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Short Communication

Organogenesis induction in rice callus by cyanobacterial extracellular product

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Cyanobacteria or blue green algae are prokaryotic photosynthetic microorganism that produces a wide array of substances, including plant growth regulators. In the case of growth regulators, gibberellin, auxin, cytokinin, ethylene, abscisic acid and jasmonic acid have been detected in cyanobacteria. Many substances have been added to plant tissue culture media in order to promote plant regeneration. The present research communication gives a report of the study of the effect of extracellular products of *Plectonema* sp., isolated from paddy fields on regeneration of rice. The endosperm of three rice varieties, IR 50, ASD 16 and ADT 36, were used as explants. IR 50 showed earlier and good callus induction response in MS medium. For root induction, cyanobaterial extracellular product was added instead of 2,4-D. The result showed that the number of days taken for root initiation and root growth was quicker by adding the extracellular products. Interestingly, more proliferation of roots in cyanobaterial extracellular product treatments was also observed compared to 2,4-D which might due to the production of growth regulators like auxin(s). Tremendous growth of root length and volume in short period indicate that MS with cyanobaterial extracellular product may also be used for screening of rice genotypes for water stress condition.

Key words: Callus induction, cyanobacterial extracellular products, *Plectonema* sp., rice, root induction.

INTRODUCTION

Cyanobacteria or blue green algae are the most dominant photosynthetic prokaryotic microorganisms that produce a wide array of substances. These include antibiotics, algicides, toxins, pharmaceuticals and plant growth regulators (Metting and Pyne, 1996). Among the growth regulators, gibberellin, auxin, cytokinin, ethylene, abscisic acid and jasmonic acid have been detected in cyanobacteria (Stirk et al., 1996; Gupta and Agarwal, 1973; Ordog and Pulz, 1996). There is accumulating evidence that cyanobacteria produce plant hormones or demonstrate plant hormones-like activity. Tissue culture in rice is a gateway to genetic engineering. The most important area in the field of tissue culture with proven practical application is the organogenesis.

Plant regeneration from somatic cells can be accomplished through two morphogenic pathways: somatic embryogenesis and organogenesis (adventitious shoots and roots). The pathways of regeneration dependon the media and the material used. Both pathways have been observed in rice tissue cultures (Usha Rani and Reddy, 1996). Many substances have been added to the culture media to enhance plant regeneration. Promoting factors such as organic additives, plant hormones as well as amino acids have given good results. Cyanobacterial biomass extracts

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Abbreviations: 2, 4-D, 2, 4-dichlorophenoxyacetic acid; CEP, cyanobacterial extracellular products; MS, Murashige and Skoog's medium.

Table 1. Callus induction in rice using 2,4-D.

Rice cultivar	2,4-D		
	2 mg/l	2.5 mg/l	3 mg/l
IR 50	88.0*	91.0*	85.0*
ASD 16	82.1	85.0	80.2
ADT 36	79.5	82.3	80.3
Mean	83.2	86.1	81.8
SE	0.51	0.32	0.27

Table 2. Effect of 2,4-D and cyanobacterial extracellular products (CEP) on root induction in IR 50 rice.

Treatment	Number of calli taken	Percentage of regeneration (%)	Number of days for root induction (Days)
MS + 2,4 D (2.0 mg/l)	25	12	49
MS + 2,4 D (2.5 mg/l)	25	16	47
MS + 2,4 D (3.0 mg/l)	25	32	48
MS +CEP (5.1 ml/l)	25	32	41
MS +CEP (10.2 ml/l)	25	48	37
MS +CEP (20.4 ml/l)	25	40	40

have been observed to strongly promote somatic embryogenesis (Wake, 1992). Extracts of *Plectonema* sp. stimulated somatic embryogenesis and somatic embryo development in sandalwood (*Santalum album*) (Bapat, 1996). Here, we studied the extracts of the cyanobacteria, *Plectonima* sp., isolated from paddy fields for its effect on root induction in rice callus.

MATERIALS AND METHODS

In the present investigation, the three rice varieties, IR 50, ASD 16 and ADT 36 were studied. To obtain rice calli, the endosperm from matured dehusked caryopsis was excised and embryos were sterilized by immersion in 96% ethanol for 5 min, 0.1% $HgCl_2$ for 2 min and then vigorously stirred and washed with sterile distilled water for 30 min. The embryos were then inoculated in 150 ml glass flask containing 25 ml MS medium, supplemented with different concentrations (2.0, 2.5 and 3.0 mg/l) of 2,4-D, sucrose (30 g/l) and agar (8 g/l). The pH of the medium was adjusted to 5.7 before autoclaving for 20 min at 120°C. After 10 days in dark at 26 ± 1°C, the remnants of germinated embryo were removed. Calli were then transferred to the fresh medium and subcultured from 25 to 83 days and then were transferred to regeneration medium.

Conventionally, for root induction, 2,4-D is used in MS medium. In this experiment we used cyanobacterial extracellular products (CEP) and compared with its 2,4-D for its influence on regeneration. For preparing the CEP, soil samples from different locations of rice fields showing visible blue green algal growth in water logged conditions were selected for isolation. The soil samples were serially diluted to 10^{-2} using sterile distilled water and 1 ml of 10^{-2} aliquots were inoculated to 100 ml of nitrogen free Bristol's medium in Erlenmeyer flasks. After inoculation, flasks were incubated in light chamber at 28 ± 1°C for four weeks. Then the biomass was separated from the medium (pH 7.2) by centrifugation for 20 min at 8000 x g and 15 ± 1°C. The culture medium containing CEP was sterilized by filtration. Calli chosen for the experiment had a mean fresh weight of 5.53 ± 4.22 mg. These were cultivated in test tubes containing 13 ml of regeneration media: MS + 2.5 mg/l 2,4-D (control) and MS + 10.2 ml/l CEP. One callus was inoculated in each test tube. The test tubes were maintained at 26 ± 1°C, under fluorescent light, photoperiod 16:8 at mol photons m^2 s^{-1}.

RESULTS AND DISCUSSION

Rice is the principal food crop for more than half of the world's population. For the improvement of rice, tissue culture is one of the biotechnological tools that plays an important role in the genetic engineering of rice. Success of any tissue culture depends upon the standardization of medium used for that experiment. In this experiment,
callus induction medium was standardized using three concentrations of 2,4-D (2, 2.5 and 3 mg/l) were tried for getting maximum callus induction. Among the three concentrations, 2.5 mg/l 2,4-D shows good response compared to other two concentrations. Among the rice varieties used for the callus induction, IR 50 showed significantly good response (91%) followed by ASD 16 (85%) (Table 1). After 21 days, the friable calli obtained were transferred for regeneration. Normally in any tissue culture media the organic growth regulators are used in the chemical form. But in our study, naturally available algae (*Plectonema* sp.) extract was used to determine their role and it compared favourably with normally used 2,4-D. Earlier regeneration is the primary goal of the *in vitro* studies and the number of days taken for root induction was recorded. CEP at 10.2 ml/l showed quick response with root initiation in 37 days, whereas in the case of 2,4 D, it started after 47 days (Table 2).
To standardize the media, different amounts of CEP were also tried in order to get maximum percentage of root induction. By comparing the 2,4-D and CEP, CEP showed a maximum of 48% regeneration in 10.2 ml/l and followed by 20.4 m and 5.1 ml/l with 40 and 32% response, respectively (Table 2). CEP is a natural substrate which can be used as better alternative to 2,4-D in the MS medium for rice root induction. Interestingly, we also observed better proliferation of roots in CEP compared to 2,4-D treatments, which might be due to the production of growth regulators like auxin(s) and abscisic acid (Figure 1). Further indepth research is still required to determine the specific substance in the CEP extract as well as its mode of action.

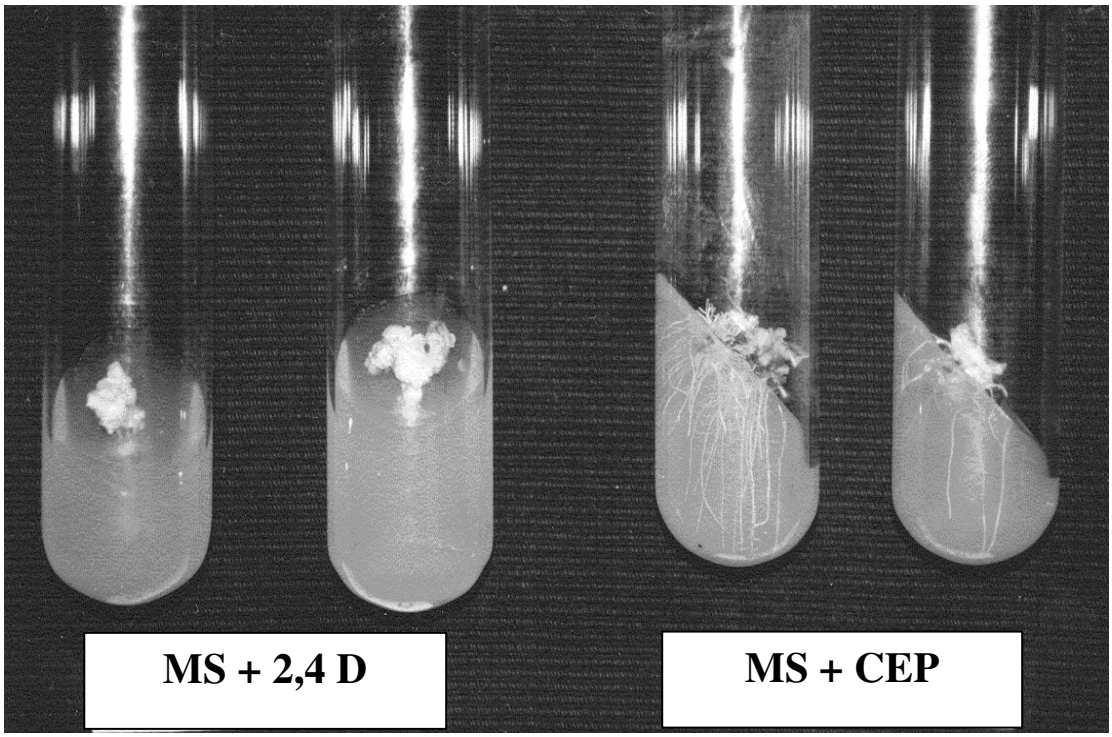

Figure 1. Rice root induction in MS medium with 2,4-D and cyanobacterial extracellular products (CEP).

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