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Use of roots transformed by *Agrobacterium rhizogenes* in rhizosphere research: applications in studies of cadmium assimilation from sewage sludges

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Abstract

The use of roots transformed by *Agrobacterium rhizogenes* in models for the rhizosphere is discussed. A list of species for which transformed root cultures have been obtained is provided and the example of studies of cadmium assimilation from sewage sludges is given to illustrate how transformed root cultures can be used in physiological tests under non-sterile conditions.

Introduction

Our objective in this paper is to comment on the usefulness of roots transformed by *Agrobacterium rhizogenes* in rhizosphere research, with the aid of an example: the study of cadmium availability in sewage sludges.

The principal difficulty that has impeded understanding of the plant root and its relationships with the soil and the organisms living in the soil is that of access. The rhizosphere is hidden from view and once disturbed no longer functions in a normal fashion. Underground plant organs are fragile and chemical interactions with the soil are complex. The rhizosphere organisms are for the most part microscopic, numerous and varied. Study of the rhizosphere would ideally take place *in situ*, using the full resources of microbiology, plant biology, ecology, and soil chemistry. However, a system as complex as the rhizosphere is difficult to approach experimentally without simplification.

Attempts to model, and thus simplify, certain functions of the plant root and its interactions with the exterior have been limited by several obstacles: e.g., roots cannot be obtained in large quantities under axenic conditions and many of the parasites of importance to the root are obligates – they cannot be cultured *in vitro* away from the root. Attempts to study roots produced through hydroponics have been limited by the fact that the conditions are not sterile and aerial plant organs are a source of complexity. Attempts at using root cultures to produce model rhizospheres [40, 54] have been limited by the generally slow growth and fragile nature of these cultures.

Roots induced by the soil bacterium *Agrobacterium rhizogenes* are amenable to culture [55]. The physiological basis for this phenomenon is not known, but it is certainly due to the presence in the plant genome of T-DNA (transferred DNA) of bacterial origin. A large plasmid (termed Ri, for root-inducing) is the source of the foreign genes responsible for the transformed phenotype, one

Table 1. Species transformed by *A. rhizogenes.*

Species	Roots [1]	Plants	Reference
Abrus precatorius	yes [2]	no	K. Soo Ko (unpub.)
Ambriosia artemisiifolia (ragweed)	yes	no	[26]
Anagallis arvensis (pimprenel)	yes	yes	[26]
Anchusa officinalis	yes	no	[26]
Antirrhinum majus (snapdragon)	yes [2]	no	[18]
	yes	yes	[26]
Arabidopsis thaliana	yes	yes	[34]
Arachis hypogaea (peanut)	yes	no	[26]
Aristolelia australisica	yes [2]	no	E. Davioud (unpub.)
Artemisia annua	yes [2]	no	E. Davioud (unpub.)
Armoracia rusticana (horse radish)	yes [2]	yes [2]	[28]
Atropa belladonna (belladonna)	yes	yes	[23]
	yes [2]	yes [2]	[22]
Atropa caucasica	yes	no	E. Knopp and A. Strauss (unpub.)
Beta vulgaris (sugar beet and red beet)	yes [2]	yes [2]	A. Yacoub and D. Tepfer (unpub.)
	yes	no	[19]
	yes	no	[26]
Bidens sulphureus	yes	no	[16]
Brassica chinensis	yes [2]	no	G.-L. Chi (unpub.)
Brassica hirta (mustard)	yes	no	[26]
Brassica napus var. *oleifera* (oilseed rape)	yes [2]	yes [2]	[31]
	yes [2]	yes [2]	[17]
Brassica oleracea (cauliflower)	yes [2]	no	[36]
	yes [2]	yes [2]	[11]
Brassica oleracea (cabbage)	yes [2]	yes [2]	J. Tourneur (unpub.)
Brassica pekinensis	yes [2]	no	G.-L. Chi (unpub.)
Brassica rapa (turnip)	yes [2]	no	[47]
Calystegia sepium (morning glory)	yes [2]	no	[49, 22]
Cassia torosa	yes [2]	no	K. Soo Ko *et al.* (unpub.)
Cassia obtusifolia	yes [2]	no	K. Soo Ko *et al.* (unpub.)
Cassia occidentalis	yes [2]	no	K. Soo Ko *et al.* (unpub.)
Catharanthus roseus	yes [2]	no	[7]
	yes [2]	no	E. Aird *et al.* (unpub.)
	yes	no	[18]
Catharanthus trichophyllus	yes [2]	no	E. Davioud (unpub.)
Centaurea cyanus (cornflower)	yes	no	[26]
Cichorium endivia	yes	no	[26]
Cichorium intybus (endive)	yes	no	[26]
	yes [2]	yes [2]	G. Touraud (unpub.)
Cinchona ledgeriana (Peruvian bark)	yes	no	[18]
Convolvulus arvensis (morning glory)	yes [2]	yes [2]	[49, 52]
Coriandrum sativum	yes	no	[26]
Crepis capillaris	yes [2]	no	[3]
Cucumis sativus (cucumber)	yes [2]	yes [2]	[56]
	yes	no	[26]
Datura chlorantha	yes	no	E. Knopp and A. Strauss (unpub.)
Datura ferox	yes	no	E. Knopp and A. Strauss (unpub.)
Datura innoxia	yes	no	E. Knopp and A. Strauss (unpub.)
	yes [2]	no	[7]
Datura metel	yes	no	E. Knopp and A. Strauss (unpub.)
Datura meteloides	yes	no	E. Knopp and A. Strauss (unpub.)
Datura rosei	yes	no	E. Knopp and A. Strauss (unpub.)

Table 1. (Continued)

Species	Roots [1]	Plants	Reference
Datura sanguinea	yes	no	E. Knopp and A. Strauss (unpub.)
Datura stramonium (jimsonweed)	yes [2]	no	[35]
	yes	no	[26]
Daucus carota	yes [2]	no	[55]
	yes [2]	no	[45]
	yes [2]	yes [2]	[49, 50, 52]
	yes [2]	yes [2]	[10]
Dianthus caryophyllus (carnation)	yes	no	[26]
Digitalis lanata	yes [2]	no	[7]
Duboisia myoporoides	yes [2]	no	[12]
Ervatamia obtusifolia	yes [2]	no	[7]
Eucalyptus qunnii (eucalyptus)	yes [2]	no	[2]
Foeniculum vulgare (fennel)	yes [2]	no	[7]
	yes	yes	[26]
	yes [2]	yes [2]	A. Attal and D. Tepfer (unpub.)
Galinsoga parviflora (quickweed)	yes	no	[26]
Gentiana lutea (yellow gentian)	yes [2]	no	E. Davioud (unpub.)
Glycine max (soya bean)	yes [2]	no	A. Yacoub and D. Tepfer (unpub.)
Gypsophila muralis (babybreath)	yes	no	[26]
Helianthus annuus (sunflower)	yes	no	[26]
	yes [2]	no	C. Attal and D. Tepfer (unpub.)
Helianthus tuberosus (Jerusalem artichoke)	yes [2]	no	C. Attal and D. Tepfer (unpub.)
Hyoscyamus albus	yes	no	E. Knopp and A. Strauss (unpub.)
Hyoscyamus aureus	yes	no	E. Knopp and A. Strauss (unpub.)
Hyoscyamus bohemicus	yes	no	E. Knopp and A. Strauss (unpub.)
Hyoscyamus muticus	yes	no	[14]
	yes [2]	yes [2]	C. Attal and D. Tepfer (unpub.)
Hyoscyamus niger	yes	no	[14]
Ipomoea batatas (sweet potato)	yes	no	[13]
	yes	no	[26]
Ipomoea aristolochiaefolia	yes	no	[26]
Ipomoea purpurea	yes	no	[26]
Kalanchoe daigremontiana	yes	no	[26]
Linum grandiflorum (flax)	yes	no	[26]
Lithospermum erythrorhizon	yes	no	[43]
Lotus corniculatus (bird's foot trefoil)	yes [2]	yes [2]	[37]
Lupinus albus (white lupin)	yes	no	[26]
Lupinus polyphyllus (lupin)	yes	no	[26]
Lycopersicon esculentum (tomato)	yes	no	[4]
	yes [2]	yes [2]	[42]
	yes [2]	yes [2]	[25]
Lycopersicon peruvianum	yes	no	[4]
Macroptillium atropurpureum (siratro)	yes	no	[5]
Malus domestica (apple)	yes [2]	yes [2]	C. Lambert and D. Tepfer (unpub.)
Medicago sativa (lucerne)	yes	no	[5]
	yes [2]	yes [2]	[46]
	yes [2]	yes [2]	[44]
Medicago tornata	yes [2]	no	C. Attal and D. Tepfer (unpub.)
Nicotiana africana	yes [2]	no	[33]
Nicotiana cavicola	yes [2]	no	[33]
Nicotiana glauca	no	yes [2]	[48]
Nicotiana hesperis	yes [2]	no	[33]
	yes	yes	[57]

Table 1. (Continued)

Species	Roots [1]	Plants	Reference
Nicotiana plumbaginifolia	yes [2]	yes [2]	[21]
Nicotiana rustica	yes	no	[19]
	yes	no	[41]
Nicotiana tabacum (tobacco)	no	yes	[1]
	yes [2]	yes [2]	[49, 50, 51, 52]
	yes [2]	yes [2]	[9]
	yes [2]	yes [2]	[8]
Nicotiana umbratica	yes [2]	no	[33]
Nicotiana velutina	yes [2]	no	[33]
Panax ginseng	yes	no	K. Soo Ko *et al.* (unpub.)
	yes [2]	no	[59]
Petunia hybrida (petunia)	yes	yes	[30]
Phaseolus vulgaris (bean)	yes [2]	no	C. Attal and D. Tepfer (unpub.)
	yes [2]	no	[18]
Pimpinella anisum (anis)	yes	no	[26]
Pisum sativum (pea)	yes [2]	no	[6]
Polygonum aviculare (knotweed)	yes	no	[26]
Polygonum convolulus (corn bindweed)	yes	no	[26]
Polygonum hydropiper	yes	no	[18]
Populus tremula × *Populus alba* (poplar)	yes	no	J. Carr and F. Le Tacon (unpub.)
Populus trichocarpa × *Populus deltoides*	no	yes [2]	[38]
Psophocarpus tetragonolobus (winged bean)	yes	yes	C. Attal and D. Tepfer (unpub.)
Raphanus sativus (radish)	yes	no	[47]
Rheum palmatum (rhubarb)	yes	no	[26]
Rumex crispus (yellow dock)	yes	no	[26]
Scopolia carniolica	yes	no	E. Knopp and A. Strauss (unpub.)
Scopolia japonica	yes	no	[15]
	yes	no	[24]
	yes	no	[27]
Scopolia straminifolia	yes	no	E. Knopp and A. Strauss (unpub.)
Sesbania rostrata	yes	no	[26]
Silene armeria (catchfly)	yes	no	[26]
Sinapis alba (white mustard)	yes	no	[26]
Solanum laciniatum	yes	no	[18]
Solanum launatum	yes [2]	no	[7]
Solanum nigrum (nightshade)	yes [2]	yes [2]	[58]
	yes	no	[26]
Solanum sysembrifolium	yes [2]	no	[7]
Solanum tuberosum (potato)	yes [2]	no	[36]
	hes [2]	yes [2]	[31, 32]
	yes	no	[29]
	yes [2]	no	[39]
	yes [2]	yes [2]	[20]
Spergula arvensis (spurry)	yes	no	[26]
Tagetes erecta (marigold)	yes	no	[26]
Tagetes patula (marigold)	yes	no	[15]
Trifolium pratense (red clover)	yes	no	[5]
Valerianella locusta	yes	no	[26]
Vicia sativa (common vetch)	yes	no	[26]
Vigna aconitifolia (moth bean)	yes	yes	K. Sukhapinda and E. Shahin (unpub.)
Vigna unguiculata (cowpea)	yes	no	[26]

[1] Stable, axenic root cultures

[2] Biochemical confirmation of transformation obtained.

attribute of which is facility of culture (see [53] for further discussion). The roots of well over 100 dicot species have been transformed and cultured. Table 1 gives a list that is representative of the diversity of the species transformed, but is not complete, the true number being difficult to determine because such results often remain unpublished. Transformed root cultures have been used in a number of rhizosphere applications, including the culture of obligate parasites and the study of root secondary metabolites and exudates. These aspects are reviewed in [53]. In the present article a novel use for such cultures is described with the intent of illustrating the diversity of the applications possible with transformed roots.

The assimilation of substances from the soil by the root is conditioned by the physical and chemical nature of the soil, the presence of microorganisms, and the release of substances by the plant. The availability, or the potential for assimilation, of a given substance in a given soil is thus difficult to predict. We have used transformed root cultures to model cadmium uptake from polluted sewage sludges with the objective of developing a simple test for cadmium availability and establishing an experimental system with which to study the chemistry and physiology of cadmium assimilation.

The problem of heavy metal contamination requires urgent attention. Cadmium is liberated as a by-product of metal mining and refining; is a frequent contaminant in the phosphates incorporated into fertilizers; is used in many manufacturing processes and appears as a major contaminant of household waste (e.g., button batteries). Cadmium is highly toxic and, unlike organic pollutants, is not degraded or converted to a non-toxic form. Cadmium thus accumulates in soils and leaches into water supplies. It is also taken up by plants and is thus consumed either directly or indirectly by man. One of the important sources of cadmium and other heavy metals is the sewage sludges produced in the decontamination of liquid wastes of both domestic and industrial origin. The use of sludges as fertilizers is limited by their cadmium content.

Since cadmium is highly reactive, its interactions with constituents of the soil are key determinants of its availability to the plant. Similar levels of contamination can have different consequences depending on their availability: either remaining attached to soil constituents or entering the plant, as a function of the nature of the soil and the biological activity in the rhizosphere. The plant is a major biological determinant in the assimilation of cadmium, since the root acidifies the soil in its quest for minerals, iron in particular, and thus solubilizes heavy metals such as cadmium. The nature of this process is difficult and costly to study in the soil using whole plants. We have therefore explored the use of transformed roots as a substitute experimental model.

Transformed roots in axenic culture tend to grow well, and (like roots in nature) they condition the medium through selective uptake and excretion, and thus are generally resistant to unfavourable culture conditions. We have used transformed morning glory roots (*Calystegia sepium*) as models for studying cadmium uptake because these roots survive for long periods in non-axenic conditions after their removal from organ culture. They can be placed directly into the soil, where they cease to grow but remain metabolically active, living on energy reserves accumulated during *in vitro* culture. This property is important in studies of interactions between the root and its chemical environment, since sterilizing the soil is difficult and introduces chemical alterations that are likely to alter the availability of heavy metals.

Results

In order to assay cadmium availability, dry sewage sludge was diluted with highly purified water and added to transformed *C. sepium* root cultures that had been rinsed in the same water. The control consisted of roots treated in the same manner, but the sewage sludge was replaced by sufficient cadmium nitrate to reproduce the total cadmium concentration in the sewage sludge. The roots were cultured for five days, rinsed thoroughly and cadmium assimilated by the root

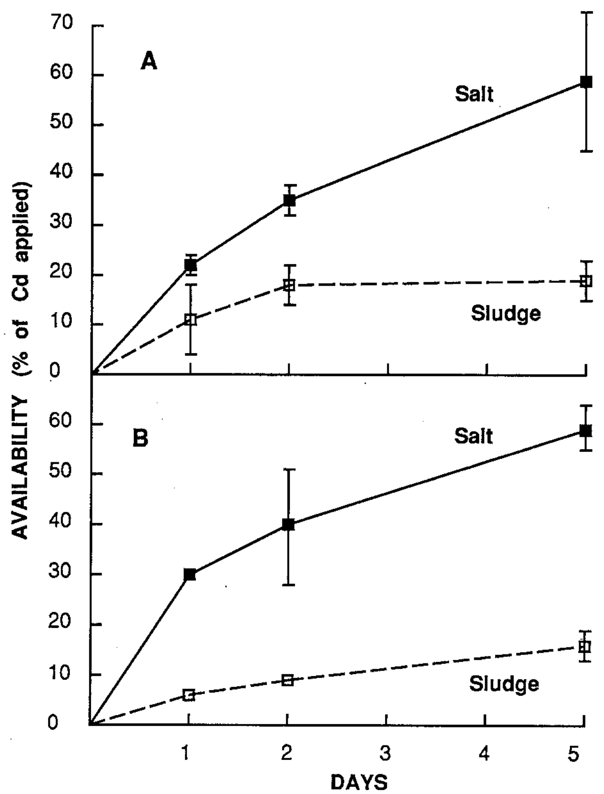

Fig. 1. Influence of time and total Cd concentration on the availability of Cd from a sewage sludge, as compared with Cd applied as $Cd(NO_3)_2$. Initial total Cd concentration: A, 1 mg/l; B, 2 mg/l. (Bars = SD of the mean, n = 3. Bars not shown if coefficient of variation <2%.)

was determined by atomic absorption. Representative results (Fig. 1) show that the cadmium was less available in the sewage sludge than in the salt. The difference represents the affinity of the sludge for the contaminating cadmium when plant roots are present. Sludges of different origin respond differently in this bioassay for cadmium availability (data not shown), indicating that transformed roots can be used to distinguish between the availability of cadmium in different sludges. We are considering the potential of this model in a general test for heavy metal availability and the possibility that it could be used to study the biophysics of cadmium assimilation.

Discussion

Molecular and biochemical approaches to physiological problems often require large amounts of homogeneous material. Transformed roots not only provide sufficient material, but they are resistant to stress, providing the flexibility necessary in experiments that pose physiological questions. In the example giving above, transformed roots placed in conditions where they depend on accumulated energy reserves, yet continue to absorb cadmium from a sewage sludge. Similar uses might include studies of drought, anoxia, starvation for minerals, etc. Transformed roots can be produced in a large variety of dicot species (Table 1). It should be noted that microorganisms can be introduced into such a model. Results from this experimental system must, however, be verified under natural conditions using whole plants growing in complex soils. Model systems may improve our understanding of the rhizosphere and, in the present example, allow us to establish limits in the recycling of sewage sludges.

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