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Original Article

PRODUCTON OF DOUBLE HAPLOID POPULATION IN TWO INDICA RICE (*Orysa sativa* L.) CROSS SAFRI-17XIR-64 AND MTU1010 VARIETY

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Abstract: Anther culture based double haploid (DH) production is a technology which can significantly reduce the time period required for development of new crop variety. In the present investigation an attempt was made to develop DH using anther culture in rice (Oryza sativa L.). One cross Safri-17xIR-64 and one variety MTU1010 was subject for the study. Parameter like media composition, hormonal treatment etc was standardized for efficient development of DH. The anther were excised and plated on to N6 (Chu.,1978) media supplemented with 3% maltose 0.8% agar , 2 ml/l 2,4-D and the pH was maintained of 5.8. In cross Safri-17xIR64 the callus induction percent was 0.49% and in variety MTU1010 callus induction percent is 0.40%.The induce callus was transfer in 16 different media (T1 to T16) for regeneration. Then green callus was transfer to green plant regeneration media. Treatment no.T15 was found to be best as it has produce 106 number of green plant & treatment no.T1 has produced 58 albino plants in Safri-17xIR-64. In MTU1010, T1 has generated 9 green plants and 5 albino plants.

Key words: *Oryza sativa*, Double haploid, Anther culture

INTRODUCTION

The development of anther culture techniques for production of rice was a major advance in the field of rice breeding in the last few decades and anther culture is one of the most intensively investigated areas of *in vitro* culture methods of rice as doubled haploid approach can effectively reduce the time required for varietal improvement [1]. However, indica rice cultivars are generally recalcitrant to culture and also the green plant regeneration potential is limited. Since higher levels of callus induction and green plant regeneration is a pre-requisite for utilization of anther culture in breeding programs. Detailed studies on the various factors that govern the culture response of anthers under in vitro condition of indica rice are crucial. To raise the yield ceiling in rice, which has become static after the green revolution, hybrid rice was considered as an option to increase productivity per unit area. However, despite its yield advantage over inbreeds, the hybrid rice technology has not found favor with the farmers of India. The main reasons for its low adoption include the seed cost and the quality of the produce. Since doubled haploid approach can effectively address the problems associated with hybrid rice through production of high yielding doubled haploids with uniform grain quality, the basic studies on indica rice anther culture assumes great significance. Low temperature shock has been reported to enhance the androgenic response in several species including rice [2,3].The growth regulators, mainly the auxins and cytokinins are known to control the dedifferentiation and differentiation processes in the in vitro cultures of crop plants. The rate of success can be enhanced by improving the composition of

tissue culture medium, especially by manipulating plant growth regulators [4]. This study was an attempt to evaluate the efficiency of the anthers from the elite indica rice cross Safri-17xIR-64 and variety MTU1010 using phytohormones in the culture media on both callus induction and green plantlet regeneration.

MATERIALS AND METHODS

The experimental materials were cross Safri-17XIR-64 (Resistant to Blast and Drought) and variety MTU1010 (Resistant to blast) two elite indica rice that were developed at Indira Gandhi Krishi vishwavidyala Raipur. The boots (panicles along with the boot leaves in which they are still enclosed) panicle were collect in the morning hours from a plant. The boots were sterilized with 70% alcohol. The wrapped boots were storage at 10p C for 9 days for cold pretreatment. The middle portion of the panicle from the pretreated spikelet by 0.1% $HgCl_2$ was used for 5 minute was used for inoculation. For callus induction media N6 [5] each supplemented with 2,4-D (2mg/l), maltose (3%) pH of the medium was adjusted 5.6 to 5.8 solidified with agar-agar (0.8%) and the media were autoclaved at 121p C (15psi) for 20 min. For green callus regeneration, modified MS [6] medium supplemented with different hormone combination with maltose/ sucrose (3% and 1.5% w/v) and agar-agar (0.8%) by adjusting pH of the medium 5.6 to 5.8 (T1,T16 Table 3). The pollen embryoids/calli from the responding anthers were transferred within one week old callus onto the regeneration medium and the cultures were incubated under artificial light (~ 2000 Lux) at 25±2p C for regeneration. The green callus transfer in the shooting media MS media powder 4.41 g and sucrose (3%) adenine sulphate 0.18 gm / l (ADS), Inositol 100 mg/l, BAP 4 ml /l and IAA 1 ml/1 and adjust ph 5.8 ,8 gm /l (0.8%) agar use for shoot development . The green plantlets of around 1 cm in length were transferred to rooting in simple MS medium without hormone for root formation. The plants with well-formed roots were transferred to pots in the green house.

RESULTS AND DISCUSSION

Callus induction of anther:-After cold pretreatment of boots, the panicles were take out and sterilized with 0.1 % $HgCl_2$ for five minutes then wash for five times distilled water . The anther were dusted on petri plate containing solidified N6 media supplemented with maltose 3%, agar 0.8 % and 2mg/ l 2,4-D. Each petri plate was dusted with excised anthers as shown in (Fig. A). Then the petri plates were incubated in 25±2p C inside a BOD incubator for callus induction. Low rate of callus induction and plant regeneration is generally observed in anther culture with indica rice [7]. Obtained frequency of 3.53 of callus induction in N6 medium and 1.12% in plant regeneration. 2,4-D in N6 media was used for inducing callus from anther of cross Safri-17xIR-64. Total 21,151 anthers dusted on the plates, 125 callus were regenerated (Fig. B). Sripichitt et al. [8] concluded that callus induction of indica varieties were definitely poor (1.7- 4.4%) as compared to japonica variety (17%). Callus regeneration percent was found to be very low in cross Safri-17xIR-64 with 0.49% (Table 1). In variety MTU1010, 13,783 anthers dusted on the plate, only 12 callus were induced out of it. Callus induction percent was found to be very low in MTU1010 variety with 0.40% (Table 2). The low percentage of callus induction and green plant regeneration has limited the application of anther culture techniques in indica rice breeding programmes [9].

Influence of phytohormones on regeneration:- The callus generated from the anther of cross Safri-17xIR-64 was transferred to 16 different treatment (T1 to T16; Table 3) to work out the best media composition which can convert white callus to green for green plant generation. On an average 212 callus was subjected to 16 different treatments. Out of 16 treatments, 7 (T1, T3, T4, T9, T11, T15, T16) treatments responded for greening of the callus (Table 4). In T3 14.28 % callus greening was observed with 15 days of incubation. Auxin: cytokinin (NAA: KN: BAP) in a ratio of 1:1:3 (0.25: 0.25: 0.75 mg/l) had stimulated highest regeneration (20.00-22.00%) [10]. In rest treatments no response was observed.

In T9 and T15 has also converted 8.33% of callus into green callus (Fig.1C). However, as the green callus of T9 which transferred to proliferative media the callus turned out to brown and no plant generated. The present anther culture results were sowing similar low rate of callus induction (Table 1) and plant regeneration as observed in anther culture with indica rice (Table 4) [7] The frequency of obtained 1.12% in plant were regeneration. In case of T15 which has converted 8.33 % of callus into green has

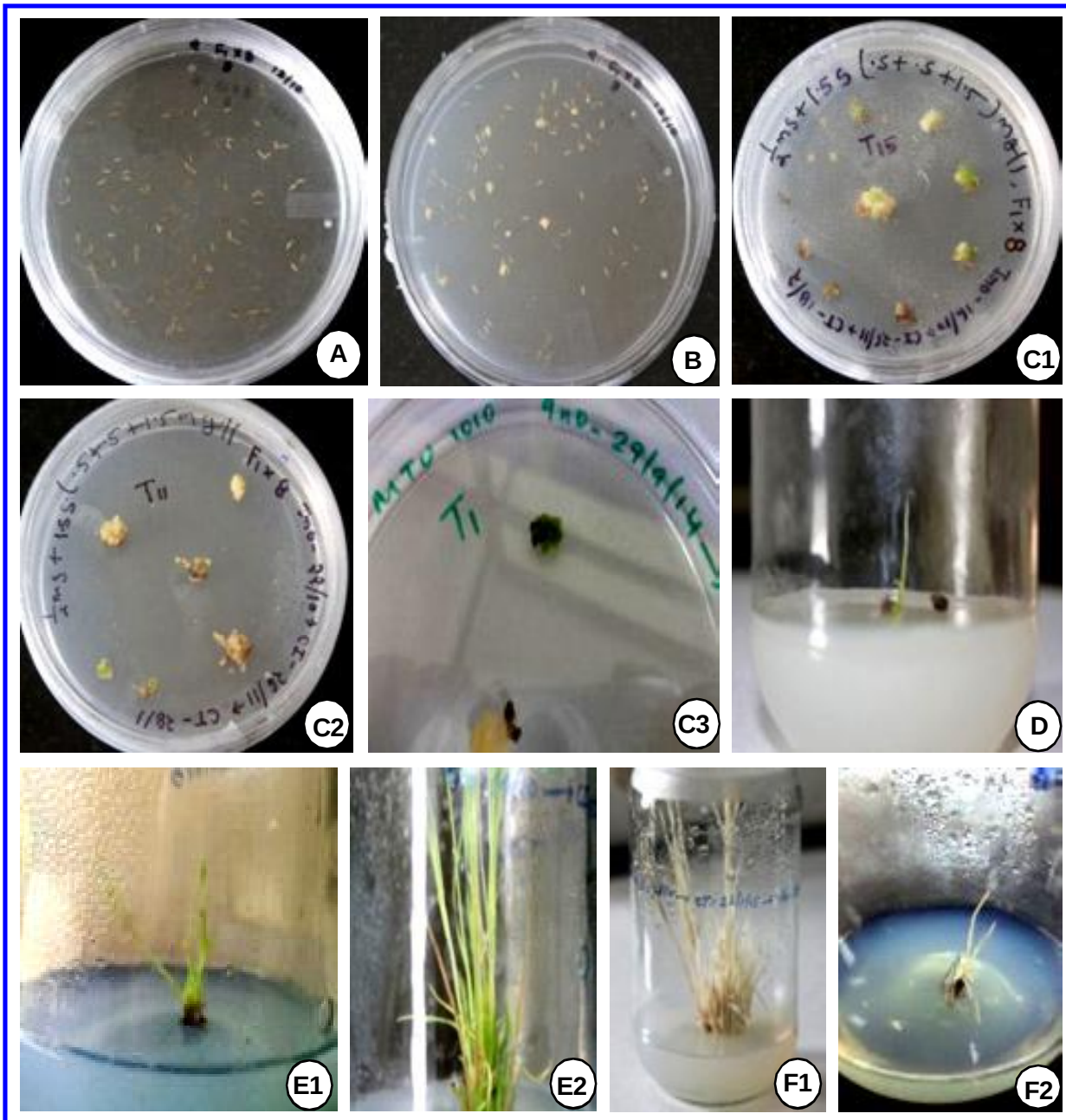

Fig. A: Dusting of anthors, **Fig. B:** Callus induction, **Fig. C 1,2,3:** Callus greening, **Fig. D:** Shooting, **Fig. E 1,2:** Green plant, **Fig. F 1,2:** Albino plant

Table 1: Callus induction in cross safari-17 x IR-64

S. No.	Total No. of anther inoculated	Avg. no. of anther dusted/plate	Total no. of callus induced	Callus Indu-ction%
1	998	66.53	0	0
2	1133	80.92	0	0
3	1561	104	6	0.38
4	1816	90.8	0	0
5	1877	93.85	1	0.005
6	1890	94.5	37	1.95
7	1918	95.9	1	0.05
8	2328	89.53	0	0
9	2410	96.4	18	0.74
10	2423	96.92	14	0.57
11	2797	93.23	48	1.71
Total	21151	91.14±9.24	125	0.49±0.71

Table 2: Callus induction in variety MTU1010

S. No.	Total No. of anther inoculated	Avg. no. of anther dusted/plate	Total no. of callus induced	Callus Induc-tion%
1	927	57.93	10	1.07
2	996	76.61	0	0
3	1013	67.53	0	0
4	1247	83.13	0	0
5	1255	83.66	0	0
6	1260	84	0	0
7	1365	91	1	0.072
8	1445	96.33	0	0
9	1520	76	1	0.065
10	2755	91.83	0	0
Total	13783	80.80± 11.69	12	0.40±0.33

S. No.	TREATMENT	S. No.	TREATMENT
A	0.25:0.25:0.75	C	01:01:02
T1	1/2 MS + 1.5M + 0.8 AGAR	T9	1/2 MS + 1.5M + 0.8 AGAR
T2	1/2 MS + 3M + 0.8 AGAR	T10	1/2 MS + 3M + 0.8 AGAR
T3	1/2 MS + 1.5S + 0.8 AGAR	T11	1/2 MS + 1.5S + 0.8 AGAR
T4	1/2 MS + 3S + 0.8 AGAR	T12	1/2 MS + 3S + 0.8 AGAR
B	0.5:0.5:3.0	D	0.5 : 0.5: 1.5
T5	1/2 MS + 1.5M + 0.8 AGAR	T13	1/2 MS + 1.5M + 0.8 AGAR
T6	1/2 MS + 3M + 0.8 AGAR	T14	1/2 MS + 3M + 0.8 AGAR
T7	1/2 MS + 1.5S + 0.8 AGAR	T15	1/2 MS + 1.5S + 0.8 AGAR
T8	1/2 MS + 3S + 0.8 AGAR	T16	1/2 MS + 3S + 0.8 AGAR

Table 3: Effect of different concentration growth hormone in MS Media for callus regeneration. A, B, C, D = hormone conc. In mg/ l (NAA: KN: BAP). M = %maltose, S = % sucrose , A = % Agar

Rice sample	Combinations (NAA:Kn:BAP)mg/l	Treatment	Calli plate	Calli regenerated	Regeneration %	Green plant regeneration	Albino plant regeneration
Safri-17XIR-64	0.25:0.25:0.75	T_1	13	1	7.69	0	58
		T_3	14	2	14.28	0	0
		T_4	15	1	6.66	0	0
	01:01:02	T_9	12	1	8.33	0	0
		T_{11}	14	1	7.14	78	0
	0.5:0.5:1.5	T_{15}	12	1	8.33	106	0
		T_{16}	14	1	7.14	0	0
MTU1010	0.25:0.25:0.75	T_1	2	1	0	9	5

Table 4: Influence of different hormonal combination on the regeneration potential cross Safri-17xIR-64 and MTU1010 variety

also regenerated 106 numbers of green plants and T11 has produced 7.14 % green callus with 78 green plants (Fig.1E) and T16 has produce 7.14 % of green but no plant were generated out of it. Out of 7 responding treatments for green callus generation only 3 treatments has produced green plant these are treatment number T1, T11, and T15. T1 has generated 7.14 % of green callus and responded for 58 albinos plant were generated (Fig.1F). In variety MTU1010 on an average 28 callus was subjected to16 different treatment. Out of 16 treatments, Treatments T1 has responded into greening callus. In T1 50 % callus greening (Fig. 1C) was observed with 16 days of incubation. Rest of other treatments no response observed. T1 has converted 50 % of callus into green plant & producing 9 numbers of green plants and 5 albinos (Table 4)

CONCLUSION

*I*n cross Safri-17xIR-64 callus induction percentage was 0.49, in variety MTU 1010 callus induction percent was 0.40. In cross Safri-17xIR-64 best callus greening occurred (14.28%) in T3 treatment .In cross Safari-17xIR64 treatment no. T15 was the best response with 106 green plants. In variety MTU 1010 callus greening was 50% in treatment T1, 49 green plants regenerated along with 5 albino plants.

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