Journal of Agricultural Science and Technology B 8 (2018) 97-104
doi: 10.17265/2161-6264/2018.02.003

Effects of Cultivar and Explant Sources on Callus Induction and Plant Regeneration in Rice (*Oryza sativa* L.)

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Abstract: *In vitro* callus induction and plant regeneration potentiality were studied from mature embryo of three Indian rice (*Oryza sativa* L.) groups at Field Crops Department, Agricultural Faculty, Ondokuz Mayıs University, Samsun, Turkey. The study was done by using callus induction MS medium having different concentration of four. The present research was conducted according to the design of randomized blocks trial. A total of 696 calluses, 193 plants and 917 seeds were obtained from Indica group; 2,110 calluses, 103 plants and 235 seeds were obtained from Japonica group; 1,243 calluses and 13 plants were obtained from Javanica group. With regard to number of calluses obtained from each explant source, 52 calluses were obtained from whole-plant explants, 1,668 calluses from root explants, 629 calluses from shoot explants, 649 calluses from the 1st node explants, 240 calluses from the 2nd node explants, 269 calluses from the 1st internode explants and 12 calluses from the 2nd internode explants. With regard to number of plants obtained from each explant source, 27 plants were obtained from whole-plant explants, 195 plants from shoot explants, 43 plants from the 1st node explants, 40 plants from the 2nd node explants and four plants from the 1st internode explants. With regard to number of seeds, 823 seeds were obtained from shoot explants and 329 seeds were obtained from the 2nd node explants. Germination rate of harvested seeds was over 90%. The establishment of this regeneration system is essential for the development of a genetic transformation system for commercial rice cultivars.

Key words: Rice, *Oryza sativa* L., callus induction, plant regeneration.

1. Introduction

Rice (*Oryza sativa* L.) is a monocotyledonous plant with the second greatest place with regard to cultivated land and production. It is the basic nutrient source of more than half of the world population [1]. New biotechnological methods have been developed as an alternative to classical plant breeding programs to improve rice yields. These methods are usually complementary or supporting methods to conventional ones [2]. Despite great efforts, desired yield increases have not been achieved yet in rice with conventional farming systems. For sustainable agriculture and quality life, conventional farming systems should be supported with alternative production techniques to have sufficient and quality yields [3].

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Plant regeneration from somatic tissue and organs is a significant issue in numerous fields, especially in gene transfer. Thusly, as the results of researches carried out up until now, plant regeneration from somatic tissues has become a routine in several dicotyledonous plants. However, several researches were carried out with various monocotyledonous plants, including rice, for plant regeneration from the root [4, 5], mature embryo [4, 6-9], immature embryo [8, 10, 11], coleoptile [12, 13] and young flower organs [7, 14]. But, desired success levels have not been achieved yet.

Callus induction and plant regeneration are influenced by several factors, like nutrient media [15, 16], chemical concentrations in nutrient media [7, 17] and plant genotype [14, 18]. Therefore, there is a need for new and comprehensive researches for healthy and fertile plant regeneration from somatic tissues of

monocotyledonous plants [19, 20].

From this point forth, the present study was conducted to develop a system for healthy and fertile plant regeneration from various somatic tissues and to integrate the system to be developed into gene transfer studies.

2. Materials and Methods

The present research was conducted at Field Crops Department of Ondokuz Mayıs University (Samsun, Turkey) in 2008-2009, to develop a system for healthy and fertile plant regeneration from rice somatic tissues. Whole-plant, root, shoot, 1st and 2nd node, 1st and 2nd internode explants of 12-day old rice seedlings belonging to Indica (Pusur, Shoni), Japonica (Taipei-309, Toyonishiki) and Javanica (Aus-38, Baldo) rice groups were used in experiments. The study was conducted in randomized blocks trial design with three replications.

For seedling production from which the explants are to be taken, surface-sterilized seeds were sown in MS0 nutrient media. For surface sterilization, seeds were placed into 100 mL Erlenmeyer flasks and subjected to pre-treatment in a water bath at 52-57 °C for 15 min [21]. Pre-treated seeds were shaken with 70% ethanol for 1 min, passed through sterile water three times, treated with a couple drops of Tween-20 containing 20% NaOCl for 20 min and passed through sterile water 3-5 times for rinsing. Seeds were then placed in Petri dishes (9 cm in diameter) with sterile dry papers to remove the water over the seed surfaces [19, 20, 22, 23].

Sterilized seeds were sown in MS0 nutrient media, explants were taken from the grown seedlings on the 12th day, explants were then transferred to glass Petri dishes (15 cm in diameter for whole-plant explants and 9 cm for the other explants) with a nutrient media containing 2.5 mg/L 2.4-D, 3% sugar, 0.76% agar (pH = 5.8). Five explants from each explant source were transferred to Petri dishes in three replications. Explants transferred to nutrient media were then kept at 28 °C in dark incubators until callus induction (Fig. 1). Throughout the incubation period, explants were transferred to fresh LS 2.5 media in every three weeks.

Following the callus induction, calluses were transferred to MS media containing 0.5 mg/L 6-Benzylaminopurine (BAP) and naftalen asetik asit (NAA), 3% sugar, 0.76% agar with pH = 5.8. Transferred calluses were subjected to 12 h light-12 h dark photoperiods at 26 °C in a climate cabin until shoot induction (Fig. 2a).

The shoots formed in nutrient media were then transferred to magenta boxes containing 0.5 mg/L indole-3-acetic acid (IAA), 3% sugar, 0.76% agar with pH = 5.8. The magenta boxes with shoots were subjected to 12 h light-12 h dark photoperiods at 26 °C in a climate cabin until sufficient root and shoot development (Fig. 2b).

The plantlets with complete root and shoot development were transferred to glass jars with tap water for 1-2 d to adapt them to external conditions,

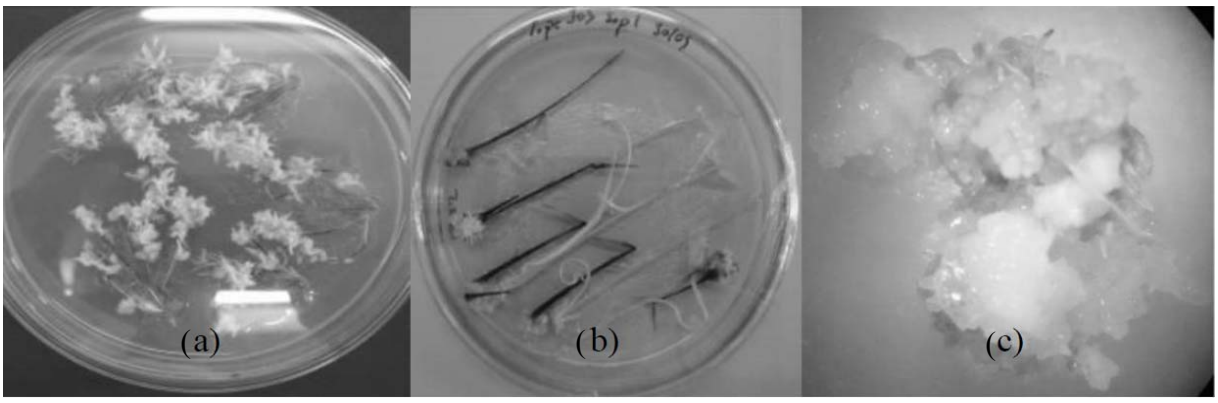

Fig. 1 Root explants (a), stem explants (b) and developing cali general views (c).

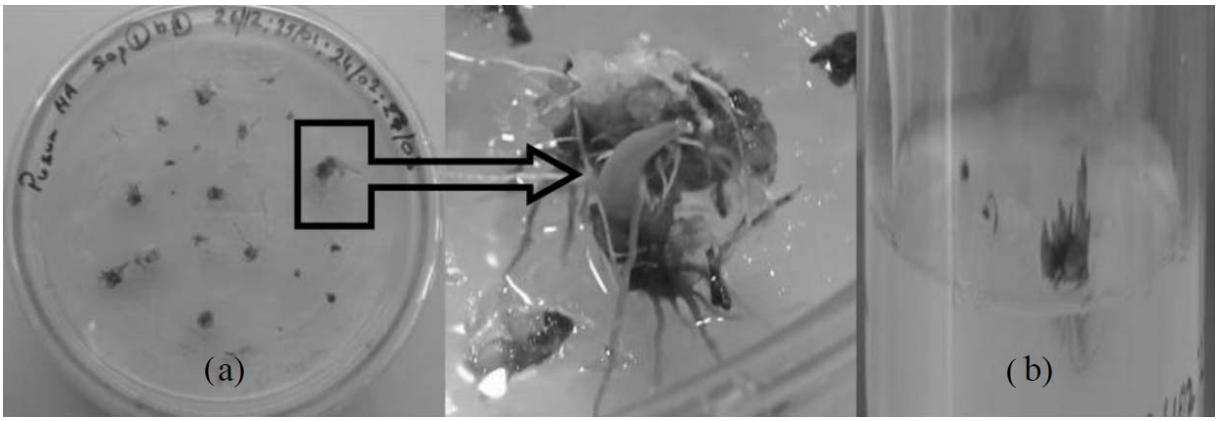

Fig. 2 The shoot induction from stem explants (a) and general view of the shoot transferred rooting media (b).

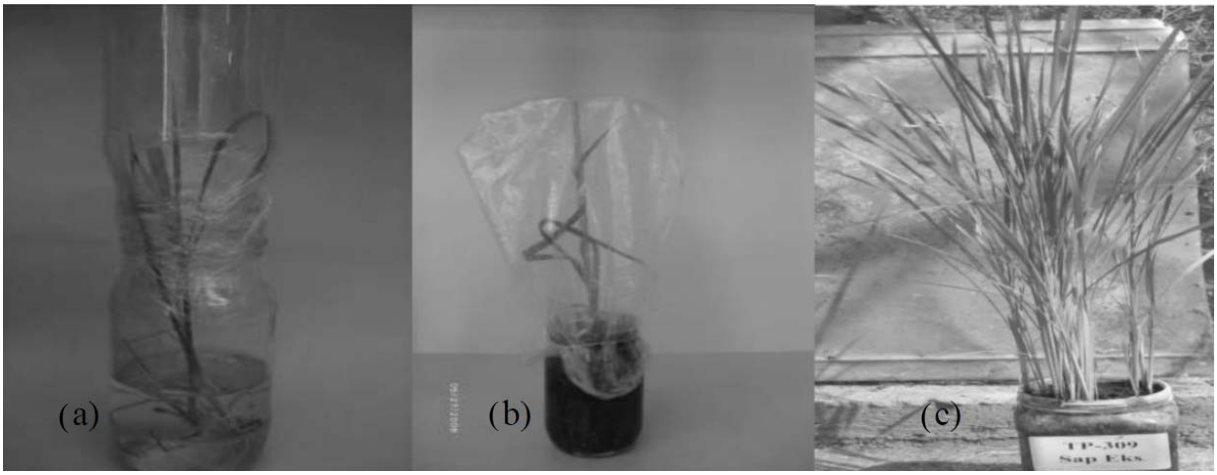

Fig. 3 Plantlets in rooting (a) and compost media (b) and general views of plantlets in panicle formation stage (c).

and jars were covered with polyethylene bags to provide a moisture balance. Two days later, plants were transferred to other glass jars containing 1:1:1:2 yard soil/river sand/perlite/turf mixture, jars were covered again with polyethylene bags to preserve a moisture balance and plants were misted with tap water for 10 d (Fig. 3a). Two-three weeks later under laboratory conditions, plantlets were transferred to plastic jars (15 cm in diameter) containing clay-loam soil (Fig. 3b). Daily water requirements of the plants were met and yellow leaves were removed. To meet nitrogenous fertilizer need of the plants, 10.75 mg liquid ammonium sulphate was applied to each jar as to have 12 kg pure substance per decare. Plants developing in plastic jars and with panicles were covered with isolation bags to prevent seed loss, and the plants with physical maturity were manually harvested (Fig. 3c).

The frequency of callus induction and plant regeneration (%) was measured using Eqs. (1) and (2):

$$\text{Callus induction} = (\text{Formed callus number}/\text{Total number of explant}) \times 100 \quad (1)$$

$$\text{Plant regeneration} = (\text{Formed number of plant}/\text{Total callus number}) \times 100 \quad (2)$$

3. Results

3.1 Number of Callus

The data obtained from seven different explant sources transferred to nutrient media were provided in Table 1. A total of 4,049 calluses were obtained in nutrient media. With regard to number of calluses from each explant source, 582 calluses were obtained from whole-plant explants, 1,668 calluses from root

explants, 629 from stem explants, 649 from the 1st node explants, 240 from the 2nd node explants, 269 from the 1st internode explants and 12 from the 2nd internode explants. With regard to number of calluses from each rice groups, 696 calluses were obtained from Indica group, 2,110 from Japonica group and 1,243 from Javanica group. With regard to number of calluses from each rice cultivars, 437 calluses were obtained from Pusur and 259 calluses were obtained from Shoni cultivar of Indica group; 733 from Taipei-309 and 1,377 from Toyonishiki cultivar of Japonica group; 720 from Aus-38 and 523 from Baldo cultivar of Javanica group (Table 1, Fig. 4).

3.2 Number of Plants

The data on plant regeneration from the calluses transferred to differentiation ambient were provided in Table 1. A total of 309 plants were obtained from these calluses. With regard to number of plants obtained from each explant source, 27 plants were obtained from whole-plant explants, 195 from stem explants, 43 from the 1st node explants, 40 from the 2nd node explants and four from the 1st internode explants. With regard to number of plants obtained from each rice groups and cultivars, 193 plants were obtained from Indica group, 103 from Japonica group and 13 from Javanica group; 168 plants were obtained from Pusur and 25 from Shoni cultivar of Indica group, 75 from Taipei-309 and 28 from Toyonishiki cultivar of Japonica group, six from Aus-38 and seven from Baldo cultivar of Javanica group (Table 1, Fig. 5).

Table 1 The data obtained from the research of the number of callus, plant and seeds.

Consisted	Explant sources	Group and varieties of rice									General total
		Indika			Japonica			Javanica			
		Pusur	Shoni	Total	Tp	Ty	Total	A-38	Baldo	Total	
Numbers of callus	Plant	13	135	148	96	185	281	105	48	153	582
	Root	226	0	226	203	604	807	285	350	635	1,668
	Stem	117	36	153	172	203	375	75	26	101	629
	1st node	81	65	146	174	210	384	93	26	119	649
	2nd node	0	12	12	76	0	76	79	73	152	240
	1st internode	0	11	11	12	175	187	71	0	71	269
	2nd internode	0	0	0	0	0	0	12	0	12	12
Total		437	259	696	733	1,377	2,110	720	523	1,243	4,049
Numbers of plants	Plant	10	0	10	10	7	17	0	0	0	27
	Root	0	0	0	0	0	0	0	0	0	0
	Stem	136	0	136	46	6	52	6	1	7	195
	1st node	22	0	22	0	15	15	0	6	6	43
	2nd node	0	25	25	15	0	15	0	0	0	40
	1st internode	0	0	0	4	0	4	0	0	0	4
	2nd internode	0	0	0	0	0	0	0	0	0	0
Total		168	25	193	75	28	103	6	7	13	309
Number of seeds	Plant	0	0	0	0	0	0	0	0	0	0
	Root	0	0	0	0	0	0	0	0	0	0
	Stem	638	0	638	185	0	185	0	0	0	823
	1st node	0	0	0	0	0	0	0	0	0	0
	2nd node	0	279	279	50	0	50	0	0	0	329
	1st internode	0	0	0	0	0	0	0	0	0	0
	2nd internode	0	0	0	0	0	0	0	0	0	0
Total		638	279	917	235	0	235	0	0	0	1,152

Tp = Taipei-309; Ty = Toyonoshiki; A-38 = Aus-38.

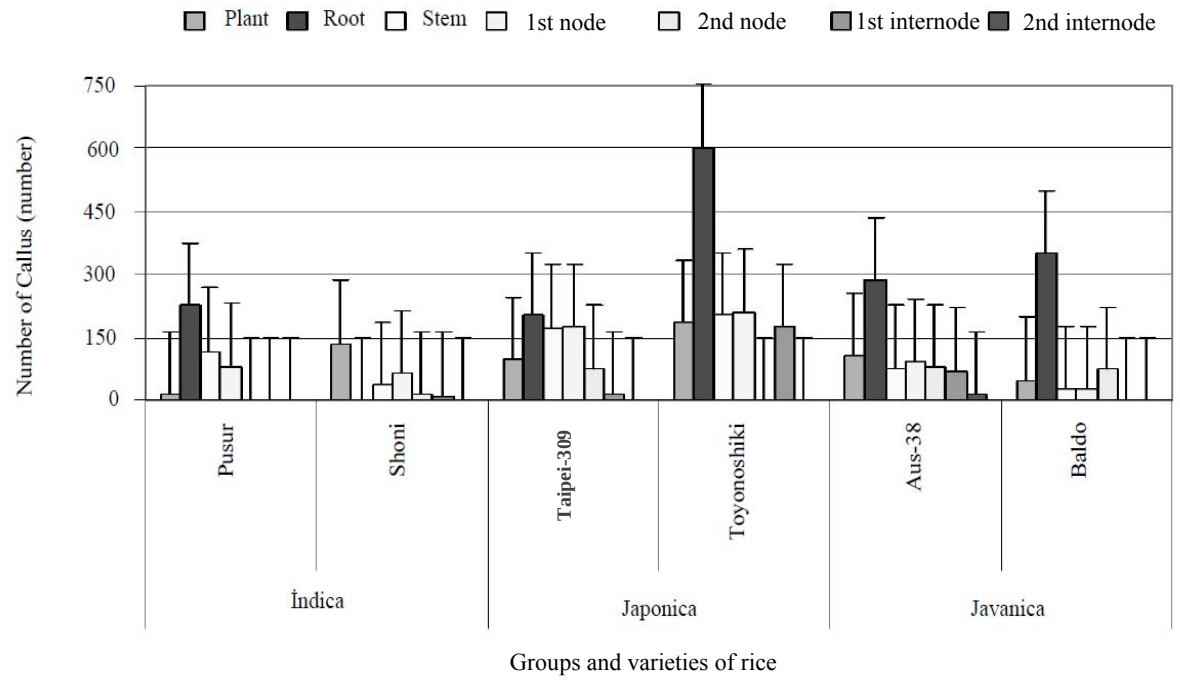

Fig. 4 Induction callus varieties and distribution of rice groups.

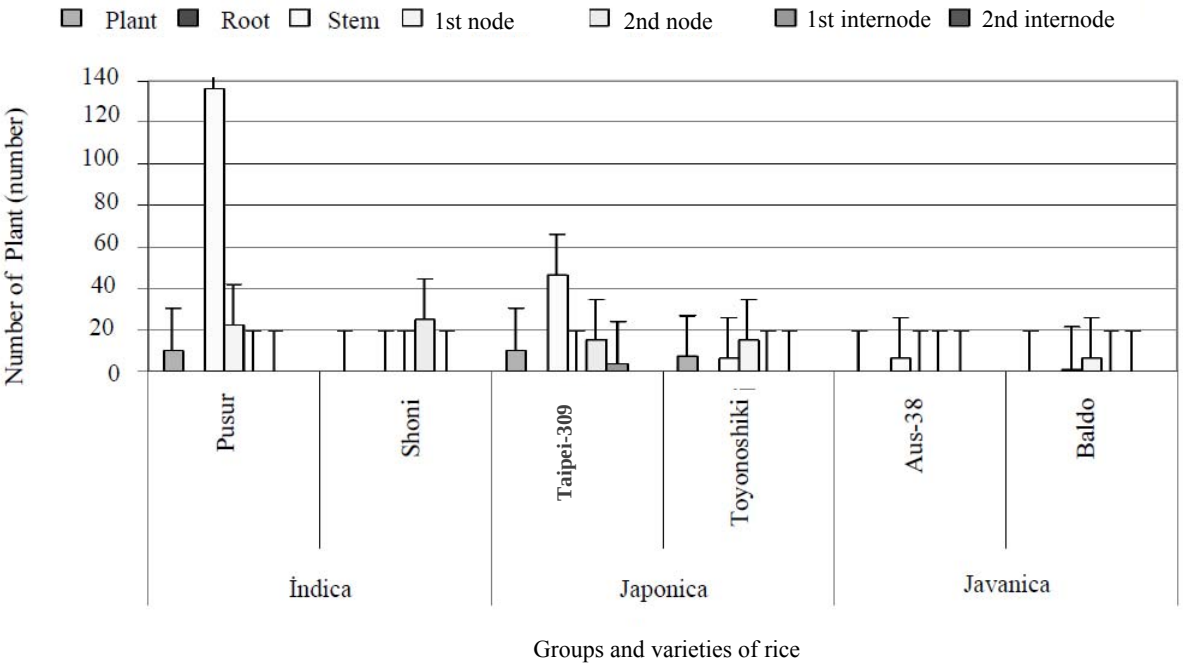

Fig. 5 Number of plant varieties and distribution of rice groups.

3.3 Number of Seeds

A total of 1,152 seeds were obtained from the harvested plants. With regard to number of seeds obtained from each explant sources, 823 seeds were obtained from shoot explants and 329 seeds were obtained from the 2nd node explants. With regard to number of seeds obtained from each of rice groups

and cultivars, 917 seeds were obtained from Indica group and 235 seeds were obtained from Japonica group; 638 seeds were obtained from Pusur, 279 seeds were obtained from Shoni cultivar of Indica group and 235 seeds were obtained from Taipei-309 cultivar of Japonica group (Table 1).

4. Discussion

Current findings were parallel to the results of Abe and Sasahara [24], indicating the highest callus induction capability for cultivars of Japonica group. Present findings also comply with the results of Rachmawati and Anzai [25], again indicating the highest callus induction capability for rice cultivars of Japonica group, but do not comply with the findings indicating that this group was followed by Indica and Javanica groups. Such incompliances were mainly because of different cultivars used in these two studies. Thusly, Hoque et al. [26] reported higher callus induction potential for a cultivar of Indica group than for a cultivar of Japonica group. The argument indicating that callus induction potential of the rice did not depend on groups, but resulted from the differences among the genotypes, Kyozuka et al. [27] proved those findings.

Current findings comply with earlier findings indicating that the differences in plant regeneration potential of Indica, Japonica and Javanica rice groups were mainly resulted from the differences among the genotypes rather than the differences among the groups [28], donor plant genotype was a quite significant factor in plant differentiation [29], genotype was more significant in plant differentiation than nutrient media [30].

As to conclude, it can be stated based on current findings that primarily the rice group, then the cultivar and explant source played significant roles in callus induction and plant regeneration from different explant sources. Despite high callus induction rates, plants were not able to be obtained from some explant sources and such a case may be related to explant age, since these explants turned into brown shortly after placing them into differentiation ambient and lost their vigor. Therefore, age of explants transferred to nutrient media can be brought forward (less than 12 d), and faster division activity of younger plants may be used as an advantage in differentiation process. Similarly, more efficient callus induction and plant regeneration potential of young explants were also reported by several researchers [12, 22, 31, 32]. Besides the type, amount of plant growth regulator in culture ambient has significant impacts in morphogenesis [33-35]. Such impacts might have also yielded different outcomes in different studies.

The following points were drawn from the present study: (1) present callus induction ambient was a proper ambient; (2) nutrient media should be supplemented with auxin to improve callus stimulation and plant regeneration frequency; (3) explant age should be lower than 12 d; (4) to reduce plant die-outs, roots should be kept in water for 2-3 d while transferring the plants to outside and then plants should be kept in well-moisture balanced ambient for 1-2 weeks.

5. Conclusions

In present study, shoot and the 2nd node explants yielded fertile and healthy seeds and plants. In this way, the system desired to be integrated was developed. This system can be integrated into gene transfer studies and may eliminate several problems, especially sterility problem, resulted from long-duration stays under *in vitro* conditions.

Acknowledgments

The authors would like to thank the Ondokuz Mayıs University (Samsun, Turkey) for their support to the plant biotechnology lab in the field crops section.

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