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Nutrients Accumulation and Vascularization of Agrobacterium Tumours Induced on Launaea capitata Spreng. Plant

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Agrobacterium tumefaciens strain (AGRO1) was isolated from stem tumours gathered from Launaea capitata Spreng. Plant. The isolate was used to infect the stems of the host plant then tumours induced were detached and analyzed by atomic absorption spectrophotometry. The tumours were found to contain higher concentrations of some micro- and macroelements as well as sugars in comparison to healthy or infected stems. Cross sections made through the tumours showed globular and extensively branched tumour bundles, consisting of both xylem and phloem which were functionally connected to the vascular system of the host stem. This sophisticated pattern of vascularization was discussed in relation to the ability of the tumoural tissue to accumulate high concentration of minerals and sugars.

Keywords: Agrobacterium tumefaciens, tumours, minerals concentration, histology

INTRODUCTION

Agrobacterium tumefaciens (Smith and Townsend, 1907; Conn, 1942) infects dicotyledonous broad-leaved plants from almost 100 different families causing crown gall disease throughout the world (Pionnat et al., 1999). The disease gains its name from the large tumour-like swellings (galls) that typically occur at the crown of the plant; just above soil level and causes major crop losses in agriculture which are most marked in horticultural and ornamental crops (Kado, 2002). The other related bacterium species, Agrobacterium rhizogenes, induces adventitious root formation at the site of infection in a large number of plants (Sawada et al., 1993). During the infection process a segment of the Ti (tumor-inducing) or Ri (root-inducing) plasmid, called T (transferred)-DNA, is exported from Agrobacterium to the plant cell nucleus where it is integrated into the chromosomal DNA and expressed. The T-DNA transfer and integration processes involve a large number of bacterial and host factors, and finally results in genetically transformed plant cells.

Agrobacteria suppress plant defense mechanisms via the chromosomally encoded degradation of hydrogen peroxide (Xu and Pan, 2000) and by Ti or Ri plasmid-related functions (Veena et al., 2003). Transformation of plant cells results in elevated hormone (auxin and cytokinin) production. Both hormones trigger abnormal proliferation leading to tumourous growth or abnormal rooting (Costacurta and Vanderleyden, 1995; Tzfira and Citovsky, 2008). Tumours produce and secrete specific
amino acids and sugar derivatives, called opines. These opines serve as selective nutrients for the invading bacterium and promote conjugal transfer of its Ti plasmid. Vigorous growth of tumours requires rapid nutrient supply from the shoot and root (Malsy et al., 1992). Tumours are generally regarded as strong metabolite sink for their host plants (Agrios, 2004). In comparison with the untransformed tissue of the host, tumours showed an increase in the cell wall and vacuolar invertase activity (Weil and Rausch, 1990). Both cell wall and vacuolar invertases have an important role in growth processes in sink tissues (Sturm et al., 1995). Solute accumulation was also determined for sugars (such as sucrose, glucose and fructose), total amino acids, cations, including Fe$^{2+}$ and K$^+$ and anions (Pradel et al 1996; Mistrik et al., 2000).

Aloni et al. (1998) confirmed the hypothesis that A. tumefaciens-induced galls produce ethylene that controls vessel differentiation in the host stem. They found that infection via A. tumefaciens results in high rates of ethylene evolution from the developing crown galls which ensure water-supply priority to the growing gall over the host shoot. However, Veselov et al. (2003) reported that the development of A. tumefaciens-induced tumour primarily depends on the excessive production of auxin and cytokinin which are responsible for characteristic patterns of vascularization in both tumour and host tissues. They also investigated the involvement of additional phytohormone signals such as ethylene, jasmonic acid and abscisic acid in the vascularization required for rapid tumour proliferation.

MATERIALS AND METHODS

The ability of Agrobacterium tumefaciens-induced tumours on the stems of Launaea capitata Spreng. to accumulate certain minerals and total available sugars was studied in relation to tumour anatomy and compared with their respective infected and uninfected stems.

Mineral content

Mineral constituents (Fe, Mg, Ca, K, Na, Cu, Co, Mn and Zn) of tumours, infected and healthy ( uninoculated control) stems were determined by Atomic Absorption Spectrometry. Samples for atomic absorption analysis were prepared according to the method of Perkin Elmer manual (1993). In this method plant tissue (either tumour, healthy or infected stems) was ashed in a muffle furnace at 500°C over night. The ashed content of each sample was cooled, dissolved in 5 ml of 20% HCl. The solution was heated to dissolve the residue and then filtered through an acid wet filter paper. The filter paper was washed into 50 ml volumetric flask, the solution was diluted to volume with deionized water and mixed well. Then the samples were ready for analysis by Atomic Absorption.

Total available carbohydrates

Total available carbohydrates (CHO) were determined using the Manual Clegg Anthrone Method (Clegg, 1956). In this method, one gram of air-dried sample was transferred to a graduate 100 ml stoppering measuring cylinder. Ten millilitre of distilled water were added to the sample and stirred with a glass rod to disperse the sample thoroughly. Thirteen millilitre of 52% Perchloric acid reagent were added and stirred frequently for 20 minutes. The contents were diluted to 100 ml with sterilized water, mixed thoroughly and filtered into a 250 ml graduated flask. Ten millilitre of the sample extract was diluted to 100 ml and one ml of diluted filtrate was pipetted into a test tube. One ml of 0.01% (w/v) standard glucose solution was prepared in a second test tube. A third tube containing one ml of distilled water was also prepared as a blank. To each tube, five ml of freshly prepared Anthrone reagent (0.01g Anthrone’s powder in 100 ml of Conc. H$_2$SO$_4$) was rapidly added. Each tube was stoppered, placed in a boiling water bath for exactly 12 minutes, cooled to room temperature and the contents were then transferred to a one cm$^3$ cuvette. The absorbencies of samples and standards were read at 630 nm against the blank. The total available CHO was calculated according to the following equation: Glucose (%) = 25 x b/a x w

Where: $b \equiv$ absorbency of diluted sample. $a \equiv$ absorbency of diluted standard. $w \equiv$ weight of sample (g).

Histological study

Cross sections for microscopic examinations were made through the tumours produced on the stems of Launaea capitata Spern. The cross sections were made to pass through the tumour to the uninfected side of the stem. Sections through infected and healthy stems were also made. Sectioning and staining were made according to the method of John (1958). In this method, wax-embedded stem or tumour segments were fixed for one hour in one part of formalin plus one part acetic acid in 18 parts of 50% ethanol. Following dehydration in ethanol, the segments were dehydrated in tertiarybutanol and then transferred to molten paraffin. Serial sections, 10μ thick, were made by a microtome (Feica Tc 65), stained with safranine and with fast green and were then mounted on glass slide for microscopic observation.
RESULTS AND DISCUSSION

The tumours produced on Launaea capitata Spreng. as a result of infection by Agrobacterium isolate AGRO1 were carefully detached and analyzed by Atomic Absorption spectrophotometry for macro- and microelements. Healthy and infected stems were similarly analyzed and the results are shown in Figure 1. Results in this figure can be summarized in the following points:

a. With the exception of Fe, the concentrations of all elements determined were higher in the tumorous tissue than in both infected and healthy stems.

b. With the exception of Fe and K, the infected stems accumulated higher concentrations of all elements than did healthy stems.

c. The percentage of total available sugars in the infected stems was higher than in the healthy stem. In the later it was higher than in the tumour.

The presented results demonstrate a general tendency of tumours induced on Launaea capitata plants to accumulate high concentrations of trace elements (Cu, Co, Mn and Zn) and to a lesser extent of the macroelements Fe, K, Mg and Ca. Results shown here are comparable with the results obtained by Malsy et al. (1992) for sugars, and with the results of Pradel et al. (1996) for K+. High solute accumulation in crown galls of Kalanchoë daigremontiana was also reported by Marx and Ullrich (1988). Malsy et al. (1992) reported that induction of crown galls by A. tumefaciens reverses assimilates translocation and accumulation in Kalanchoë daigremontiana. However, Senger et al. (1983) reported that the secretion by tumour cells of a vascular permeability factor that promotes the accumulation of ascites fluid. In the light of these results, tumours may be considered as a strong sink tissue with abnormal anatomy that guarantees an efficient flow of water and nutrients into tumourous tissue. Vascularization is essential for efficient assimilate import from the host plant into the tumour parenchyma cells via the symplastic pathway (Malsy et al., 1992; Pradel et al., 1996, 1999). In addition, acquisition of inorganic ions is enhanced by the auxin- and ethylene-dependent proliferation of vessel numbers at the host/tumour interface and disruption of the tumour epidermis and cuticle. To clarify this, histological investigations were carried out to compare the anatomy of tumours with those of normal stems. The sections obtained are shown in Figure 2, 3 and 4. The sections revealed the presence of numerous enlarged cells which radially surround the tumour. These cells, which are absent from uninfected stems, seemed to originate from the loosely packed cortical cells and to have divided on all planes resulting in excessively branching arched and unorganized vascularization. In addition, epidermal disruption is also evident (Figure 2). Enlarged cells with reticulated lignified vessels also appeared in abundance (Figure 3) and the number of both xylem and phloem vessels increased. It is also evident in Figure 2, 3 and 4 that the number of sieve elements has increased considerably in the phloem bundles of the tumours than in the phloem of the uninfected stems. The abnormal pattern of vascularization reported here appears to be a key step in establishing solute flow into plant tumours. The increased number of narrower vessels appeared to accelerate water flow through the tumour at the expense of the host shoot. These findings are in line with the gall constriction hypothesis suggested by Aloni et al. (1995) and Schurr et al. (1996). It is generally reported that, specific xylem parenchyma cells form at sites of considerable auxin and cytokinin concentrations (Veselov et al., 2003). The high level of phytohormones (auxins, cytokinins and ethylene) reported by various investigators in tumourous tissue...
Figure 2. T.S. through a tumour of a 30-day old *Launaea capitata* Spreng. seedling showing numerous vascular bundles.

Figure 3. T.S. through a tumour of a 30-day old *Launaea capitata* Spreng. seedling showing lignified vessels with enlarged cells.

Figure 4. T.S. passing across the stem to the tumour showing: A. normal vascularization in the stem, B. abnormal vascularization in the tumour (note the continuity of the vascular system at the stem-gall interface).


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