

In vitro culture of *Secale cereale* L. explants—callus formation and organ differentiation

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Abstract

Explants originating from seeds, embryos, seedlings and maturing plants of rye were cultured on a modified Murashige and Skoog medium (MS) and a Schenk and Hildebrandt medium (SH). Depending on various combinations and concentrations of NAA (α -naphthaleneacetic acid), IAA (β -indoleacetic acid), 2,4-D (dichlorophenoxyacetic acid), 2,4,5-T (trichlorophenoxyacetic acid), 2,4-D (dichlorophenoxyacetic acid), 2,4,5-T (trichlorophenoxyacetic acid = 2 iP), ZEA (zeatin), and GA₃ (gibberellic acid) mature embryo, isolated radicles and lateral roots formed callus tissue which was only capable of rhizogenesis. Leaf segments of 3 and 5-day-old seedlings produced callus and roots, however the rachis of a spike of 7 mm in length was capable of forming shoots which later developed into plants.

INTRODUCTION

Rye has been rarely used as an experimental material in tissue culture. Usually callus tissue of various organs of rye were capable of producing only roots while plants were very rarely obtained. Anther cultures resulted in haploid plants and immature scutellum gave green plants with ploidy level corresponding to the ploidy of explants. (Malępszy, 1975; Thomas, Wenzel 1975; Thomas et al., 1975; Wenzel, Thomas, 1974; Rybczyński, 1979).

In our laboratory, tissue culture of rye has been established, and this paper gives more details on morphogenetic abilities of several explants of plants during their ontogenesis.

MATERIAL AND METHODS

Material used in the culture was obtained from grains, embryos, seedlings and maturing plants of *Secale cereale* L. (cv. 'Strzekęcińskie Ja-

re'). The whole procedure concerning sterilization of grain and isolation of embryos, radicles and lateral roots were described previously (Rybczyński, 1978a).

The leaves from 3 and 5-day old seedlings cultured individually in sterile conditions were divided into 4 or 5 segments and spikes of 7 mm in length were isolated from maturing plants cultured in greenhouse.

The explants were cultured on modified Murashige and Skoog (1962) medium (MS) with the exception of radicles which were cultured on modified Schenk and Hildebrandt (1972) medium (SH). The media were supplemented with 2,4-D; 2,4,5-T; IAA; NAA; GA₃; KIN; IPA; ZEA; BAP; vitamins, yeast extract and sucrose. All media were solidified with 8.0 g/l Difco Bacto agar and adjusted to pH 5.6—6.0 before autoclaving. Cultures were initially kept in darkness and later were transferred to a 16 hours light/8 hours dark cycle at $22 \pm 1^\circ \text{C}$.

RESULTS AND DISCUSSION

In the previous paper (Rybczyński, 1978b) scutellum isolated from immature rye embryos formed tissue with different morphogenetic abilities, depending on the 2,4-D concentration and time of the culture. Generally the callus was capable of root differentiation.

In these experiments, callus was obtained from isolated mature embryo cultures in the presence of 2.0 mg/l 2,4-D and 0.5 mg/l KIN on modified MS medium. The callus was produced in the mesocotyl region of the explant (Fig. 1). At the same time, the coleoptile was morphologically anomalous and leaf primordia developed into normal leaves. Rich overgrowth of callus and rhizogenesis in a long term culture were observed as well.

Isolated radicles 1-2 mm in length and lateral root primordia were cultured separately, as it was difficult to define their reaction in isolated embryo culture in the presence of 2,4-D.

In the same conditions, isolated lateral root primordia of rye cultured on MS medium supplemented with 2,4-D and KIN, both at a concentration of 0.5 mg/l and with 3.0 g/l yeast extract, produced very vigorous callus tissue. Rhizogenesis of this callus occurred rarely.

On modified SH medium, corresponding to 2,4-D concentrations from 0.5 to 10.0 mg/l the radicles were capable for forming callus. In a long term culture the addition of 2.5 mg/l 2,4-D appeared to be the best concentration as far as callus development was concerned. 0.5 mg/l 2,4-D mainly stimulated rhizogenesis of the callus and 10.0 mg/l stimulated only the first stages of callus initiation (Fig. 2 and 3).

PLATE I

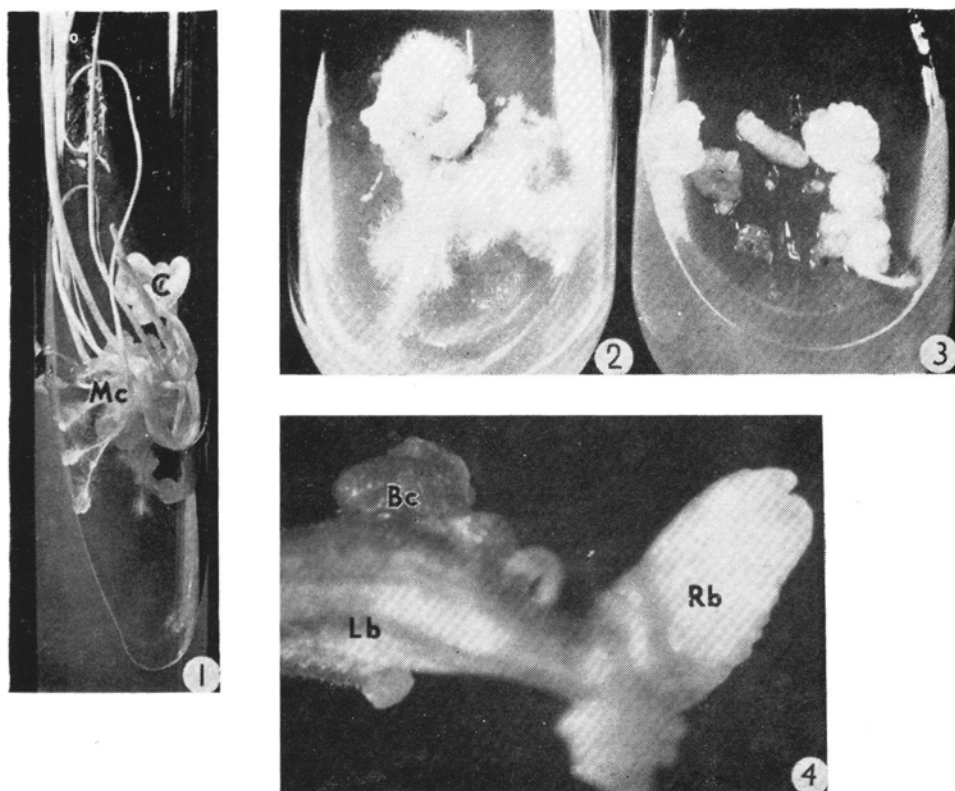


Fig. 1. Mature rye embryo culture.

Formation of mesocotyl callus. Development of shoot elements and callus formation of redifferentiated roots on MS medium supplemented with 0.5 mg/l KIN and 1.0 mg/l 2,4-D after 6 weeks of culture. (1.5 X); Mc — mesocotyl callus; C — coleoptile

Fig. 2-3. Radicle producing callus on SH medium containing various concentrations of 2,4-D after 11 weeks of culture. (2.5 X).

Fig. 2. 0.5 mg/l 2,4-D; Fig. 3. 2.5 mg/l 2,4-D.

Fig. 4. Development of callus and root primordia from second leaf segment originating from 5-day-old rye seedlings after 1 week of culture.

Modified MS 50% medium supplemented with 3.0 mg/l 2,4-D and 0.5 mg/l KIN. (18 X). Bc — green callus of leaf blade; Lb — segment of leaf blade; Rb — root differentiated from cut surface of leaf blade.

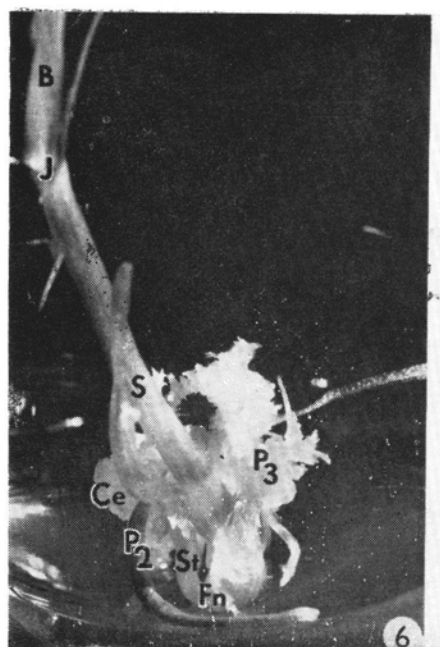
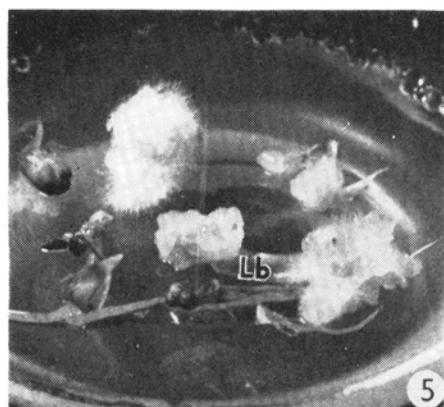


Fig. 5. Callus formation and its rhizogenesis on the cut surface of leaf segments originating from 3-day-old rye seedlings after 8 weeks of culture

Modified MS 70% medium supplemented with 3.0 mg/l 2,4-D and 0.5 mg/l KIN. (12 \times); Lb — segment of leaf blade.

Fig. 6. Direct differentiation of green shoots from rachis after 7 weeks cultured on modified MS medium supplemented with 1.0 mg/l NAA and 2.0 mg/l KIN (5 \times)

B — leaf blade initiating shoot elements; Ce — callus-like overgrowth of the explant; Fn — first node of regenerated plant; J — sheath-blade joint; P₂, P₃ — plantlets in different developmental stages; S — sheath; St — structure initiating differentiation of succeeding leaves of the first plantlet.

Callus originating from radicles and lateral root primordia on the media lacking 2,4-D did not possess any capacity for shoot formation.

Henke et al. (1978) found that 2.0 mg/l of 2,4-D was the optimum concentration for initiation and growth of callus derived from rice primary roots and 6.0 mg/l for induction of the basal part of the leaf. Root and shoot organogenesis was induced in both root and leaf derived calli by subculturing on MS medium lacking 2,4-D. Aging callus caused almost complete inhibition of shoot development. The addition of IPA partially restored the potential for shoot organogenesis.

In contrast to the results obtained on rice, leaf segments of rye originating from 3 and 5-day-old seedlings were able to produce strongly hydrated green callus and regenerated only roots. These results were obtained on a modified MS medium with a lower concentration (70%) of mineral salts than ordinary MS medium and with a different concentration of 2,4-D and KIN. (Fig. 4 and 5).

Better results were observed by Saalbach and Koblitiz (1979) in the culture of leaf segments of 7-day old barley seedlings. In principle, loose and colourless callus originating from mesophyll cells was obtained, and only one explant exhibited shoot formation. In these cultures the medium contained macro-salts of MS (60%) and micro-salts (50%), 10.0 mg/l 2,4-D and a dialysable component of a water extract obtained from 150 g barley seedlings/l medium.

On the other hand plantlet formation and differentiation of epidermal tissue in green callus culture from excised leaves of *Lilium* were obtained on modified SH medium. This medium was supplemented with IAA, NAA, 2,4-D and BA in different concentrations and combinations (Kato, Yasutake, 1977). All attempts to induce callusing from 1 to 5-week-old wheat segments proved unsuccessful (Bhojwani, Hayward, 1977).

The development of monocotyledonous plants results from the function of primary and intercalary meristems. Meristematic activities and morphogenetic potentials of the plant undergo inhibition during maturity, however rachis originating from maturing plant of wheat appeared to be the source of callusing.

Callus originating from wheat rachis and its segments was capable of shoot and root differentiation. Dudits et al. (1975) and Nemet and Dudits (1976) were the first to use "T" medium in wheat rachis cultures supplemented with 2,4-D, IAA and ZEA. Gosch-Wacker et al. (1979) used various basal media containing 1.0 to 2.0 mg/l 2,4-D for callus induction and proliferation. The callus obtained from wheat rachis, isolated from the plant at a later stage up to first microspore mitosis, showed high root differentiation of the media supplemented with these 2,4-D concentrations during early subculture up to 6 to 10 months. In older calli spontaneous root regeneration decreased. When

2,4-D was replaced by IAA and ZEA, shoot differentiation was induced during the first to fourth subculture.

In our experiments the rachis originating from a spike of 7 mm in length was capable of callus formation. Later the callus differentiated shoots. Similarly, direct shoot differentiation was observed. Callus formation was stimulated by 2,4,5-T and IPA. The basal medium (MS) supplemented with both 2,4-D and ZEA in the same concentration 1.0 mg/l stimulated differentiation of several shoots, which were preceded by callus formation. 1.0 mg/l NAA and 2.0 mg/ KIN, however, appeared to be the most suitable growth substance for the inducement of direct shoot formation. Fig. 6 presents three leaves in different development stages.

In conclusion, the above described experiments allowed for defining the conditions necessary for obtaining rye callus from different organs of the plant and showing different morphogenetic potentials (Table 1).

Table 1

Results of the *in vitro* culture of different explants of *Secale cereale*

Explant	Growth substances	Results
Embryo	2,4-D+KIN	mesocotyl callus (rhizogenesis)
Radicle	2,4-D	callus (rhizogenesis)
Seminal roots	2,4-D+KIN	callus (rhizogenesis)
Leaf segments	2,4-D+KIN	callus+direct rhizogenesis
7-mm rachis	NAA+KIN	plantlets+callus
	2,4-D+ZEA	callus+roots+plantlets
	2,4,5-T+IPA	callus (rhizogenesis)

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Hodowla in vitro fragmentów dojrzałych zarodków, fragmentów liści i osadki kłosowej żyta (Secale cereale L.) — tworzenie kalusa i różnicowanie organów

Streszczenie

Eksplantaty pochodzące z roślin żyta (*Secale cereale*, cv. 'Strzełęcińskie Jare') będących w różnych stadiach rozwoju, hodowano na zmodyfikowanych pożywkach Murashige i Skooga (1962) oraz Schenka i Hildebrandta (1972). W zależności od stosowanych stężeń substancji wzrostowych, w różnych

kombinacjach takich jak NAA (kwas α -naftalenooctowy), IAA (β -indoliloctowy kwas), 2,4-D (kwas 2,4-dwuchlorofenoksyoctowy), 2,4,5-T (kwas 2,4,5- tróchlorofenoksyoctowy), KIN (kinetyna), ZEA (zeatyna), IPA (izopentynyloadenina), BAP (6-benzyloaninopuryna) i GA₃ (kwas giberelinowy) dojrzałe zarodki, izolowane i hodowane oddzielnie radikule i korzenie pierwotne tworzyły tkankę kalusową zdolną tylko do tworzenia korzeni. W warunkach hodowli *in vitro* fragmenty liści pochodzące z siewek w wieku 3 i 5 dni zdolne były do tworzenia tkanki kalusowej oraz do bezpośredniego tworzenia korzeni. Różnicowanie elementów pędów stwierdzono w hodowlach osadki kłosowej pochodzącej z kłosów o długości 7 mm.