T-DNA genes to study plant development: precocious tuberisation and enhanced cytokinins in *A. tumefaciens* transformed potato

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Summary

Potato Line Mb1501B is a derivative of the cultivar Maris Bard (*Solanum tuberosum*), transformed with T-DNA from *A. tumefaciens* strain LBA1501. In culture it grew as frequently branching stunted shoots with a basal callus, lacking roots. These shoots did not form tubers. When grafted, Mb1501B shoots gradually became morphologically more normal and aerial tubers formed readily. Cultured Mb1501B shoots contained 100-200-fold higher concentrations of the biologically-active cytokinins zeatin, zeatin riboside and their corresponding side-chain o-glucosides than untransformed Maris Bard shoots. Cultured Mb1501B shoots contained approximately a 3-fold lower concentration of indole acetic acid (IAA). In grafted Mb1501B plants a 3-10-fold higher concentration of the active cytokinins was found compared with untransformed plants and no difference in IAA concentration.

Introduction

Virulent strains of *Agrobacterium tumefaciens* induce tumours (crown-galls) on many dicotyledonous plants following infection of wounded plant tissue (9, 32). Such tumours are characterised by the production of tumour-cell specific opines and by hormone-independent growth when cultured in vitro, free from the inducing bacteria and removed from the plant (see for reviews 5, 16). Both these characters are genetically determined by the stable integration of the transferred DNA (T-DNA)-segment from a large *Agrobacterium* tumour-inducing (Ti) plasmid, into the host genome of transformed plant cells (7, 33, 36). These observations have two major implications: (1) *Agrobacterium* can be used to efficiently and stably introduce DNA into plant cells, which has resulted in the construction of advanced plant genetic engineering vectors and (2) T-DNA genes themselves, particularly the hormone related genes, provide specific and novel genetic tools to study whole plant development.

Mutagenesis studies have determined the map position and some insight in the function of T-DNA genes involved in opine production and plant hormone-independent growth (13, 15, 19, 25, 29). Two T-DNA genes coding for transcripts 1 and 2 (37) are involved in the synthesis of auxins. The auxin gene for tr2 codes for an hydrolase capable of converting indole-3-acetamide to indole-3-acetic acid (17, 31). It has been suggested that the auxin gene for tr1 codes for a monoxygenase capable of converting L-tryptophan to indole-3-acetamide (17, 31). The T-DNA gene for tr4 (37) codes for an enzyme involved in the first committed step in cytokinin biosynthesis (1, 2, 4, 13, 19, 21, 25). In agreement with this, enhanced concentrations of the auxin IAA and of cytokinins were detected in tobacco tumours (1).

Mutant *A. tumefaciens* strains inactivated for either of the auxin genes induce shoot producing tumours in tobacco (13, 25) and other plants like potato (26). Such shoot producing tobacco tu-
mours contained less auxin than wild-type tumours (four fold less) but thirty fold more cytokinin (1). The increase in cytokinin content of shoot-producing tobacco tumours was not due to enhanced expression of the T-DNA cytokinin gene (35).

Transformed shoots were regenerated from the tobacco tumours. Usually they did not form roots and had a distinct morphology which reverted to normal following grafting (34, 39). The plants formed flowers and T-DNA was transmitted through meiosis (20, 28, 38, 39).

The observations that the endogenous cytokinin concentration was increased in primary shoot-producing tobacco tumours and that transformed tobacco plants were regenerated prompted us to examine similarly transformed and regenerated potato shoots and plants. One objective was to study the feasibility of using T-DNA genes to specifically modify plant development in potato. The formation of potato tubers is an agronomically important process which can be induced by addition of cytokinins to potato shoots growing in vitro (27). In general, it seems likely therefore, that potato plants containing wild-type and engineered T-DNA genes open a new and powerful approach to study the influence of hormone-related genes on the molecular control of the complex processes of growth, development and tuberisation in potato.

In this paper the plants transformed with shoot inducing Ti T-DNA have been used to examine the changes in plant development and hormone content brought about by the hormone related T-DNA genes from A. tumefaciens. In the accompanying paper (6) the corresponding underlying molecular events directly related to T-DNA structure and expression have been examined.

Materials and methods

Plants and growth conditions

Transformed potato line Mb1501B (26) and shoot cultures of potato cultivar Maris Bard were maintained by micropropagation on Murashige and Skoog medium (23) solidified with 0.9% agar without added plant hormones but with 20 g/1 sucrose (MS20). The cultures were grown at 25°C, 16 h day length, in glass jars with transparent petri dish lids. Growth conditions in growth chambers were 12 h day length; 18°C day temp and 15°C night temp; 80–90% humidity. Transformed Mb1501B shoots were grafted usually onto stem segments of untransformed potato shoot cultures that had one leaf. This stem segment plus mini-scion was maintained for approximately two weeks on MS20 so that from the axillary bud a shoot plus roots developed and the scion could establish itself firmly. The mini-construct was then potted up in soil and grown in a growth chamber, initially covered with a polythene bag.

Extractions and purification of auxins and cytokinins

Auxins and cytokinins were extracted from homogenized fresh shoot tissue with methanol overnight at -15°C. Only differentiated shoot tissue of Mb1501B was extracted not the undifferentiated callus tissue at the base of the shoots (see Fig. 1). For the growth chamber grown material all the leafy tissue, excluding the aerial tubers, of the Mb1501B scion shown in Fig. 2d (39.2 gram) was extracted. For Maris Bard the entire Maris Bard scion in Fig. 2d was extracted (13.6 gram) plus an additional, 23.1 gram of two other Maris Bard plants to obtain an equivalent fresh weight of tissue for the extraction.

Approximately 1 000 dpm 3H-dihydro zeatin (6.34 Ci mmole-1) and 1 000 dpm 2 14C-IAA (49 mCi mmole-1) per g. fwt. tissue was added as internal standards. This enabled the estimation of overall recovery and was used as a marker for the relative position of known cytokinin standards on HPLC. The combined methanolic extracts were reduced to an aqueous phase and the ether soluble acids removed for IAA analysis (8). The pH 8.0 butanol solubles (cytokinins) were applied to a SP-Sephadex C-25 cation exchange column and the basic fraction passed through a Sep Pak C18 cartridge. The cytokinin containing fraction was resolved by analytical HPLC (18) (4.5 X 250 mm column of Hypersil 5-ODS eluted with a shallow linear gradient, 4%-14% at 0.4% min-1, of acetonitrile in water pH 7). Fractions (2 ml) were collected every minute, an aliquot removed for counting and remainder dried down in 50 ml conical flasks for subsequent bioassay. The ether soluble acids (IAA) were purified by DEAE Sephacel anion-exchange chromatography and quantitatively determined by an HPLC native fluorescence method (8).