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The anatomy of tissue cultured red raspberry prior to and after transfer to soil

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Abstract. The leaf, petiole, stem and root anatomy of an aseptically cultured red raspberry clone (*Rubus idaeus* L.) was studied before and 5 weeks after transfer to soil under controlled environmental conditions. Tissues persistent from culture showed little or no change with time in soil; they grew minimally and slight secondary wall deposition occurred. New organs formed in successive weeks after transplantation showed a graded increase in potential size and development. Some features, such as collenchyma formation, rapidly returned to control levels; this was seen in new leaves expanding in the first week after transplantation. Other features, such as sclerenchyma formation, did not occur in leaves expanding during the first 2 weeks after transplantation, even when these were a month or more in age. Some sclerenchyma was seen in leaves expanding in the third week after transplantation, increasing in later-formed leaves. Increasing the light intensity of transplant accelerated the return to control-type organ size and appearance. During acclimatization transitional forms of leaves, petioles, stems and roots develop that ranged anatomically from culture- to control-type. This trend is analagous to the normal developmental sequence of organ formation as it affects the potential for development of successily formed organs.

Introduction

Little is known of how the anatomy of micropropagated plantlets is affected by the culture environment or how anatomy of transplants is modified during acclimatization to the soil environment. Evaluation of structural changes taking place during acclimatization is prerequisite to comprehension of this process and necessary to the development of more efficient transplant protocols [27]. In reports describing foliar anatomy and surface features of plantlets and/or transplants [1–5, 11–15, 21, 24, 25–28] leaves were sampled at random 2–6 weeks after transplantation. No information was given of the stage in the acclimatization process at which these leaves were formed and none was given of petiole, root or stem anatomy. In the present study leaves formed in culture were labeled at the time of transplantation and new leaves labeled weekly thereafter so petioles, leaves and subjacent stem tissue could subsequently be identified as to period of development (measured in weeks from the time of transplantation).

Structural features of the petiole, leaf, stem and root anatomy of red raspberry plantlets grown in aseptic culture, prior to and after transplantation, were examined. The way in which light intensity affected anatomy of cultured plantlets and the survival and anatomy of transplants was of interest. Also of interest was assessment of persistent organs (leaves, petioles, stem retained from culture) and changes in anatomy of new organs formed during the acclimatization process in soil.

Materials and methods

Red raspberry plants used in these experiments were a clone derived from one meristem tip, from a new selection (BC72-1-7), 'Haida' x 'Canby', developed by the British Columbia red raspberry breeding program. Plantlets were cultured in 500 ml jars containing 30 ml of red raspberry rooting medium [6], modified in that 25 g/L glucose replaced 30 g/L sucrose. Plantlets were subdivided and subcultured at 4 week intervals into new medium or transferred to soil at this time.

Plantlets were incubated at low and high light intensity (25 and 80 $\mu Es^{-1}\ m^{-2}$). Twelve rooted plantlets with all leaves tagged were transferred to soil at each light intensity. Plantlets from 25 $\mu Es^{-1}\ m^{-2}$ culture were transplanted to 40, 80 and 120 $\mu Es^{-1}\ m^{-2}$ in soil. Plantlets from 80 $\mu Es^{-1}\ m^{-2}$ culture were transplanted to 80, 120 and 160 $\mu Es^{-1}\ m^{-2}$ in soil. The transplants were kept under conditions of elevated relative humidity by closing the flats with transparent covers and misting them regularly. Cultures and transplants were incubated under controlled temperature (27 ± 2 °C) and lighting (16 h photoperiod with 3:1 cool white:warm white fluorescent lighting.

At weekly intervals transplants were examined, and any new leaves expanded during that week were tagged. After 5 weeks in soil transplants had markers of six different colours indicating the period of tissue formation. In this way leaves, petioles and subjacent stem tissue could be evaluated for changes occuring in successive weeks during acclimatization. Root samples collected from transplants after they had been 5 weeks in soil were a mixture of those persistent from culture and new ones formed at various times after transplantation.

Anatomical features were examined from plantlets, persistent and new tissues of transplants and 2 year old field control plants. Petiole (portion subjacent to the leaf), leaf (mid-tip area), stem (subjacent to the petiole) and root samples were cut into small sections, fixed in formalin-acetic acid-alcohol, dehydrated in an ethanol series and wax-embedded. These were sectioned (8–10 μm), stained and counterstained in safranin and fast green and mounted in permount [20]. Micrographs were taken from the prepared slides and the prints examined. The general shape, size and integrity of each organ was noted and support tissue described [17].

Results and discussion

Of 36 plantlets transplanted from low light intensity culture ($25\,\mu Es^{-1}\,m^{-2}$) 33 survived. The 3 mortalities occurred in transplants to $120\,\mu Es^{-1}\,m^{-2}$. This and previous unsuccessful attempts to transplant from $25\,\mu Es^{-1}\,m^{-2}$ culture to $160\,\mu Es^{-1}\,m^{-2}$ in soil suggest that $120\,\mu Es^{-1}\,m^{-2}$ is probably the upper limit to which plantlets incubated at $25\,\mu Es^{-1}\,m^{-2}$ can be transplanted successfully. Few plantlets (5/36) from high light intensity ($80\,\mu Es^{-1}\,m^{-2}$) culture survived transplantation to soil conditions and were therefore not sampled. The pronounced differences in survival rate between transplants from cultures incubated at low and high light intensity are not explicable on the basis of anatomy, which was similar in the two groups. This difference in survival rate may be explained, in part, by the presence in the lower light intensity cultures of greater pigment content [5], and possibly smaller leaf size which may reduce the amount of water stress [4, 5] and deformation when desiccation occurs [27] following transplant.

Petioles of red raspberry control plants were surrounded by four to five cell layers of collenchyma tissue beneath the epidermis and developed sclerenchyma caps six to eight cell layers deep in the phloem tissues of the three leaf traces. Petioles of in vitro plantlets had diameters only one quarter to one third that of control plants, were fragile, with thin cell walls, lacked collenchyma and possessed few phloem fibers (Figure 1a,b). Persistent leaf petioles of transplants did not develop collenchyma or additional sclerenchyma but those of new leaves, even those formed the first week after transplant had one to several layers of collenchyma cells. New leaf petioles had thicker cell walls, especially noticeable in the vascular tissues, compared to those of plantlets. The petioles of successively formed new leaves progressively increased in diameter and had an increased number of vascular elements of both xylem and phloem (Figure 1c). New leaf petioles formed the third week after transplantation had noticeably greater levels of phloem sclerenchyma than those formed in the first two weeks.

Mature leaves of red raspberry control plants were usually trifoliate, while those of both plantlets and transplants were unifoliate. The amount of leaf dissection in *Ranunculus*, which has a leaf shaped similar to red raspberry, is apparently related to temperature, with clefts or notches in the primordial leaf margins that cause lobing in the mature leaf appearing at reduced temperatures [10]. Effects of elevated temperature exposure may persist for several months [9] and may account for this suppression in the new leaves of red raspberry transplants regardless of the light intensity to which they were transferred. The epidermal cells of cultured leaves were often somewhat collapsed. This has been observed in leaves of in vitro sweetgum plantlets [27], and is likely related to elevated relative humidity [8]. Epidermal cell distortion or collapse was frequently noted in new leaves of transplants at all

light intensities. Leaves of plantlets and transplants were smaller, thinner and had less compact chlorenchyma tissues than controls. The ratio of plantlet palisade cells to epidermal cells was usually 1:1 or 2:1 as it was in very young control leaves. In older control leaves this ratio increases to approximately 5:1 as epidermal cell expansion and palisade cell division proceed. The sizes of the new leaves of transplants, the palisade:epidermal cell ratio, and the compactness of the parenchymatous tissues were greatest, although the sizes of the substomatal cavities were smallest, in those transplanted to the highest light intensity ($120\,\mu Es^{-1}\ m^{-2}$). Size of the substomatal cavities has been correlated to the amount of water stress: large where relative humidity is high [16]. Red raspberry control leaves had thick-walled collenchyma cells in the abaxial veinal areas. Leaves in culture and those of transplants lacked collenchyma.

Stems of control red raspberry plants had a continuous cylinder of collenchyma in the cortex beneath the epidermis, a thick layer of sclerenchyma in the phloem of the stele and both thin and thick-walled pith cells (Figure 1d). Stems of in vitro plantlets were smaller in diameter than control plant stems. They had little collenchyma in the cortex, very few sclerenchyma fibers and no thick walled pith cells (Figure 1e). The transplants had new stem growth with intermediate amounts of collenchyma in the cortex, some sclerenchyma fibers in the phloem and some thick walled pith cells (Figure 1f). Transplants at the higher light intensities had larger stem diameters and greater support tissue development. The base of the plant, location of the persistent portion of the stem changed little from the culture type even 5 weeks after transplant. Stem tissue persistent from culture increased only slightly in diameter through cell expansion and wall deposition. This may be a limiting feature in transplant development. It is possible that the vascular cambium, in a later stage of development, increases the amount of support tissues. It is also possible that in the case of red raspberry, where new canes are constantly produced from shoot bases and roots, the canes arising from culture derived tissue do not feature prominently in the transplant's future.

Roots of control red raspberry plants were brown in colour and had a multilayered periderm. Roots of cultured plantlets were smaller in diameter, white or pinkish in colour and had little periderm (Figure 1g, h). They were covered with fine root hairs and had a delicate appearance. Roots of transplants were small to intermediate in size, brown and had intermediate periderm development (Figure 1i). The small, delicate roots of cultured red raspberry plantlets and those 5 weeks after transplant suggest some differences in their physiology compared to control roots. This is supported by evidence of Poole and Conover [18] that roots of *Dieffenbachia maculata* (Lodd.) transplants were not yet acclimatized 6 weeks after transplant to soil. These differences may limit transplant development until control type roots are produced by the transplants at some later stage.

In leaves, stems and petioles the amount of support tissue and wall

deposition was greatest in the higher light intensity transplants. This was apparent from greater ease of sectioning of the firmer tissue and greater staining intensity and thickness of some cell walls such as the vessel elements of the zylem. This is supported by data showing significantly more dry weight accumulation in leaves at the higher transplant light intensities [5]. Despite the progresssively younger age of the tissue tested, the compactness of the leaf tissues, amount of wall deposition and diameter or size of transplant organs showed a distinct increase that was inversely related to time of organ appearance after transplant.

Scarcity of collenchyma tissue in culture and reduced amount in the new leaves and petioles of transplants compared to controls may be explained in part by results of Razdorskii [9] and those of Venning [22] cited by Esau [8]. They found that in developing plants exposed to mechanical stresses, collenchyma wall thickenings began earlier and became more massive than in plants unexposed to such stresses. In culture mechanical stresses are few as there is little or no air movement in stationary cultures. Even the first new leaves of transplants develop some collenchyma tissues, the later formed leaves have much more. The implication is that control levels of collenchyma cell wall development cannot be expected in culture and also may be inhibited by the intermediate environment into which the transplants are placed, as both are relatively tranquil in terms of mechanical stress.

Scarity of sclerenchyma fibers and secondary wall development is probably related to the elevated relative humidity of both the culture environment and the intermediate transplant environment. Eberhardt [7; cited by 23] found reduced sclerenchyma and secondary wall formation (including lignification) in both roots and stems of a wide number of species subject to conditions of high relative humidity.

Persistence of in vitro features in organs appearing after transplant implies that organs formed after transplant but which had been initiated in culture, and defined in the early stages of development (as primordia) by the culture environment do not change to control type structures. Organs formed after transplant and initiated in soil may be prevented from rapidly achieving the control type structure because they are limited in their development by:

(a) Culture environment effects which can be escaped from only gradually,

(b) The ambient intermediate type environment which enables survival through the development of transitional organs,

(c) The retention of culture type organs that make up the existing transplant structure and influence the physiological status of the rest of the plant.

Whatever the underlying reason(s) a graduated return to control type morphology occurs in red raspberry during acclimatization to the soil environment and is accelerated by higher light intensity.

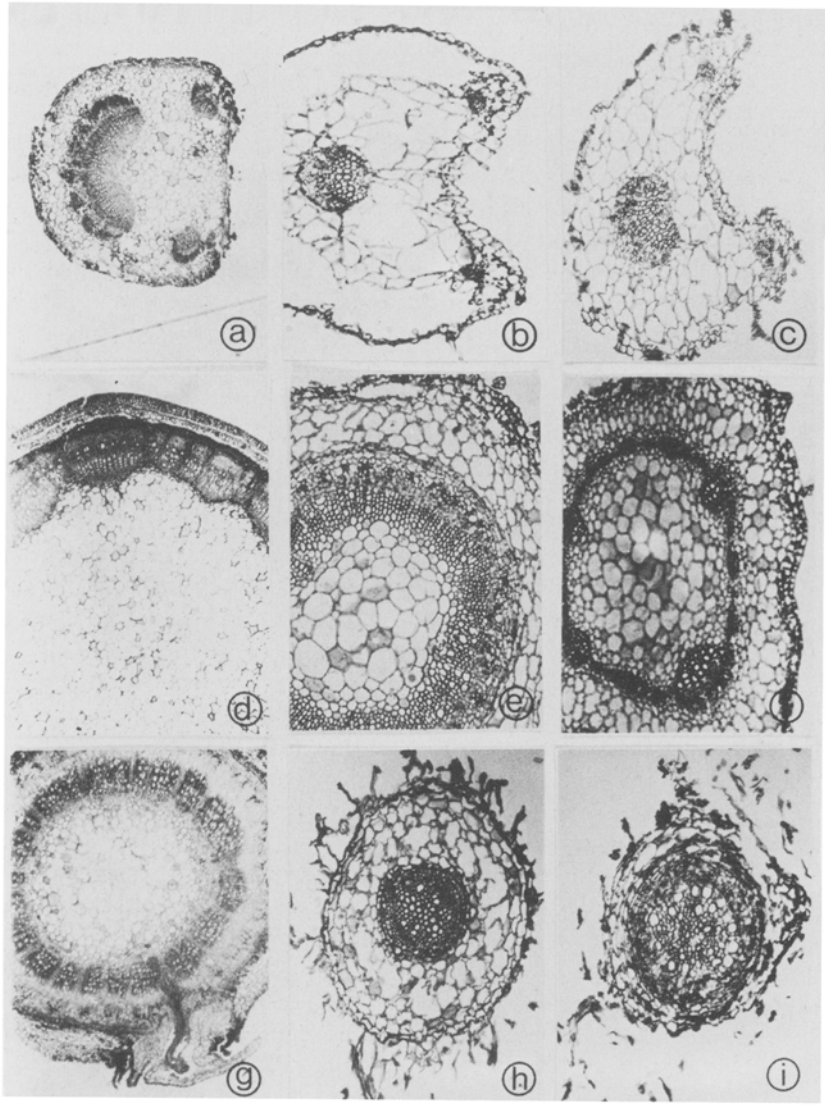

Figure 1. Photomicrographs of sectioned petioles (A–C), stems (D–F) and roots (G–I) of control plants (left), plantlets (centre) and transplants (right)

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