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IN VITRO CULTURE OF SEVERAL RICE CULTIVARS

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ABSTRACT

Tissue culture methods have been established to regenerate certain rice (*Oryza sativa* L.) cultivars, but regeneration of the rice cultivars widely grown in Arkansas has not been reported. This study has established an *in vitro* culture for the rice cultivars 'Nortai', 'Starbonnet', 'Mars', 'Tebonnet', 'Newbonnet', and 'Lemont'. Callus was induced in the dark at either 20 or 28 C from dehusked seeds cultured on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing 40 g L^{-1} sucrose, 10 g L^{-1} agar, 0.5, 1.0, or 2.0 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) and adjusted to pH 5.7. After four weeks the calli were weighed, transferred onto MS medium containing no 2,4-D, and maintained in a 12-h photoperiod (65 uE m^{-2} s^{-1}) at 25 ± 2 C to induce plant regeneration. Callus production was best when cultured on a medium containing 1.0 mg L^{-1} 2,4-D and incubated at 28 C. Plant regeneration was observed two to four weeks later. The percentage of calli regenerating platlets varied with the cultivar and the callus induction treatment. Callus induction at 20 C on a medium with a 2,4-D level less than 2.0 mg L^{-1} enhanced the regenerability of most cultivars. Regenerates were transplanted to soil and grow normally to maturity. This system can be helpful in improving rice cultivars with tissue culture techniques such as somaclonal variant selection and somatic hybridization.

INTRODUCTION

Improvement and development of superior crops to meet the needs of the world's growing population for food, fiber, fuel, medicine, and aesthetics requires the implementation of new as well as traditional techniques (Green, 1981). Rice (*Oryza sativa* L.) is the basic food of more than half of the world's population (Pathak, 1982). Traditional plant breeding has contributed greatly to the improvement of many rice varieties. Plant tissue culture, a technique of growing plant tissues *in vitro* in a nutrient medium under aseptic conditions, is an economical means for vegetative propagation that is becoming increasingly important as a tool in plant breeding.

Mutant selection, anther and pollen culture, and somatic hybridization are techniques that may be useful in rice improvement. The feasibility of these approaches is solely dependent upon the availability of a tissue culture system for the regeneration of a particular cultivar. Systems for *in vitro* regeneration of a number of rice cultivars have been established (Heyser *et al.*, 1983; Oard and Rutger, 1988; Raghava and Nabors, 1984; Siriwardana and Nabors, 1983); however, cultivars that are widely grown in Arkansas have not been included in these studies. Since genotypes differ in their ability to regenerate, our aim was to investigate suitable culture systems for the regeneration of several rice cultivars that are grown in Arkansas.

MATERIALS AND METHODS

Dehusked seeds of the cultivars 'Nortai', 'Starbonnet', 'Mars', 'Tebonnet', and 'Newbonnet' (cultivars developed by USDA/ARS and the Arkansas Agriculture Experiment Station), and 'Lemont' (a semidwarf cultivar developed by the USDA-ARS and the Texas Agriculture Experiment Station) were surface-sterilized in 95% ethanol for 30 sec, immersed in 2.6% hypochlorite for 20 min, and rinsed three times with sterile water. The mature embryos were cultured on MS medium (Murashige and Skoog, 1962) supplemented with 0.4 mg L^{-1} thiamine HCl, 100 mg L^{-1} inositol, 40 g L^{-1} sucrose, and 0.5, 1.0, or 2.0 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D). The medium was adjusted to pH 5.7 and solidified with 10 g L^{-1} agar. The cultures were incubated in the dark at either 20 or 28 C for callus induction. After four weeks the calli were separated from the original explants and weighed with a digital microscale. To induce morphogenesis, the calli were transferred to MS medium without 2,4-D. The cultures were maintained at 25 ± 2 C in a 12-h photoperiod of cool-white fluorescent light (65 uE m^{-2} s^{-1}).

A three-factor factorial experimental design with 10 seeds per treatment was used to investigate temperature, hormone concentration, and cultivar response. The efficacy of the treatments was based on callus fresh weight and subsequent plant regeneration frequency. Regenerated plants were transplanted to potting soil, acclimated, and then transferred to a greenhouse.

RESULTS AND DISCUSSION

CALLUS INDUCTION

One week after initial incubation, callus growth was visible. Callus originated from the region subtending the primary shoot that arises initially as the seed begins to germinate. Germination soon ceased, but the callus continued to proliferate (Fig. 1). Callus growth was influenced by incubation temperature and 2,4-D concentration; those grown at 28 C yielded 0.090 g, significantly more than the 0.048 g of those cultured at 20 C at all 2,4-D concentrations. Fresh weights of the calli incubated

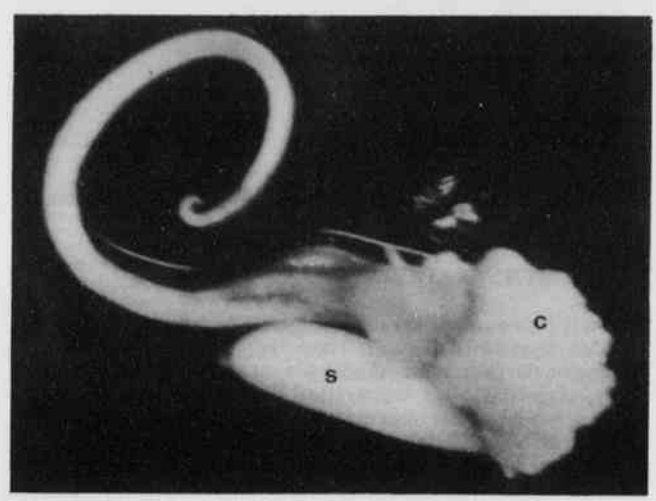

Figure 1. Rice callus (C) induction accompanied with seed (S) germination at initial stage.

on the media with 0.5, 1.0, and 2.0 mg L^{-1} of 2,4-D were 0.069, 0.086, and 0.052 g, respectively, at both temperatures. Calli induced by 0.5 and 1.0 mg L^{-1} 2,4-D were not significantly different in weight, but those induced by 2.0 mg L^{-1} 2,4-D were significantly smaller than calli induced by 1.0 mg L^{-1}.

Callus growth differed significantly among the six cultivars (Table 1). Under the conditions of this experiment callus weight for Lemont was significantly less than that of all other cultivars. Furthermore, Nortai, and Starbonnet produced the most growth.

Table 1. The Response of Rice Cultivars to Callus Induction Treatments

Cultivar	Callus Weight (g)[1]
Nortai	0.091a[2]
Starbonnet	0.087a
Mars	0.076ab
Tebonnet	0.072ab
Newbonnet	0.057bc
Lemont	0.031c

[1]Callus weight averaged over both induction temperatures (20 and 28 C) and 2,4-D concentrations (0.5, 1.0, and 2.0 mg L^{-1}).
[2]Means followed by a common letter are not significantly different at 5% (LSD = 0.027).

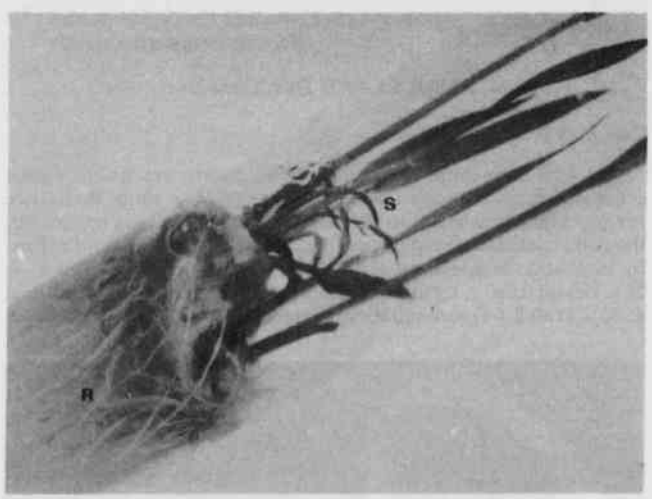

Figure 2. Plant regeneration from rice callus showing shoot (S) and root (R).

PLANT REGENERATION

Two to four weeks after callus-incubation on regeneration medium, plantlets regenerated (Fig. 2). These regenerated plantlets grew to maturity in the greenhouse. Shoot and root systems developed concurrently, suggesting plant regeneration via somatic embryogenesis, a phenomenon frequently observed in regeneration of monocotyledonous species (Nabors *et al.*, 1983). The percentage of calli that regenerated plantlets varied greatly depending on cultivar and callus induction treatments (Table 2). Generally, callus induction at the lower temperature of 20 C enhanced the regenerability of all cultivars except Starbonnet. Concentrations of 2,4-D in the callus induction medium lower than 2.0 mg L^{-1} were more effective in producing regenerable calli.

Table 2. The Influence of 2,4-D Concentration, Callus Induction Temperature, and Cultivar on Regeneration Percentage.

	% Plant Regeneration					
	20 C			28 C		
	Concentration of 2,4-D (mg/L)					
Cultivar	0.5	1.0	2.0	0.5	1.0	2.0
Nortai	25	0	0	0	0	0
Starbonnet	34	25	0	67	25	0
Mars	0	25	25	0	0	0
Tebonnet	40	67	0	0	25	0
Newbonnet	75	50	34	0	0	25
Lemont	20	40	0	0	0	0

Cultivar response to callus induction did not correspond to plant regeneration. Nortai was superior in the amount of callus produced but showed the lowest plant regeneration frequency. A specific tissue culture system may be required not only for different species, but also for various cultivars of a single species. The necessity to define a unique set of conditions for the optimum culture of each rice cultivar was evident from the wide range in callus fresh weights produced from the six cultivars tested and from their differential responses in regeneration. Variability in callus weights, as well as in plant regeneration frequency among cultivars, could be attributed to genotypic variation.

CONCLUSIONS

This system demonstrates, for the first time, the suitability of these Arkansas and Texas rice cultivars for *in vitro* culturing. The regeneration of a given cultivar may be enhanced by particular modifications of the medium. This finding will allow the application of various tissue culture techniques to augment traditional breeding programs for rice improvement, such as the selection of favorable mutants at the cellular level.

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