

A Selective Tissue Culture Medium for Growth of Compact (Dwarf) Mutants of Apple*

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Summary. Three strains of 'McIntosh' apple (*Malus domestica* Borkh.) with growth habits ranging from the standard parent type to extremely compact (dwarf) were grown in vitro as meristem-tip cultures on Murasige and Skoog medium containing a range of concentrations of benzyladenine (BA). All strains exhibited a similar optima (3 to 6 μ M BA) for maximum shoot proliferation and culture weight increase. However, tolerance to supra-optimal concentration of this cytokinin was related to growth habit. For example, at 10 μ M BA shoot production rates as a percent of the maximum rates were 90%, 20% and zero for the extreme compact, moderate compact and standard strains, respectively. Comparisons among field trees and meristem-tip cultures of all three strains revealed similarities in growth and development.

Key words: Apple – Cytokinin – Dwarf – Mutant – Tissue culture

Introduction

A compact or, in some cases, a dwarf growth habit is a characteristic of many modern crop cultivars and has contributed to important yield advances in recent years. In apple, compact (often referred to as spur-type) strains are characterized by an upright growth habit, shorter internodes, fewer but thicker shoots and a higher ratio of reproductive to vegetative growth (Lapins 1969, 1974; Eaton and Lapins 1970).

Compact or dwarf selections of other species also share many of these characteristics (Borlaug 1968). The compact growth habit can vary from extreme to slight in apple (Lapins 1974) and is a commonly reported spontaneous mutation (Lapins and Fisher 1974; Lane and Meheriuk 1976). Some compact strains have become commercially important because they possess this desirable growth habit, which permits more effi-

cient orchard management, yet they retain the desirable fruit characteristics of the parent strain. Such has been the case for 'McIntosh', a popular North American cultivar.

Compact growth mutants have not yet been discovered for all important apple cultivars and attempts to induce this mutation using ionizing irradiation has not yet shown great success (Lapins 1973; Lacey and Campbell 1979). The use of more subtle mutagens within a meristem-tip culture system could prove to be a superior approach, particularly if a selective medium could be devised.

In work of a similar nature, solutions containing specific toxins have been used to select plants resistant to certain pathogens or to determine their disease reaction (Wheeler and Luke 1955; Byther and Steiner 1972; Matern et al. 1978). In addition, plants resistant to pathogenic toxins have been regenerated from callus (Behnke 1980), and immature embryos (Gengenbach et al. 1977) grown in media containing toxins and protoplasts (Shepard et al. 1980). Selective media have also been used to obtain tissue cultures tolerant to auxinic herbicides (Chaleff 1980) and to high abscissic acid levels (Wong and Sussex 1980) by increasing the concentration of these growth regulators in the media.

Materials and Methods

Meristem-tip cultures of three strains of apple (*Malus domestica* Borkh. cv 'McIntosh') varying in their degree of compactness were established using previously described procedures (Lane 1978). The three strains were 'Summerland Red McIntosh' (standard growth habit), 'Macspur', and 'McIntosh Wijcik' (extremely compact). Both compact strains arose as spontaneous bud mutations on 'Summerland Red McIntosh' trees growing in the field.

Two experiments were conducted to determine the relative response of the three strains to the level of benzyladenine (BA) in the culture medium. In the first, six concentrations of BA (0.1, 0.5, 1.0, 5.0, 10, and 25 μ M)

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Fig. 1. Apple meristem-tip cultures grown on medium containing sub-optimal concentration of $0.5 \mu\text{M}$ BA (left) and supra-optimal concentration of $25 \mu\text{M}$ BA (right). The three strains are 'Summerland Red McIntosh' (top), 'Macspur' (center) and 'McIntosh Wijcik' (bottom)

in Murashige and Skoog (1962) medium minus indole-3-acetic acid and kinetin were used to establish limits and determine the generalized response. A second experiment with BA concentrations of 3.0, 6.0, 10 and $15 \mu\text{M}$ further defined it.

To initiate each experiment, small shoot tips (10–15 mg fresh weight) were taken from mother cultures grown in Murashige and Skoog medium containing $5.0 \mu\text{M}$ BA. Incubation was for 38 (Experiment 1) or 42 days (Experiment 2) in 250 ml flasks containing 75 ml of medium. The culture flasks were held in a growth chamber programmed for 16 hr days and 28°C and 22°C day and night temperatures, respectively.

Each flask was an experimental unit and in each experiment all treatments were replicated eight times. At the end of each experiment shoots at least 1 cm long were counted and the entire culture was weighed after drying at 65°C for two days.

In an effort to compare the growth (weight increase over time) and development (release of axillary buds from dormancy) of the cultures with that of trees of the same strains growing in the field, measurements were taken on 26 'McIntosh Wijcik' trees varying in age from 5 to 8 years, 30 'Macspur' trees 5 to 9 years old and 11 'Summerland Red McIntosh' trees 9 years old. The increase in trunk diameter per year was linear with age and is highly correlated with tree size (Westwood and Roberts 1970). Twenty representative 2-year-old shoots were collected for each strain to determine the number of axillary buds formed in 1979 which grew into shoots in 1980.

Results

The relative growth of the three strains of apple depended on the concentration of BA in the medium. In Experiment 1, designed to determine the high and low limits of BA permitting growth of any of the strains, only the 'Summerland Red McIntosh' cultures remained

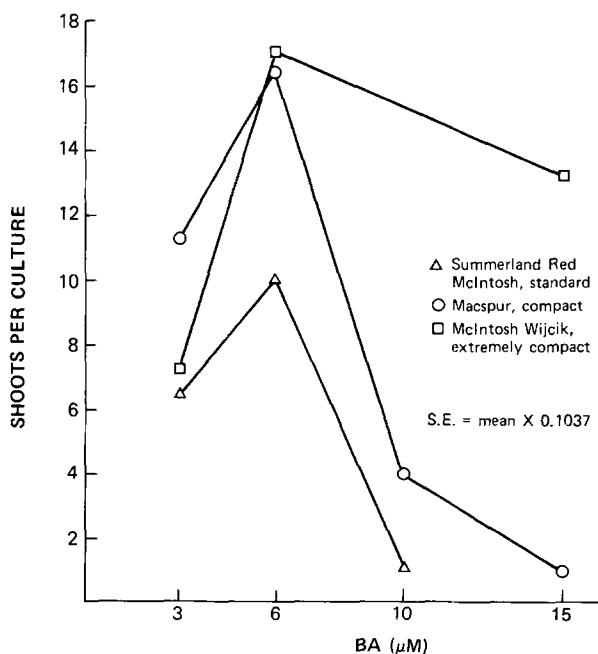


Fig. 2. Number of shoots produced by meristem-tip cultures after 42 days growth by three strains of apple with standard, compact or extremely compact growth habit as influenced by BA concentration

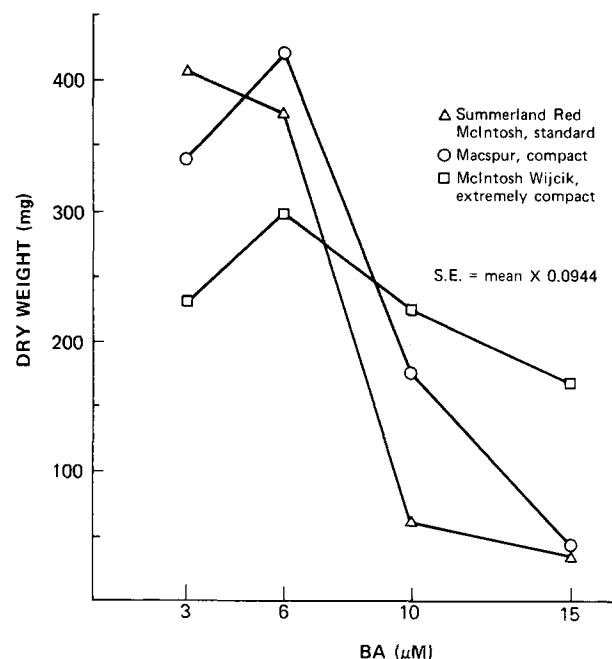


Fig. 3. Dry weight of meristem-tip cultures after 42 days growth of three strains of apple with standard, compact or extremely compact growth habit as influenced by BA concentration

healthy at 0.5 μM BA. Conversely, only 'McIntosh Wijkik' continued to grow at 25 μM BA (Fig. 1). All cultures grew best at 5.0 μM BA.

Based on the number of shoots initiated which grew to longer than 1 cm, maximum growth for all three strains occurred at 6 μM BA in Experiment 2 (Fig. 2). At this BA concentration the two compact strains produced more shoots than 'Summerland Red McIntosh'. At concentrations greater than optimum, BA reduced growth dramatically for 'Summerland Red McIntosh' and 'Macspur' and less so for 'McIntosh Wijkik'. At 10 μM BA 'Summerland Red McIntosh' did not develop any new shoots, 'Macspur' developed about 20% of number of shoots observed for 6 μM BA, while the comparable value for 'McIntosh Wijkik' was 90%. At

15 μM BA only 'McIntosh Wijkik' continued to proliferate.

When growth was measured as culture weight gain 'Summerland Red McIntosh' grew optimally at 3 μM BA whereas 6 μM BA was optimal for the two compact strains (Fig. 3). At the optimal concentration the dry weight of the 'McIntosh Wijkik' cultures was about 300 mg compared to over 400 mg for the other strains. However, the extent to which growth was suppressed by supra-optimal BA levels clearly favored growth of the two compact strains. At 10 μM BA the 'Summerland Red McIntosh' cultures were reduced to 16% of their maximum weight, 'Macspur' cultures were reduced to 45% of optimum while 'McIntosh Wijkik' cultures weighed 77% of the 6 μM BA value.

While growth and development of apple meristem cultures would not necessarily be expected to parallel that of field grown trees, similarities in the important areas of branching behavior and weight increase would strengthen the argument that cytokinin plays an important role in the manifestation of the compact growth habit in 'McIntosh' apple. At the optimal BA concentration, the 'Summerland Red McIntosh' cultures produced significantly fewer shoots per culture than either compact strain (Fig. 1). Similarly, the percent of axillary buds on 1979 field shoots of 'Summerland Red McIntosh' which broke dormancy and grew in 1980 was lower than for either compact strain (Table 1). In the field observations 'Macspur' was intermediate between the extreme compact and the standard strain.

The relative weight gain of cultures in vitro also approximated the behavior of field trees as estimated by increases in trunk diameter over time. Trunk diameter is highly correlated with tree weight and under irrigated agriculture conditions tends to increase linearly with age. The field trees we observed showed average annual increases of 1.9, 1.8, and 1.3 mm for 'Summerland Red McIntosh', 'Macspur', and 'McIntosh Wijkik', respectively. 'McIntosh Wijkik' appears to grow at a rate about 68% that of 'Summerland Red McIntosh'. The comparable figure arising from the tissue culture data was 73.5% (Table 1).

Table 1. A comparison between field grown trees and meristem-tip cultures of three strains of 'McIntosh' apple in which shoot number (proportion of axillary buds developing into shoots) and weight (rate of increase) were measured. The shoot number data are presented as a percentage of the extreme compact's value and the weight data as a percent of the standards

Strain	Growth habit	Relative shoot no.		Relative weight	
		in vitro	in vivo	in vitro	in vivo
'McIntosh Wijkik'	Extreme compact	100	100	73.5	68
'Macspur'	Compact	97	76	103	95
'Summerland Red McIntosh'	Standard	59	66	100	100

Discussion

The optimum media concentration of BA for shoot production and dry matter accumulation was similar for the three 'McIntosh' strains and in agreement with the optimum for apple reported by others (Jones et al. 1977; Snir and Erez 1980). This agreement indicates that optimum BA concentration for growth of apple meristem-tip cultures in vitro is stable and largely free from minor influences of environment. We are the first, however, to report that mutants of one commercial cultivar differ in their tolerance to supra-optimal cytokinin concentrations. An approximately three fold increase in BA concentration above the optimum was sufficient to selectively allow growth of only the extreme compact strain. Although this difference is small compared to tolerance to toxins acquired by plant cells in other selective media it was enough to easily separate the three genotypes based on growth in vitro where all cultures were dependent on supplied BA.

The responses of the strains to different BA concentration provides an interesting clue to the physiological mechanism of growth compaction in apple. In previous studies involving the 'McIntosh Wijcik' growth type, Lee and Looney (1977, 1978) observed lower gibberellin-like activity and lower abscisic acid levels in shoot tips compared to standard 'McIntosh'. Endogenous cytokinin and auxin levels are yet to be documented. However, a characteristic of the compact growth type is a strong tendency for axillary buds to break dormancy and grow into short shoots (Table 1) and it has long been known that dormant axillary buds respond to topically applied cytokinins (Sachs and Thimann 1964; Williams and Stahly 1968). It is also theorized that cytokinins moving up the stem from the root system function to control plant growth and development (Skeene 1975; Torrey 1976). Therefore, it is tempting to predict that cytokinins play a key role in defining the compact growth type in 'McIntosh' apple. However, the fact that all three strains shared a common cytokinin optima in the culture media renders this conclusion premature. The strains probably differ in their ability to metabolize the BA excessive to their growth and development requirements, or alternatively, counter the high cytokinin levels with higher or lower levels of other hormones to maintain a balance conducive to continued growth and development. For example, Sachs and Thimann (1967) have shown that auxins and cytokinins interact in the control of apical dominance.

The observation that shoot cultures of the compact strains tolerate substantially higher levels of BA in the culture medium could form the basis of a selection procedure. Appropriate adjustment of BA concentration would favour growth of the desired strain and prevent any cultures with a back mutation from, for example, compact to standard growth habit from predominating if cultures were being used for propagation. Conversely, it could be adjusted to favour growth of mutants with increased tolerance and perhaps provide a source of new strains with compact growth habit. To date in vitro mutation breeding of apple is only an interesting con-

cept but description of a selective medium for an important physiological trait such as growth habit is encouraging.

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