Progress in Tissue Culture of Wild Rice

Introduction

Successful production of callus from tissues of cereals has lagged behind the achievements in tissue culture of many other crops. Cereals typically respond poorly to the same conditions that produce vigorously growing morphogenic cultures from dicotyledonous plants. Although callus initiation has been achived with most cereals, morphogenesis and regeneration have proven more difficult (Evans et al., 1981). In some cereals, most notably corn (*Zea mays* L.) and rice (*Oryza sativa* L.), callus initiation has been accomplished and plants are routinely regenerated via organogenesis or embryogenesis (Green and Phillips, 1975, Nishi et al., 1968).

In wild rice (*Zizania palustris* L.) the initiation and maintenance of callus cultures has been difficult and no reports of success with this system have been published. As with many cereal cultures, wild rice callus is prone to slow growth, production of non-regenerable cell types and necrosis. The necrosis of cultured tissue is particularly severe in wild rice, and its causes are unknown.

An efficient culture system must be developed before *in vitro* techniques and somaclonal variation can be used for improvement of wild rice. The ability to initiate callus from organized plant tissue and regenerate plants from callus is a basic requirement, and must become routine before advanced selection schemes can be applied. The rate at which cultures grow and regenerate plants is also important because somaclonal variation continues to occur as long as cells are in culture. In older cultures the accumulation of mutations eventually results in deleterious variation (eg: albinism, sterility or lethality). It is therefore necessary to identify and maximize the factors promoting efficient initiation and rapid growth of callus.

Successful culture of cereals has often been the result of subtle modifications, such as changes in the culture medium or methods of selection and preparation of tissue. Many variables are known to affect initiation and growth of callus, and often only certain combinations of factors will produce satisfactory results. In this study, several of the most important factors have been investigated.

Explant Tissue

Introduction.

In the Gramineae immature embryos are often the best explant tissue for induction of callus (Cummings et al., 1976, Dale, 1980). Other tissues, such as root tips, intercalary meristems, and immature inflorescences have been successfully cultured in a few systems (Wernicke and Brettell, 1980). In all cases the frequency of callus initiation and overall quality of the cultures were dependent on the type of explant and the age and morphological development of the tissue. The effects of explant source and morphological development on callus initiation by wild rice explants were investigated.

Materials and Methods

Embryos, immature inflorescences and root tips of cultivar K2 were used as explants. For embryo culture, seeds were surface sterilized in 1% sodium hypochlorite for 5-7 minutes and rinsed in sterile distilled water. Embryos were excised, surface sterilized for 3-5 minutes with 0.5% sodium hypochlorite, rinsed twice in sterile distilled water, and soaked for 2-4 minutes in a 5% aqueous solution of Penicillin G (Parke-Davis, Detroit, Mi., 48232). The embryos were categorized by size (in mm) as follows:

a)	large-mature	8-11mm
b)	large-immature	8-11mm
c)	medium	5-8mm
d)	small	2-5mm
e)	micro	2mm or less

Immature inflorescences (5-15mm) and root tips (2-4mm) were excised from seedlings at growth stages 33 and 10, respectively (Percich et. al, 1988), sterilized in 2.5% sodium hypochlorite for 10 minutes and rinsed twice in sterile distilled water. Immature inflorescences were categorized as small (5-10mm) or large (10-15mm). Root tips were not categorized by size. All explants were cultured in 20 x 60mm petri plates containing 25 ml of medium. The culture medium consisted of Murishige-Skoog (MS) basal salts (Gibbco laboratories), MS vitamins, and sucrose (3% w/v) (Murishige and Skoog, 1962) supplemented with 1.0 mg/l 2,4-dichlorophenoxy acetic acid (2,4-D) and solidified with agar. Two-hundred explants (40 plates with five explants per plate) were used for the immature inflorescences, root tips and for each size category.of embryos. Callus initiation was observed after eight weeks.

Results

Embryos from the large-immature and large-mature size categories initiated the greatest number of calli (Table 11). There were no observable differences in the quality of callus derived from different embryo sizes. None of the immature inflorescence or root tip cultures showed any growth. These cultures were therefore discarded and the data omitted from Table 11. Success with mature embryos indicates that stored seed can be used as a source of explant material. This is a great advantage since supplies of immature embryos depend on the availability of living plants at a particular growth stage.

Table 11
Effect of embryo size on callus initiation from wild rice embryos.

Size	Number of calli	% initiation	Mean/plate
large-mature (8-11mm)	65	32.5	1.625 A
large-immature (8-11mm)	59	29.5	1.475 A
medium (5-8mm)	24	2.0	0.600 B
small (2-5mm)	11	5.5	0.275 BC
micro (< 2mm)	2	1.0	0.050 C

Means followed by the same letter do not differ significantly (P=.05) according to Duncan's multiple range test.

Exogenous Hormones

Introduction

Substances with plant hormone activity must be added to tissue culture media to induce or promote the desired type of growth. Auxins and cytokinins are the two classes of hormones most often used in plant tissue culture (Evans et al., 1984). The hormones selected, their concentrations and the combinations in which they are used determine the type of growth exhibited by the culture. The effects of each hormone differ greatly between plant species, making it necessary to establish the optimum hormone balance for each system by trial and error. The effects of hormone concentration and some combinations of hormones on callus initiation were tested in cultures of wild rice.

Materials and Methods

Three auxins were tested; 2,4-D (2,4-dichlorophenoxy acetic acid), NAA (naphthlene acetic acid), and Picloram (4-amino 3,5,6-trichloro-picolinic acid). The auxins 2,4-D and NAA were also tested in combination with the cytokinin BAP (benzyl-amino purine) Large-immature embryos of cultivar K2 were cultured (using the methods described previously) on standard MS media with various hormone concentrations. Forty embryos (eight plates with five embryos/plate) were cultured for each hormone treatment. Callus initiation was observed after eight weeks.

Results

The greatest number of calli were initiated on medium with 0.5 mg/l 2,4-D (Table 12). Picloram induced fewer calli by comparison and promoted slower callus growth with a greater tendency for necrosis. NAA concentrations up to 5 mg/l failed to initiate callus, but concentrations of 2-5mg/l were effective for regenerating plants from whole embryos without an intervening callus stage. BAP was detrimental to callus initiation when combined with 2,4-D and inhibited regeneration of plants from embryos when combined with NAA.

Table 12
Effects of various hormone levels and combinations on callus initiation from immature embryos of wild rice.

Hormones	Concentration mg/l	#calli	% initiation	Mean/plate
2,4-D				
	0.5	17	42.5	2.25 A
	1	7	17.5	0.88 B
	5	4	10.0	0.50 B
2,4-D + BAP				
	1; 0.1	2	5.0	0.25 B
Picloram				
	1	1	2.5	0.12 B
	2	3	7.5	0.38 B
	5	7	17.5	0.88 B
NAA				
	1	0	0	0
	2	0	0	0
	5	0	0	0
NAA + BAP				
	1; 0.1	0	0	0

Means followed by the same letter do not differ significantly (P=.05) according to Duncan's multiple range test.

Variety

Introduction

In most tissue culture systems, the explant genotype has been shown to have a significant effect on the induction of callus and the ability to regenerate plants (Green and Phillips, 1975). Differences in culturability between varieties are often distinct. In many cases, especially with open pollinated crops, there are also differences in culturability within varieties. In most systems it has been useful to first determine which varieties are most suitable for tissue culture and then make selections for best callus type within that variety . Varieties of wild rice were compared for their ability to induce callus.

Materials and Methods

Two-hundred mature embryos (40 plates with five embryos/plate) from each of the varieties K2, Voyager, Netum and M3 were cultured on MS medium supplemented with 0.5mg/l 2,4-D using the methods described previously. Callus initiation was observed and recorded after six weeks of culture.

Results

Numbers of calli derived from each variety were not significantly different (P=.05). The variety Netum initiated the most calli and and these calli suffered the least amount of necrosis. The results are summarized in Table 13.

Table 13
Effect of variety on callus initiation from mature embryos of wild rice.

Variety	Number of calli	Percent initiation	Mean/plate
K2	48	24.0	1.20
Voyager	39	19.5	1.00
Netum	54	27.0	1.35
M3	45	22.5	1.15

Gelling Agent.

Introduction

There are few gelling agents which can be used to solidify tissue culture medium. Agar is the most commonly used gelling agent. It has been satisfactory for many plant species, but has proved to have toxic effects in a few cases. Agarose is a more refined and much more expensive type of agar. It has been used in cases where a more purified gelling agent was necessary for plant tissue growth. Gelrite is a relatively new product which has not yet been widely used, but has some promise as an alternative to agarose. The effects of different gelling agents on the initiation of wild rice callus were compared.

Materials and Methods

Four batches of medium utilizing different gelling agents were prepared. Standard MS medium supplemented with 1mg/l 2,4-D was solidified with Bacto-agar (Diffco), Phyt-agar (Gibbco Laboratories), Sea-palque agarose (FMC Corporation), or Gelrite (Scott Laboratories). Two- hundred mature embryos (40 plates with five embryos/plate) of cultivar Netum were cultured (using the methods described previously) on each of the four media. Callus initiation was observed and recorded after six weeks.

Results

The greatest number of calli were initiated on medium solidified with agarose (Table 14). Agarose and Gelrite media initiated significantly more calli than Bacto-agar. Also, those calli initiated on medium solidified with Bacto-agar or Phyt-agar were more prone to tissue browning and necrosis.

Table 14
Effect of culture medium gelling agents on callus initiation from mature embryos of wild rice.

Gelling agent	Number of calli	Percent initiation	Mean/plate
agarose	121	60.5	3.03 A
Gelrite	117	58.0	2.92 A
Phyt-agar	105	53.0	2.62 AB
Bacto-agar	88	44.0	2.22 B

Means followed by the same letter do not differ significantly (P=.05) according to Duncan's multiple range test.

Carbon Source

Introduction

Plant cells require a carbon source when grown in culture and this need is generally met by the addition of sucrose (2-3% w/v) to the medium

(Gamborg, 1984). The use of carbon sources other than sucrose has been beneficial in some tissue culture systems. Combinations of sucrose and maltose, dextrose, ribose or lactose have sometimes promoted greater callus initiation and more rapid growth as compared with sucrose alone (Evans et al., 1984). The effects of different carbon sources on the initiation of wild rice callus were compared.

Materials and Methods

Six types of medium were prepared. Five of the media were amended with dextrose, lactose, glucose, maltose, or ribose. The media contained standard MS ingredients, 1 mg/l 2,4-D, half the normal amount of sucrose (1.5% w/v) and an equimolar amount of one of the other sugars. The sixth medium was the same except that it contained only sucrose (3% w/v) as a carbon source. Using the methods described previously, 100 mature embryos (20 plates with five embryos/plate) of cultivar Netum were cultured on each of the six media. Callus initiation was observed after six weeks.

Results

Media ammended with maltose or dextrose initiated significantly more calli than medium ammended with any of the other sugars or with sucrose alone (Table 15). Lactose, glucose and ribose-ammended media did not differ significantly from sucrose alone.

Table 15
Effect of carbon source on callus initiation from mature embryos of wild rice.

Carbon source	Number of calli	Percent initiation	Mean/plate
maltose + sucrose	30	30	1.65 A
dextrose + sucrose	28	28	1.60 A
sucrose	22	22	1.10 B
lactose + sucrose	20	20	1.00 B
ribose + sucrose	20	20	1.00 B
glucose + sucrose	19	19	0.95 B

Means followed by the same letter do not differ significantly (P=.05) according to Duncan's multiple range test.

Conclusion

Initiation and growth of morphogenic callus from mature embryos of wild rice can now be routinely accomplished. Modifications of the culture medium, particularly the hormone and gelling agent components, have had dramatic effects on the initiation, growth and re-differentiation of callus. In comparison with other systems, the growth rate of wild rice callus is slow. It is hoped that further refinements of the culture medium and selection of vigorously growing callus tissues will increase the growth rate of wild rice calli.

Literature Cited

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