Journal of the Minnesota Academy of Science

Volume 28 | Number 1 Article 7

1960

The In Vitro Growth of Roots of Various Crucifer Species

Richard D. Durbin
University of Minnesota

THE *IN VITRO* GROWTH OF ROOTS OF VARIOUS CRUCIFER SPECIES[1]

RICHARD D. DURBIN
University of Minnesota, St. Paul

Plant roots were first grown as independent organs in a chemically defined medium in 1922 (Robbins, 1922). Since then, the technique of growing roots *in vitro* has been applied with great success to the elucidation of problems connected with cellular physiology and morphology. One of the possible applications of this technique is the maintenance of root tissues as a substrate for obligate parasites. It was for this purpose that the work reported herein was done.

The roots of 229 species in 51 genera, within the family Cruciferae, are attacked by an obligate fungus parasite, *Plasmodiophora brassicae* Wor. Infected root cells enlarge and proliferate; they usually do not differentiate. Sometimes adventitious buds are formed that are not normally geotropic and they often grow downward or horizontally. All this results in the root's becoming enlarged and greatly distorted; hence the name "club root" has been applied to the disease. As a preliminary to studying some of the physiological aspects of this host-parasite interaction, a survey was made to determine which species' roots could be best maintained in organ culture.

MATERIALS AND METHODS: Seeds of 22 species in 10 genera were surface sterilized by placing them in a 1% sodium hypochlorite solution for ten minutes, followed by a rinse in sterile distilled water. They were then put in Petri dishes containing potato dextrose agar and allowed to germinate at 28° C. After germination, root tips 5 mm. long were excised and placed individually into the medium. Ten cultures of each species were prepared and kept in an incubator at 24° C. Thereafter, at fourteen-day intervals root-tip segments 10 mm. long were excised and transferred to a fresh medium.

Two culture media were used initially: standard White's medium (White, 1954) and the same solution supplemented with the following compounds, which were added at the indicated rates per liter of medium: riboflavin (0.5 mg.), pyridoxine (0.5 mg.), *p*-aminobenzoic acid (0.5 mg.), pantothenic acid (2 mg.), nicotinamide (2 mg.), choline (2 mg.), inositol (4 mg.), folic acid (0.04μg.), and alkaline-hydrolyzed yeast nucleic acid (5 mg.). Fifty ml. aliquots of each medium were dispensed into 125 ml. Erlenmeyer flasks and autoclaved at fifteen pounds pressure for twenty minutes.

[1] Paper No. 4386, Scientific Journal Series, Minnesota Agricultural Experiment Station. Contribution from the Department of Plant Pathology and Botany, Institute of Agriculture.

RESULTS: Roots of the following species were not carried past the second subtransfer because of a poor growth rate in both media: *Arabis alpina L.* (mountain rock-cress), *Aubrieta deltoidea* DC (aubrieta), *Lobularia maritima* Desv. (sweet alyssum), *Cheiranthus cheiri* L. (wallflower), *Brassica caulorapa* Pasq. (kohlrabi), *B. oleracea* L. var. *botrytis* L. (cauliflower), *B. oleracea* L. var. *capitata* L. (cabbage and savoy cabbage), *B. fimbriata* DC. (dwarf Siberian kale), *B. naprobrassica* Mill. (rutabaga), *B. rapa* L. (turnip), *B. oleracea* L. var. *acephala* DC. (Georgia collards), *B. oleracea* L. var. *italica* Plenck. (sprouting broccoli), *B. napus* L. (rape), *Lepidium sativum* L. (pepper grass), *Nasturtium officinale* R. Br. (watercress), *Matthiola incana* R. Br. var. *annua* Voss (stock), and *Malcolmia maritima* R. Br. (Virginia stock).

Roots of the following species have been carried through six subtransfers: *Brassica oleracea* L. var. *gemmifera* Zenker. (Brussels sprouts), *B. juncea* Coss var. *crispifolia* Bailey (southern curled mustard), *B. kaber* (DC.) L. C. Wheeler var. *pinnatifida* (Stokes) L. C. Wheeler (wild mustard), and *Raphanus sativus* L. (radish). Of this group, only the roots of radish and wild mustard grew fast enough to be considered as possible laboratory substrates for *P. brassicae*. As with the first group of species, there was no significant difference between the two media with respect to root growth. Therefore, in all subsequent experiments only standard White's medium was used.

The growth rates of roots from the following twelve varieties of

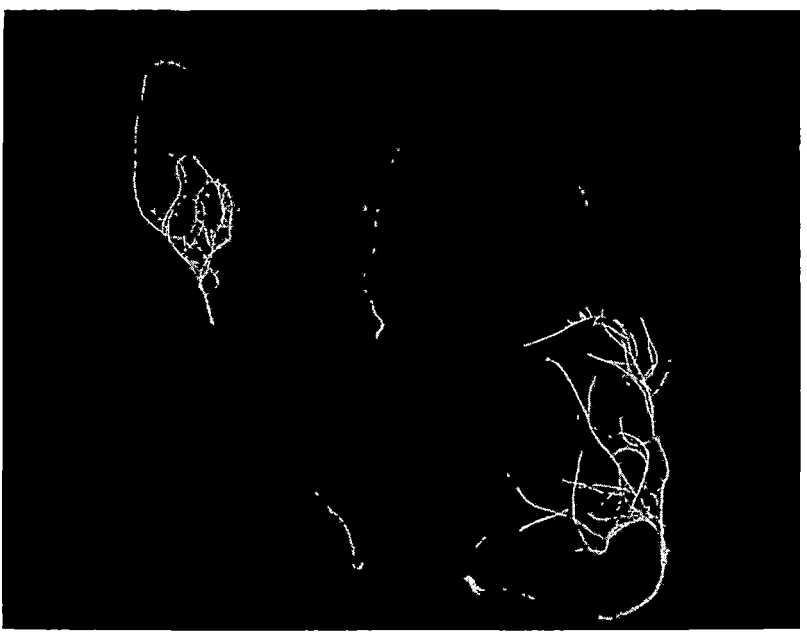

Fig. 1. Roots of six crucifer species grown *in vitro* for 28 days. *Top, left to right*: wild mustard, rape, turnip. *Bottom, left to right*: kale, cabbage, radish.

radish were compared: Cavalier, Cherry Belle, Crimson Giant, Champion, French Breakfast, Icicle, White Globe, Scarlet Turnip, Scarlet Globe short top, Scarlet Globe medium top, Long Black Spanish, and Round Black Spanish.

The average growth rate of the primary root ranged from 25 to 240 mm. per week among varieties. In addition, the position, time of appearance, and length of the secondary roots were distinctive for many varieties (Fig. 2). White Globe roots produced many long secondary roots, whereas the primary roots of Scarlet Turnip were thick and short with sparse secondary root formation. The growth rate of the roots was not correlated with the number of days required by the variety to reach maturity. For example, in Fig. 2 the longest and shortest roots are from varieties which require 56 days to reach maturity, while the other two roots are from varieties which mature in about 24 days. Likewise, the rate of seed germination was not correlated with the growth rate of excised roots.

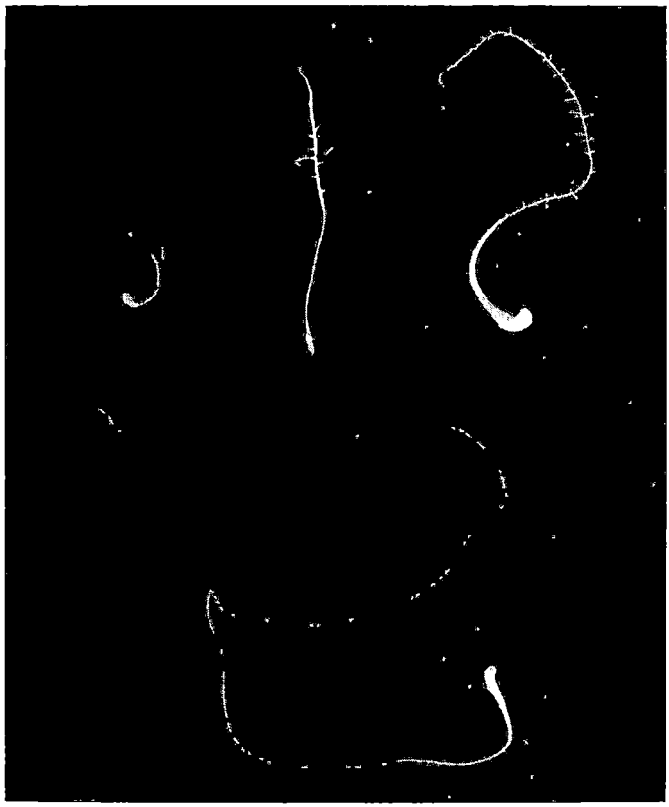

Fig. 2. Roots of four varieties of radish grown *in vitro* for 7 days. *Top, left to right*: Long Black Spanish, Scarlet Turnip, Scarlet Globe Medium top. *Bottom*: Round Black Spanish.

In an attempt to improve the growth of cabbage roots, the following materials were added at the indicated rates per ml. of standard White's medium: kinetin (10, 1, 0.1 μg.), yeast extract (5 mg.), 2, 4-dichlorophenoxyacetic acid (0.01 μg.), naphthaleneacetic acid (1 μg.), phytone (5 mg.), yeast extract (5 mg.) and 2, 4-dichlorophenoxyacetic acid (0.01 μg.), kinetin (1 μg.) and gibberellic acid (5 μg.), cabbage seed extract (10 m.), gibberellic acid (5 μg.), and cabbage leaf extract (20 mg.). Of these, only the addition of yeast extract or phytone to the medium increased the growth rate. However, the stimulation was not great and the roots, as a consequence, were not subtransferred more than three times.

Discussion: The inability of the roots of most crucifer species to grow *in vitro* is not surprising in light of the results obtained by other workers. White (1938) found that of the six species he tried, only the roots of radish and black mustard would grow. All attempts by others to culture cabbage roots have failed. As in the present work, the problem has been attacked largely by adding "growth factors" to the medium, based on the assumption that something needed for growth is lacking. It may well be that the answer lies in another direction, and that what we should be trying to do is counteract toxic materials produced by the root itself. The increase in root growth obtained by Boll (1960), when he added antimetbolites to the medium, is a case in point.

We all recognize the principle that clones within a single taxonomic species vary. Yet, in the literature concerning tissue culture, very little mention is made of the influence of the variety and in some cases even the variety used in the study is not stated.

Considering the results obtained from a comparison of the root growth of different radish varieties, the questions of how and whether a particular species' roots grow *in vitro* may depend almost as much on the horticultural variety as the species.

Summary: Attempts were made to grow *in vitro* the roots of 22 species of crucifers. Of these only 4 species' roots, (radish, brussels sprouts, wild mustard, and southern curled mustard) were successfully carried through more than three passages. The root growth of 12 horticultural varieties of radish was also compared; there were large differences among varieties in growth rate and morphology of the roots. Addition of many materials to White's medium did not improve the growth of cabbage roots.

LITERATURE CITED

BOLL, W. G. 1960. Ethionine inhibition and morphogenesis of excised tomato roots. *Plant Physiol.* 35:115-122.

ROBBINS, W. J. 1922. Cultivation of excised root tips and stem tips under sterile conditions. *Bot. Gaz.* 73:376-390.

WHITE, P. R. 1954. *The cultivation of animal and plant cells.* Ronald Press Co., New York.

WHITE, P. R. 1938. Cultivation of excised roots of dicotyledonous plants. *Amer. Jour. Botany* 25:348-356.