotypes. Callus induction varied from 0 to 19.22%. Paw San Taung Pyan Hmwe (tropical japonica) had the highest callus induction (19.22%) among the tested all. Yebaw Sein depicted the highest callus induction (6.78%) among the tested indica rice genotypes. Plant regeneration from callus varied from 0 to 25.15%. In tropical japonica genotypes, Bay Kyar Taung Pyan gave the highest value (25.15%) in green plants regeneration, while Hnan Kar gave the highest value (13.33%) among indica genotypes. In this experiment, both MS media supplemented with 0.5mg.L-1 BAP and 1mg.L-1 BAP showed maximum green plant regeneration although maximum green spot formation occurred only on media supplemented with 1mg.L-1 BAP.

Key words; anther culture, rice genotypes, carbon sources, plant growth regulators, callus

Introduction

Rice is an important food crop for the world. About 85% of the total world rice is produced and consumed in Asian countries. Being an important crop for many Asian countries, rice is grown in various ecosystems of Myanmar. The total area under rice in Myanmar was 7.21 million hectares in 2015-2016 and the total production was 28.21 million metric tons (MOALI 2016).

There have been some problems concerning with food security throughout the world. The global population will be 8.42 billion in year 2025. Rice consumers are increasing at the rate of 1.8% every year. The rate of growth in rice production has slowed down due to environmental degradation. Thus, it is necessary to increase rice production in the world (Mishra et al. 2015).

Many plant breeders have been used conventional methods to get elite varieties of rice. However, this method is time-consuming. The double haploid production techniques shorten the time required for the development of new rice cultivars. Anther culture is a simple and efficient technique for the

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production of double haploids (Gueye and Ndir 2010).

There are three types of rice- javanica, japonica, and indica. Most of the rice varieties grown in Myanmar are indica varieties and japonica rice varieties are rarely grown. A large number of good-quality rice genotypes are indigenous to Myanmar. Although some efforts have been made for anther culture on some varieties, it is still necessary to accomplish it for remaining rice genotypes.

Callus induction of indica cultivars was found to be extremely poor compared to that of japonica cultivars. Many researchers reported that different rice species, subspecies or genotypes behaved differently in response to anther culture, and genotype is the main important factor for the success of anther culture (Niroula and Bimb 2009).

Carbon source in the medium plays an important role in callus induction. Sucrose is most commonly-used carbohydrate for all types of tissue cultures. Maltose has been known to be a better carbon source than sucrose for androgenesis of many plant species. The effect of maltose and sucrose on androgenesis is variety specific (Swapna 2000).

The success of plantlet regeneration under in vitro anther culture depends on types and concentrations of different growth regulators - especially auxin and cytokinin. A number of experiments have shown that proper combinations and ratios of hormones gave good induction effects. Combination of kinetin, NAA, IAA and BAP was most effective in enhancing plant regeneration efficiency in rice anther culture (Htwe et al. 2011).

In this regard, the experiment was conducted with the following objectives -.

- To evaluate callus induction ability of the selected rice genotypes on the media supplemented with two different carbon sources
- To investigate suitable BAP concentrations on plant regeneration of anther derived calli

Materials and Methods

This experiment was conducted at Plant Tissue Culture Laboratory, Department of Horticulture and Agricultural Biotechnology, Yezin Agricultural University. Seeds of 19 rice genotypes (14 indica and 5 tropical japonica) (Table 1) obtained from the Seed Bank, Department of Agricultural Research (DAR) were grown under natural field conditions with regular water supply and recommended cultural practices.

Table1.Nineteen selected rice genotypes used in the experiment

No.	Genotypes	Life Span
	Indica	
1	Yar-8	120-125
2	Thee Htat Yin	115-120
3	Shwe Pyi Htay	127
4	Thee Htat-3	125-130
5	Hmaw Bi 3	125-130
6	Hmaw Bi 2	140-145
7	Sin Thwe Lat	135
8	Thu Kha Hmwe	137
9	Kone Myint 2	140
10	Shwe War Htun	145
11	Lat Yone Kyi	Late Nov
12	Hnan Kar	Late Oct
13	Yebaw Sein	Mid Dec
14	Shwe Ta Soke	Early Nov
	Tropical Japonica	
15	Paw San Hmwe(Acc No. 002500)	Late Nov
16	Paw San Taung Pyan Hmwe (Acc No. 002924)	Mid Dec
17	Paw San Bay Kyar (Acc No.002925)	Late Nov
18	Bay Kyar Taug Pyan (Acc No.001208)	Mid Dec
19	Nga Kywe Taung Pyan (Acc No.002054)	Mid Dec

Rice genotypes from 11 to 19 are date-fixed.

By examining the nuclear stage of pollen, the relationship between microspore stage and panicle morphology was estimated. Then panicles were collected when the distance between the flag leaf auricle to the second leaf auricle was 7-10 cm for indica rice genotypes and 10-15 cm for tropical japonica rice genotypes. These panicles were washed thoroughly with tap water and surface sterilized

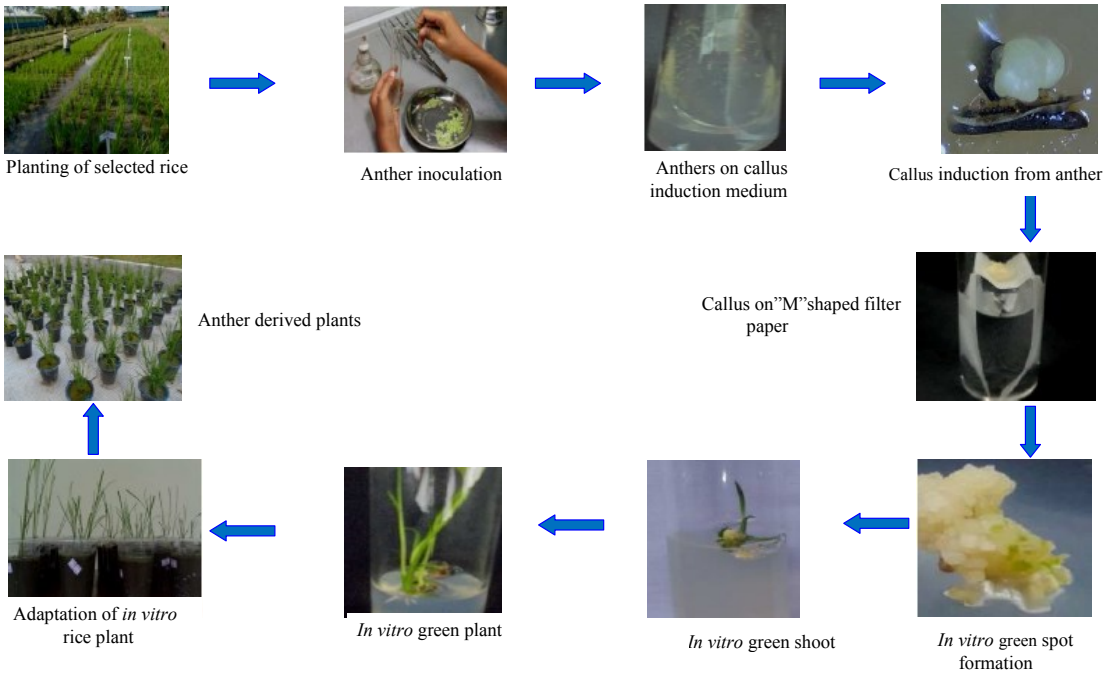

Plate 1. Protocol for anther culture of rice

Table3. Effects of maltose on callus induction in in vitro anther culture of different rice genotypes

No.	Genotypes	Callus induction %		% increase in callus induction
		Sucrose	Maltose	
	Indica			
1.	Yar-8	2.11	2.33	1.10
2.	Thee Htat Yin	0.44	0.67	1.52
3.	Shwe Pyi Htay	0.00	0.00	0.00
4.	Thee Htat-3	0.00	0.00	0.00
5.	Hmaw Bi 3	2.44	6.56	2.69
6.	Hmaw Bi 2	1.11	2.22	2.00
7.	Sin Thwe Lat	2.78	2.67	0.96
8.	Thu Kha Hmwe	0.00	1.78	(~)
9.	Kone Myint 2	0.00	0.44	(~)
10.	Shwe War Htun	0.44	1.89	4.3
11.	Lat Yone Kyi	2.67	2.67	1.00
12.	Hnan Kar	0.00	2.44	(~)
13.	Yebaw Sein	2.56	6.78	2.65
14.	Shwe Ta Soke	0.00	1.78	(~)
	Tropical japonica			
15.	Paw San Hmwe	6.89	19.11	2.77
16.	Paw San Taung Pyan Hmwe	7.89	19.22	2.44
1	Paw San Bay Kyar	6.67	11.78	1.77

with 90% ethanol. Panicles were wrapped with newspaper and incubated at 10°C for 8 to 10 days.

The cold-treated panicles were sterilized with 70% ethanol for 3 minutes and then in 2% sodium hypochloride solution for 20 minutes followed by rinsing thoroughly with distilled water for three times. Anthers at right developmental stage were cultured on N6 medium supplemented with 2 mg.L-1 2,4-D, 0.5 mg.L-1 kinetin with two carbon sources – sucrose or maltose and incubated under dark at 25±1°C. Days to induce callus formation of tested genotypes for each treatment were recorded.

Calli of 1-2 mm in diameter were pre-cultured on 'M' shaped paper bridge in liquid medium for 2 weeks and then transferred to MS solid media (regeneration media) supplemented with 1 mg.L-1 kinetin, 1 mg.L-1 NAA and (0, 0.5, 1) mg.L-1 BAP. The cultures were maintained under continuous light at 25±1°C. Green spot formation and green plant regeneration were examined.

Shoots (5cm in length) were transferred to basic MS medium for root regeneration. Well-rooted plantlets were transferred to Yoshida's nutrient solution for two weeks to allow root to develop vigorously. After two weeks, plants were transferred to small plastic cups for one week and then transplanted to pots and grown to maturity.

Data Analysis

Analysis of variance was performed using Statix (version 8) software. Treatment means were compared by LSD at 5% level.

Results and Discussion

Genotype responses and the effect of carbon source on callus induction

The variation of days to induce callus formation as affected by two different carbon sources can be observed in Table 2. It was found that there was significant difference between the two carbon sources. Sin Thwe Lat produced callus at 26 days after inoculation on both media supplemented with sucrose and maltose. It was the earliest callus producing genotype among the tested varieties. Two genotypes _ Shwe Pyi Htay and Thee Htat 3 _ did not produce callus at all till 90 days after inoculation on both sucrose- and maltosesupplemented media. Four genotypes (Thu Kha Hmwe, Khone Myint 2, Shwe Ta Soke and Hnan Kar) produced callus on maltose-supplemented media at 27, 39, 43 and 59 days after inoculation respectively although these genotypes did not produce callus at all on sucrose supplemented media till 90 days after inoculation. Herath and Bandara, (2011) reported that the time requirement for the callus initiation was also genotype-dependent and callus induction started 3 weeks after culture in their rice anther culture experiment.

The effects of maltose on the callus induction of tested rice genotypes were described in Table 3. Tropical japonica had higher response in callus induction on both media than indica. Silva and Ratnayake (2009) also reported that japonica types were more responsive than indica types in anther culture. Among the tested genotypes, Sin Thwe Lat and Bay Kyar Taung Pyan had better response to sucrose than to maltose while Thu Kha Hmwe, Khone Myint 2, Hnan Kar, and Shwe Ta Soke had specific response to maltose in callus induction. It

Table2.Days to induce callus formation of tested rice genotypes as affected by two different

No.	Genotypes	Days to induce callus	
		Sucrose	Maltose
	Indica		
1.	Yar-8	40	40
2.	Thee Htat Yin	39	40
3.	Shwe Pyi Htay	>90	>90
4.	Thee Htat -3	>90	>90
5.	Hmaw Bi 3	36	32
6.	Hmaw Bi 2	64	40
7.	Sin Thwe Lat	26	26
8.	Thu Kha Hmwe	>90	27
9.	Kone Myint 2	>90	39
10.	Shwe War Htun	34	34
11.	Lat Yone Kyi	52	48
12.	Hnan Kar	>90	59
13.	Yebaw Sein	44	44
14.	Shwe Ta Soke	>90	43
	Tropical japonica		
15.	Paw San Hmwe	48	45
16.	Paw San Taung Pyan Hmwe	40	40
17.	Paw San Bay Kyar	44	38
18.	Bay Kyar Taug Pyan	51	44
19.	Nga Kywe Taung Pyan	68	68

may be due to different requirement for carbon source of tested rice genotypes to induce callus. Swapna (2000) also reported that the effect of maltose and sucrose on androgenesis was variety-specific. In the experiment, maltose increased anther response of callus formation in most of the tested rice genotypes _ not only in indica but also in tropical japonica genotypes. This result is similar to Yi et al. (2003), who found that maltose increased anther response not only in indica but also in japonica. Callus induction of Paw San Hmwe on maltose has 2.77 times higher than that of sucrose. This may be due to the fact that fructose from the cleavage of sucrose inhibits the androgenesis (Last and Brettell 1990).

Effects of BAP concentrations and genotypes on plant regeneration

After 2 weeks on regeneration medium, green spots started on some calli while others turned to brown color. Callus browning may be due to non-embryogenic callus or the sucrose-supplemented medium. Darachai et al. (2010) reported that the medium containing sucrose promotes the ethylene production in plant tissue and ethylene can cause the browning of callus in tissue culture. Most of the differentiated calli produced either green or albino plants while some produced both. Green spot formation and green plant regeneration are important for the success of in vitro anther culture.

Table4. Effects of genotypes and BAP concentrations on green spot formation, green plant regeneration and number of green plants per culture

No.	Genotypes	Green spots formation %	Green plants regeneration %
	Indica		
1.	Yar 8	6.67 c	2.22 fg
2.	Hmaw Bi 3	4.45 cd	0.00
3.	Hmaw Bi 2	5.93 cd	0.00
4.	Lat Yone Kyi	17.0 b	11.11 cd
5.	Hnan Kar	15.55 b	13.33 c
6.	Yebaw Sein	1.48 d	0.74 g
7.	Shwe Ta Soke	6.67 c	5.93 ef
	Tropical Japonica		
8.	Paw San Bay Kyar	8.89 c	7.41 de
9.	Bay Kyar Taung Pyan	28.18 a	25.15 a
10.	Nga Kywe Taung Pyan	23.70 a	20.00 b
	LSD (0.05)	4.55	4.75
	BAP concentrations ($mg.L^{-1}$)		
	0	7.11 c	5.11 b
	0.5	12.89 b	10.00 a
	1	15.56 a	10.67 a
	LSD(0.05)	2.49	2.60
	Pr>F Genotypes	0.0001	0.0001
	PGRs	0.0001	0.0001
	Genotypes*PGR	0.0001	0.0005
	CV %	40.71	52.55

Effects of genotypes and BAP concentrations on green spot formation and green plant regeneration were described in Table 4. Among 19 tested genotypes, green spot formation occurred on 10 genotypes and green plant regeneration was found only on 8 genotypes. Aung et al. (2015) also founded that only three of 12 tested japonica genotypes produced green plants although all genotypes induced calli.

Green spot formation and green plant regeneration were significantly different among genotypes. The highest green spot formation (28.18%) was found in Bay Kyar Taung Pyan among tropical japonica rice genotypes. Lat Yone Kyi gave the highest green spot formation (17%) among indica rice genotypes. In green plant regeneration, Bay Kyar Taung Pyan (tropical japonica genotype) gave the highest green plant regeneration (25.15%) among all the tested rice genotypes and Hnan Kar produced maximum green plant regeneration (13.33%) among indica rice genotypes. Thus, it can be said that green plant regeneration was a genotype-dependent character. This was also reported by many researchers who studied on regeneration of cereal anther culture (Kaushal et al. 2014).

There was a significant difference among the effect of BAP concentrations on green spot formation and green plant regeneration. The highest concentration of BAP (1mg.L-1) gave maximum green spot formation out of the BAP concentrations tested. It was recorded that 1 mg.L-1BAP and 0.5 mg.L-1BAP resulted in 10.67% and 10.00% green plant regeneration respectively. The lowest green plant regeneration was found in the treatment without BAP. Thus, addition of BAP to the medium is more suitable for green plant regeneration than without BAP. Rout et al. (2016) reported that higher concentration of BAP with low concentration of

kinetin was found effective for shoot regeneration of anther culture. In this experiment, BAP was applied together with 1 mg.L-1 kinetin and 1 mg.L-1 NAA.

Significant interaction effects between genotypes and BAP concentrations were found in green spot formation and green plant regeneration. Green spot formation and green plant regeneration of tested rice genotypes were different depending on BAP concentrations. Green plant regeneration of tested rice genotypes ranged from 0 to 25.15 % depending on BAP concentrations (Table 4). Ranjana et al. (1998) found that plant regeneration frequency varied from 0 to 26.86% depending upon the genotypes and the constituents of the media used.

Conclusion

According to the results of this study, tropical japonica genotypes induced more callus than indica ones. Paw San Taung Pyan Hmwe was the highest in callus induction among tropical japonica. Maltose has superior effect on callus induction in most of the tested rice genotypes to sucrose.

In green plant regeneration, Bay Kyar Taung Pyan showed the highest percentage among tropical japonica and Hnan Kar gave the highest percentage among indica. In the case of BAP concentrations, combination of 0.5 mg.L-1 BAP with 1 mg.L-1 kinetin and 1 mg.L-1 NAA, gave good results for most of the tested rice genotypes for green plant regeneration.

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