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Potentiality of Leaf Sheath Cells for Regeneration of Rice (*Oryza sativa* L.) Plants

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Summary. Regeneration of rice plantlets (*Oryza sativa* L.) from calli originated from leaf sheath cells was made possible. This was possible in tissues initially grown in media containing 2.4-D (2.4-dichlorophenoxyacetic acid) at low temperature and illumination. The slow growing tissues were subsequently subjected to growth conditions at an elevated temperature and higher illumination with addition of kinetin and IAA and without 2.4-D. The suitability of leaf sheath cells for protoplast technology is indicated by this success.

Key words: Regeneration – Leaf sheath cells – Complete plantlet formation of rice

Introduction

The rapid advancement of genetic modification of plant cells has established the prospect of protoplast technology as an useful tool in crop improvement. However the complete success of the technology in most of the cases rests heavily on the development of a reproducible method for the regeneration of the entire plant after the initial manipulation has been caused at the protoplast level. Hence, regeneration forms an integral part of the programme. Earlier we claimed success in obtaining regeneration of rice protoplasts isolated from leaf sheath cells where previously only a limited success of differentiation in the calli in terms of rhizogenesis had been possible to achieve (Deka and Sen 1976). Since then, no further progress has been made on inducing differentiation on protoplast derived callus tissue to form root and shoot although some important observations have been accumulated indicating that the protoplasts derived from various genotypes of cultivated rice have a plating efficiency varying from none to a rarely seen maximum of about 30%. Unfortunately most of the genotypes do not respond favourably. However, it may well be confirmed that the leaf sheath cells yield by far the best protoplasts for plating (Sen et al, in preparation). Having failed to induce differentiation in leaf sheath protoplast derived calli, it was necessary for us to ask whether the leaf-sheath cells are totipotent or not. This communication deals with the results of our study in that direction and indicates that they do contain totipotent cells, although the number varies with genotype, as revealed through the response of the relevant cells to given cultural conditions.

Materials and Methods

Leaf sheath pieces (5 mm in length) were collected from aseptically grown 40 days (*Oryza sativa* L.) seedlings of various cultivars (viz., 'Jaya', 'Taichung Native 1', 'IR8', 'Lal Nankada', 'Ratna', 'Padma', 'Meghna', 'T 141', 'GEB 24', 'Jhingasaal', 'Lathisaal') for callus induction. For callus growth, the following basal culture media were used: 1. MS: Murashige and Skoog, (1962), 2. B5: Gamborg, (1968), 3. NN: Nitsch and Nitsch, (1969), 4. SH: Schenk and Hildebrandt, (1972). These media were furthermore either supplemented with or normally contained following growth regulators viz., 2.4-D (2.4-dichlorophenoxyacetic acid at 1 and 2 mg/l level); IAA (indole-3-acetic acid at 1 mg to 5 mg/l level); NAA (naphthalene acetic acid at 1 mg to 2 mg/l level). Media were solidified with 0.7% agar. Growth conditions varied from complete darkness to continuous light (1000 lux) at 25°C. After our preliminary trials it was found out that basal medium supplemented with 2.4-D (2 mg/l) promptly yielded callus. All calli lines that form the experimental material of this study (Table 1) are derived after supplementing the media with 2.4-D at 2 mg/l concentration level. Induction of differentiation was attempted on B5 medium with or without 2.4-D, IAA and kinetin at 30°C with light (4000 lux at 16/8 photoperiod).

Results

Of all the four media tried, B5 in combination with 2.4-D (2 mg/l) and darkness, yielded within 2 to 3 weeks of time the best callus growth, which appeared as a soft whitish

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Table 1. Response of leafsheath inoculum of rice to different culture medium with respect to callus formation

Genotypes/strains	MS	B5	SH	NN
'Jaya'	+	–	–	–
'Taichung Native 1'	+	+	–	–
'IR8'	–	+	–	–
'Lal Nankada'	–	–	+	+
'Ratna'	–	+	–	–
'Padma'	+	+++	+	–
'Meghna'	+	–	+	–
'T 141'	–	–	–	–
'GEB 24'	–	+	–	–
'Jhingasaal'	–	–	–	–
'Lathisaal'	–	–	–	–

Symbols: – = 0%; + = 1-4%; +++ = 8-12%

mass. The frequency of leaf sheath inoculum response of which 'Padma' gave the highest, was however only 8-12% in B5, which, incidentally turned out to be the best amongst all our attempts. Other media either failed to induce callus or their yield was not at all significant in any amount. In a single case with NN, MS and SH we did obtain the production of calli in some genotypes (Table 1). However, most of them turned brown to black accompanied by senescence in the course of time. Almost all our attempts to carry forward the calli lines obtained from other than B5 medium were failures as often their growth could not be stimulated. Thus, we ultimately settled with the calli that we obtained from five genotypes ('Taichung Native 1', 'IR8', 'Padma', 'Ratna' and 'GEB 24') grown on B5 medium. We observed that some strains of rice failed completely to respond in the callus induction medium, whereas 'Padma' responded most favourably.

The initially obtained calli of 'Taichung Native 1', 'IR8', 'Padma', 'Ratna' and 'GEB 24' were subsequently subcultured in darkness in the same basal medium with 2.4-D until a sufficient amount of calli was obtained. After 4 passages, with 4-6 weeks of growth allowed in each passage, the tissue lines were offered light (1000 lux) supplemented with an increase in the 2.4-D level (5 mg/l). This accelerated growth in all cases to a fairly moderate level. The soft whitish calli turned pale brownish yellow after a period of time. After 6-8 weeks, these calli were subjected to conditions conducive for the induction of differentiation. The differentiating conditions essentially comprised of culturing the tissue lines on B5 with or without 2.4-D, IAA and kinetin at 30°C with an increased amount of light (4000 lux at 16/8 h photoperiod). Table 2 documents the results of our observations. Calli from

Table 2. Repsonse of calli lines obtained from leafsheath tissue of different genotypes of rice in B5 supplemented with 2.4-D, IAA and kinetin

The name of genotype	No. of tubes attempted	Concentration of growth substances in mg/l			Changes observed after 4-6 weeks	No. of tubes showing changes	Result in percentage
		2.4-D	kinetin	IAA			
'Padma'	76	0.5	0.5	2.0	MG	10	13.1
	56	0.5	1.0	–	NG	–	–
	95	0.5	1.0	2.0	MG + SGP	8 + 3	8.4 + 3.2
	81	0.5	1.0	5.0	Rh	8	9.8
	47	1.0	0.1	1.0	Rh	7	14.8
	55	2.0	–	2.0	Rh	4	7.2
	55	2.0	0.1	1.00	NG	–	–
	97	2.0	1.0	–	NG	–	–
	39	5.0	–	5.0	Rh	9	23.07
	88	–	0.1	1.0	MG	8	9.1
	89	–	0.5	2.0	SGP	4	4.5
	20	–	0.5	5.00	SGP	3	15
	54	–	1.0	2.00	D + SGP	4 + 6	7.4 + 11.1
	156	–	1.0	5.00	D	16	9.75
'Taichung Native 1'	20	–	0.5	5.00	NG	–	–
	30	–	1.0	5.00	NG	–	–
'IR8'	20	–	0.5	5.00	SG	2	10
	30	–	1.0	5.00	NG	–	–
'Ratna'	20	–	0.5	5.00	NG	–	–
	30	–	1.0	5.00	D + SGP	2 + 4	6.6 + 13.2
'GEB24'	20	–	0.5	5.00	NG	–	–
	30	–	1.0	5.00	NG	–	–

NG = no growth; MG = moderate growth; SGP = slow growth with small protuberance in the callus surface; D = differentiation; Rh = rhizogenesis

Fig. 1

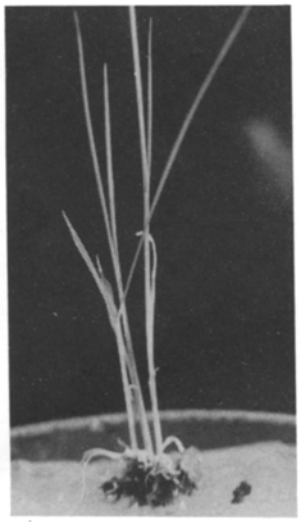

Fig. 2

Fig. 1. Differentiation of callus tissue of rice resulting in plantlet formation
Fig. 2. Upon subculturing of the plantlet, emergence of a complete plant

'Taichung Native 1', 'IR8', and 'GEB 24' did not show any meaningful change. With kinetin (1 mg/l) and IAA (2-5 mg/l) the calli of 'Padma' differentiated into throwing out small plantlets (Fig. 1) which upon careful handling grew into complete rice plants (Fig. 2). Such a response could also be seen with 'Ratna'.

The success in being able to induce differentiation in the case of 'Padma' varied in our separate attempts, although not remarkably. It was also observed that small pieces of calli did show in some cases many small globular protuberances (embryoids) over the surface of the calli mass, however in most cases only one and in some cases three plantlets emerged from a single calli mass. Obviously only a few embryoids could finally morphogenise at the final outburst for differentiation and the rest aborted. It is presumed that there exists further scope for improvement of the technique so as to be able to cut down the number of embryoid abortions which occur during the differentiating process.

Discussion

As indicated earlier, we set out in search of discovering whether leaf sheath cells contained totipotent cells which could ultimately be utilised for protoplast research. There appears to be a similar problem in corn as well (Potrykus et al 1979). We did find out that indeed the leaf sheath cells of rice contain totipotent cells which upon suitable cultural conditions give rise to complete plants. In the course of our study it became fairly clear that, similar to the regeneration capacity of the leaf sheath-derived protoplasts, the totipotency of the leaf sheath cells vary considerably relative to the genotype of the inoculum. In the present case only two genotypes from the eleven that we initially undertook responded favourably. The behaviour of the nine remained varying. As such, one may not conclude that the leaf sheath cells of these strains do not contain totipotent cells. On the other hand our results indicate that the inherent totipotency of the leaf sheath cells varied in nature and in amount; and that our limited efforts could not fully exploit the situation to any advantage. The most practical conclusion one may draw is that the genotypes play an important and decisive role with regard to callus induction and redifferentiation. The genotypes which showed the potentiality for protoplast regeneration earlier (Deka and Sen 1976) did not respond favourably in our present study.

The success that we attained in demonstrating the totipotency of at least some of the leaf sheath cells in rice is believed not to have stemmed from the use of immature embryos as the source of initial inoculum. All past successes in plant regeneration in vitro in rice (Kawata and Ishimura 1968; Maeda 1968; Niizeki and Oono 1968; Nishi et al 1968; Tamura 1968; Nakano and Maeda 1974, 1979; Mascarenhas et al 1975; Henke et al 1978; Correjo-Martin et al 1979) did not include leaf sheath as the source of inoculum. On the contrary, that the leaf sheath calli in cereals are not productive have been indicated earlier (Dale and Deambrogio 1979).

We found that B5 was the most efficient basal medium although not all rice strains responded in the same manner to it. Such a feature has earlier been experienced in barley as well (Dale and Deambrogio 1979).

The urgency for development of suitable techniques for application in protoplast research directed towards crop improvement of cereals is strong. It is true that the maximum benefit from single cell research would come from plant systems where techniques could be adapted to manipulate large homogeneous populations of totipotent cells (Thomas et al 1979). Progress as such in cereals has not been great and the going has been tough for understandable reasons. Yet with the trend of small yet significant successes that have been attained in the past (Deka and Sen 1976; Dudits and Nemet 1976; Koblitz 1976; Potrykus et al. 1977, 1979; Tsai Chi-Knei et al. 1978; King et al. 1978; Vasil and Vasil 1979, 1980; Brar et al. 1979) we hope we are leading towards a final breakthrough. We ardently hope that the leaf sheath cells that are totipotent in rice are not further eliminated selectively while protoplasts regenerate. Such a possibility can not totally be ignored. Nevertheless, the demonstration of totipotency amongst leaf sheath cells is considered to be a step forward towards our final goal.

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