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Tissue culture of horse-radish (Cochlearia armoracia L.) meristems: sterilization of buds and comparison of media

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Abstract

The attempt was made to cultivate horse-radish meristems taken from buds removed from roots. The lowest per cent of contamination was found after the buds had been soaked in 80% ethanol for 6 minutes and then in 5%, 7.5% or 10% chloramine for 30, 30 or 15 minutes, respectively. Both agar media: Murashige-Skoog and Linsmaier-Skoog, were equally good, providing a moderate number of developing plants. The Linsmaier-Skoog medium was more satisfactory when solidified with agar; the results with liquid medium and filter-paper bridges were not as good.

Tissue culture is now commonly used in biological research. It also has been widely applied in horticulture for rapid multiplication of several plant species and for the production of virusfree mother plants (Murashige 1974, 1977; Fossard and Bourne 1977; Holdgate and Aynsley 1977). It seemed therefore worthwhile to apply it for horse-radish since this plant is increasingly being cultivated by Polish vegetable growers (Lenkiewicz 1977) and is attacked by several viruses, for instance: arabis mosaic virus (AMV), cauliflower mosaic virus (CIMV), cabbage black ring virus (CBRV), turnip mosaic virus (TuMV) and, tobacco ring spot virus (TRSV), (Hickman and Varma 1968; Shukla and Schmeltzer 1972; Böning and Trojan 1967; Chenulu and Thornberry 1965; Paludan 1971, 1973; Thomas and Procter 1973; Brčak and Prohazkova 1976).

We have found only scarce literature concerning tissue culture of horse-radish. Hickman and Varma (1968) obtained horse-radish plants form tissue culture of meristems taken from 2 cm long buds. These buds were washed in 96% ethanol and 5% calcium hypochlorite for 15 minutes and grown on Morel and Müller (1964) medium.

Paludan (1971) got horse-radish plants from buds sterilized by quick washing with 96% ethanol followed by soaking in 1–5% calcium hypochlorite for 15-30 minutes and then soaked in 0.5% 8-hydroxyquinoline. The medium used was that of Murashige and Skoog solidified with agar.

The most important initial problem with this culture in Poland was to work out the method of bud sterilization suitable for our conditions. Already the first trials made in Skierniewice have shown that the methods of Hickman and Varma (1968) and of Paludan (1971) were in our conditions not satisfactory, similarly like the methods proposed by other authors. Sterilization of horse-radish buds is very troublesome since they are usually taken from roots brought from the field and are contaminated with multiple soil microorganisms.

The methods of sterilization applied most often in tissue culture work are: soaking plant parts for several minutes in a solution of sodium hypochlorite (Pow 1969; Davis et al. 1974; Jones et al. 1977), calcium hypochlorite (Churchill at al. 1973; Jeleska 1974; Dore 1974) or 0.1% or 0.2% mercury chloride (Gwóźdź 1973; Domańska i Rennert 1976). Also immersion in 50–60% ethanol (Walkey 1968; Smith et al. 1976), or application of chloramine T solution (Bajaj and Bopp 1972), as well as 8-hydroxyquinoline sulphate may be interesting in this respect (Paludan 1971; Nowak and Rudnicki 1975). Several authors have used a combination of these sterilizing techniques ((Hickman and Varma 1968; Lis 1970; Paludan 1971; Rossini 1972; Zee et al. 1977).

The another purpose of this research was to choose a suitable medium for horse-radish meristem culture.

There are several media used in tissue culture (Murashige 1974). The most commonly used for *Crucifereae* are those of Murashige and Skoog (1962) and Linsmaier and Skoog (1965). The tissues of several species grow well on a medium solidifield with agar, whereas others do better on a liquid medium with paper bridges (Murashige 1974).

MATERIAL AND METHODS

Horse-radish roots were brought from a commercial plantation in central Poland, washed in tap water, and soaked in a 10% solution of chloramine T for 20-30 minutes. They were then placed in a peat and perlit mixture (1:1) and preserved in a cold room (2°C) untile needed, i.e., for several weeks or months. The containers with roots were transferred to a warm greenhouse 20 days before the expected time of bud excision. Only buds which had started to develop after this treatment were excised from the roots. To obtain uniform buds, they were taken from the middle and lower part of the root. The external leaves

and scales were removed from the buds, and then the buds were washed in running tap water for 24 hours. Afterwards, the buds were sterilized in different ways and rinsed as will be discussed later. The sterilized buds were dissected under the stereoscopic microscope to obtain meristems with some axial tissue of joint length 0.5–1.5 mm. They were grown on different media, as shown later, but in the trials with differnt methods of sterilization, the Linsmaier-Skoog medium solidified with 0.7% agar was used. The meristems were placed in test tubes and stored on shelves lighted with "day-light" fluorescent tubes (light intensity 6 klux), at temperature 20°C, and with 16 hours of illumination.

RESULTS

1. Comparison of sterilization methods

The following substances and concentrations were used:

1) ethanol, 80%, for 3, 6, 12 and 15 minutes;

2) calcium hypochlorite, 20%, for 3, 6, 15 and 30 minutes;

3) mercury chloride, 0.1% or 0.2%, for 3 or 6 minutes;

4) chloramine T 5%, 7.5% or 10%, for 15 or 30 minutes;

5) 8-hydroxyquinoline sulphate (Chinosol), 0.5% and 1%, for 10, 15 or 20 minutes;

6) the fungicide Topsin 0.1% (thiophanate methyl WP) for 15 or 30 minutes.

The substances mentioned were used mostly in combinations. The experiments were conducted in two sets. In the first, performed January-May, 32 different combinations of treatments were applied using 30-70 meristems per treatment. Each treatment was usually repeated once, although sometimes the second repetition was several days later, with buds excised from roots of another group. The first observations of the cultures were at about 14 days after the beginning of the experiment and the final ones at about 40-56 days. The number of contaminated cultures, as well as the numbers of plantlets in different stages of development, were noted.

The best methods of sterilization in this first set of experiment were as follows (Table 1):

1) ethanol, 80%, for 6 minutes, then (whithout rinsing) chloramine T, 5%, for 30 minutes;

2) as above but chloramine T, 7.5%;

3) as above but chloramine T, 10%, for 15 minutes.

The per cent of contaminations after 20 days was very low with these methods of sterilization (0%-4%) and the per cent of plantlets transferred into pots was, after 40 days, rather high (52-64%).

Table 1

The influence of different methods of sterilization on the development of meristems and on the number of contaminated cultures. Experiment begun May 3-11, 1977

Method of sterilization	Results after days:									
	20					40				
	contaminated	necrotic	healthy nondeveloped	developing	transplanted into pots	contaminated	necrotic	healthy nondeveloped	developing	transplanted into pots
Ethanol 80% for 6 minutes, chloramine T 5% for 30 min	4.1	0	0	62.6	33.3	18.7	0	0	29.3	52.0
Ethanol 80% for 6 minutes, chloramine T 7.5% for 30 min	0	0	0	66.0	34.0	7.1	0	0	28.6	64.7
Ethanol 80% for 6 minutes, chloramine T 10% for 15 min	0	0	0	74.5	25.5	9.8	0	0	27.5	62.7

The second, small-scale experiment was conducted October-December 1977, using 15 meristems per treatment. Only the three best methods of sterilization, mentioned in Table 1, were used. All meristems were put on agar the same day. After 21 days of incubation in the same conditions as those applied during the first experiments, the per cent of contaminations was initialy 12-17% and after 42 days it increased to 22-26%. The per cent of developed plants was 28-33%. This result — less satisfactory than that obtained during the spring (see Table 1) — may be explained by the use of much poorer plant material. No differences were found between the three methods of sterilization. The meristems issuing from buds sterilized in different ways behaved, in this experiment, in a similar manner.

2. Comparison of meristems developing on different media

The three media compared were those of:

1) Linsmaier and Skoog (1965)

2) Murashige and Skoog (1962) and

3) Knopp's macroelements' mixture enriched with microelements according to Heller (see Butenko 1964) and with 30 g/l saccharose, 0.5 mg/l IAA and 0.2 mg/l kinetin.

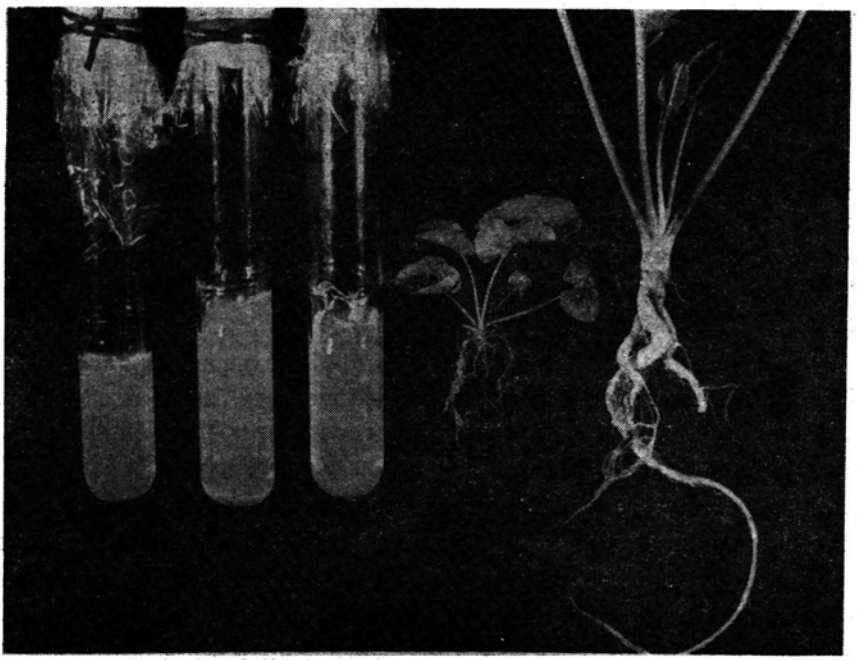

Fig. 1. Different stages of horse-radish meristem culture

Table 2

Growth of the horse-radish meristems on solidified or on liquid Linsmaier-Skoog medium

Medium	State of the meristems (or plantlets)	Per cent from the total number of meristems (or plantlets)				
		after: 14 days	21 days	35 days	42 days	56 days
Solidified with agar	contaminated	5.8	5.8	5.8	5.8	5.8
	healthy without leaves or roots	94.2	52.5	44.2	2.6	2.6
	inhibited development	0	0	0	41.6	38.8
	plantlets in test tubes	0	41.7	14.2	14.2	2.8
	plantlets in pots	0	0	35.8	35.8	50.0
Liquid medium with filter paper bridges	contaminated	0	0	0	0	0
	healthy without leaves or roots	91.7	91.7	88.3	52.5	35.8
	inhibited development	8.3	8.3	9.2	45.0	61.7
	plantlets in test tubes	0	0	2.5	2.5	0.0
	plantlets in pots	0	0	0	0	2.5

The experiment was set up with three repetitions in January-February 1978. There were 15 explants per repetition. The buds were sterilized with 80% ethanol for 6 minutes and with chloramine T, 7.5%, for 30 minutes (Table 2).

After 35 days of incubation in conditions described earlier, it became obvious that the thrid medium containing Knopp's mixture was not suitable for horse-radish meristems, whereas the other two mixtures gave equal results: the per cent of rooted plantlets was 15-18%, the per cent of not yet fully developed plantlets 24-36% and the per cent of contaminations was 15-22%. The results of this experiment differ markedly from those in Table 1 since the mother plant material was different.

Comparison of the agar and the liquid Linsemier and Skoog (1965) medium was done in September-November 1977, also with three repetitions including 12 explants per repetition. The plantlets in the liquid medium were grown on filter paper bridges (see Walkey 1968).

This experimet revealed that the meristems developed much better on the agar than on the liquid medium (Table 2). Twenty one days after the start of the culture there were 42% of the plantlets rooted on the agar medium, whereas there were none on the liquid medium. After 56 days, 50% of the plantlets were transplanted into pots from the agar medium and there were only 25% from the liquid medium.

DISCUSSION

The tissues of different plant species, as well as of different parts of a plant, usually need specific methods of sterilization before culturing *in vitro*. The most sophisticated methods of sterilization are particularly necessary for those parts which come into contact with non-sterile soil. The buds excised from field grown horse-radish roots proved to be very difficult to sterilize and the methods reported by other autors were, in this case, not satisfactory. Among many methods tried, the best ones appeared to be those shown in Table 1. These treatmnets resulted in a low per cent of contaminations and were not harmful to plant material. The methods of sterilization which included treatment with calcium hypochlorite and/or 8-hydroxyquinoline (see Hickman and Varma 1968; Paludan 1971) proved to be not satisfactory in our condition.

The two commonly used media for tissue culture: Linsmaier-Skoog and Murashige-Skoog were both satisfactory for horse-radish meristems. These media are often used for explants of other *Crucifereae* species. For example, the Linsmaier-Skoog medium was used for cauliflower curds (Crisp and Walkey 1974; Baroncelli et al. 1973), and the Murashige-Skoog medium for flower buds of broccoli (Anderson and Carstens 1976), and for horse-radish (Paludan 1971). The enrichel Knopp's medium proved to be unsatisfactory for horse-radish and will not be used in the future.

Horse-radish meristems grew much better on the agar than on the liquid Linsmaier and Skoog medium. However, it is not excluded that the liquid medium may also be satisfactory after some modifications.

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Kultury tkankowe merystemów chrzanu (*Cochlearia armoracia* L.): sterylizacja pąków i porównanie pożywek

Streszczenie

Przeprowadzono próby kultury tkankowej merystemów chrzanu wziętych z pąków wyrastających na korzeniu. Spośród wielu metod sterylizacji jakie wypróbowano, najlepszą okazało się zanurzenie pąków na 6 minut w 80% etanolu, a następnie w 5%, 7,5% lub 10% chloraminie T na odpowiednio: 30, 30 lub 15 minut. Pożywki agarowe Linsmaiera i Skooga oraz Murashige i Skooga okazały się jednakowo dobre. Pożywka Linsmaier-Skooga zestalona agarem była znacznie lepsza niż płynna z mostkiem z bibuły filtracyjnej.