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ISOLATION OF A PURE THORNLESS LOGANBERRY BY MERISTEM TIP CULTURE

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INDEX WORDS

Rubus, blackberry, raspberry, hybridberry, Loganberry, chimera, *in vitro*, plant breeding, tissue culture, thornless.

LIST OF TERMS: BAP = 6-benzylaminopurine; GA_3 = gibberellic acid; NAA = naphthalene acetic acid; hybridberry = polyploid bramble interspecific hybrids; MS = MURASHIGE & SKOOG (1962) high mineral salt medium; TL = 'Thornless Loganberry'; TL_{tc} = tissue culture-derived (non-chimeral) 'Thornless Loganberry'.

SUMMARY

'Thornless Loganberry' (TL) is a periclinal chimeral blackberry in which a layer of mutant (thornless) epidermis surrounds a core of wild-type (thorny) tiusse. Due to its chimeral arrangement, TL produces thorny adventitious root cuttings and thorny offspring. To separate the chimera into its components parts, meristems of TL were grow *in vitro* on modified Murashige and Skoog medium to yield callus and adventitious shoots. One of these shoots has survived, flowered, and produced thornless offspring from seed. The importance of this non-chimeral TL is discussed.

INTRODUCTION

'Thornless Loganberry', (TL) (*Rubus sp.*) ($2n = 6\times = 42$) is a fully fruitful sport of the 'Logan' blackberry. It was discovered in 1929 and distributed as 'Bauer Thornless' (JENNINGS, 1981). Both thorny and thornless types have been propagated asexually and by seeds to result in a 'considerable admixture of inferior [thorny] seedlings and virus-infected plants' (JENNINGS, 1981). From these mixtures both thorned and thornless Loganberry clones have been reselected ('LY59' and 'L654', respectively) (BEAKBANE, 1935; BEAKBANE & LABERN, 1960; WAY, 1966).

Unfortunately, due to chimerism, TL thornlessness genes are not available to the geneticist (DARROW, 1937). TL is a periclinal ('hand-in-glove') chimera in which a layer of mutant (thornless) epidermis (L_1) completely encloses a core of wild-type (thorny) tissue ($L_2 + L_3$). Since thorns (technically known as prickles) have an epidermal origin, the entire plant appears to be thornless.

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Under some situations the thornless condition is stable, but when the plant develops adventitious shoots, from roots (L_3) or when it freezes to the ground, the plant regenerates itself from internal tissues to yield thorny shoots (DERMAN, 1947; DARROW, 1955). In addition, *Rubus* gametes also develop from internal (L_2) regions of the shoot and therefore produce thorny seedlings. DARROW (1937) reported only thorny offspring from selfed TL flowers. Similar observations have been made by the senior author in selfed and hybrid progenies involving TL. HULL & BRITTON (1958) reported a single thornless seedling from a TL × Eldorado (8 ×) cross.

MCPHEETERS & SKIRVIN (1983) have reported a tissue culture system where 'Thornless Evergreen' (a periclinal chimera similar to TL) blackberry was separated into its component parts. Among the regenerated plants a large percentage (46.3%) were of epidermal origin. These plants were believed to be pure thornless because they produced thornless rather than thorny adventitious shoots from isolated root segments.

To use the TL thornlessness (gene(s) for bramble improvement, chimeral TL must be separated into its component parts to yield a non-chimeral thornless form.

MATERIALS AND METHODS

Shoot tips of 'TL' ('L654') were gathered from a glasshouse grown plant at Lincoln, New Zealand, in November, 1981. These tips were disinfested with 70 per cent ethanol for 30 seconds; 0.32 per cent sodium hypochlorite for 20 minutes; and three washes in sterile water. The meristem (0.5 mm) was excised and transferred to ANDERSON's (1980) medium. The meristems developed calli which were sub-cultured regularly. In May, 1982, the calli were moved to a new basic medium which consisted of $\frac{1}{2}$ strength MS (1962) macro salts, full strength MS micro salts and iron; the vitamins of Gamborg's B5 medium (Gamborg, et al., 1968); sucrose (30 g/litre); and agar (0.6%). This medium was supplemented with kinetin (0.5 mg/litre) and NAA (0.1 mg/litre) and/or various levels of GA_3 (1, 5, 10, or 20 mg/litre) and BAP (0.05, 0.1, 0.5 or 2.05 mg/litre). The pH was adjusted to 5.5 prior to autoclaving. The media were dispensed into 100 ml erlenmeyer flasks (25 ml/flask) and sterilized by autoclaving at 121 °C for 15 min.

The cultures were maintained at 22 ± 3 °C under diffuse light, 16 hr day length, for the first four weeks, when light intensity was increased to 10000 lux. Callus growth and differentiation were monitored on a regular basis. Callus was subcultured onto identical media. Rooted adventitious shoots were moved directly to soil and placed under intermittent mist within a plastic-enclosed humidity chamber in a glasshouse. As the plants hardened, they were removed from the mist and grown to maturity.

To investigate the internal genotype of regenerates, root cuttings were obtained, placed in sand, and stratified for 2 months @ 4 ± 1 °C. They were then brought into a glasshouse for adventitious shoot evaluation.

The mature pot-grown plants were maintained in a screenhouse where they received natural chilling. In the spring of 1984, the plants were forced to flower in the glasshouse and selfed to investigate the possibility of the thornless character being expressed sexually. Seeds were harvested, extracted, and dried. They were scarified and stratified according to the procedures followed by the Scottish Crop Research Institute (personal communication, D. H. Jennings): seeds were scarified in concentrated sulphuric acid for 30 minutes, rinsed in running water, and immersed in 1% calcium hypochlorite

(w/v) which was dissolved in a saturated solution of calcium hydroxide for one week. At the end of that period, the seeds were washed in running water and immediately mixed with sand and sown into a 15 cm diameter pot filled with 10 cms of ground pumice and 2.5 cms of peat-sand. The pots were watered and placed in sealed plastic bags. The pots were moved to a screenhouse and covered by shade cloth to prevent heat accumulation. The seeds were stratified by natural winter chilling. In the early spring, the pots were transferred to a heated glasshouse for germination. Germinated

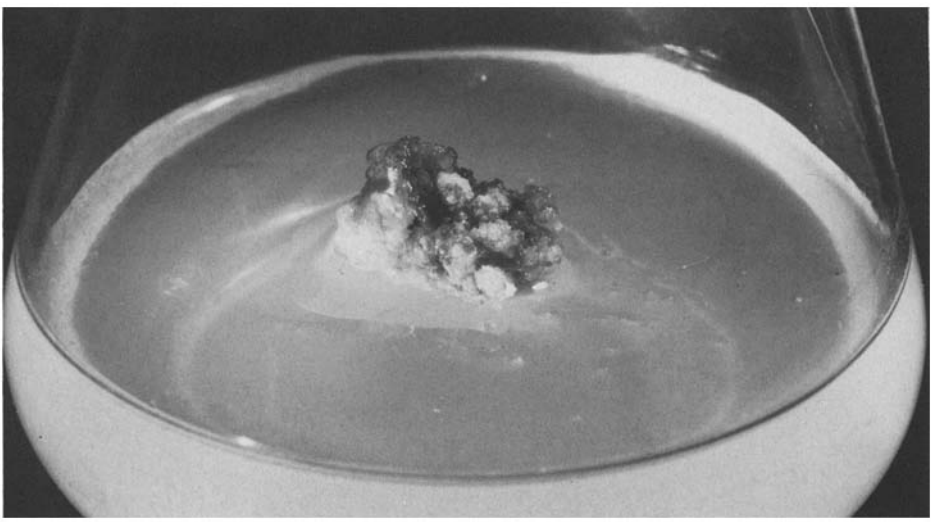

Fig. 1. Callus which has developed from a meristem of 'Thornless Loganberry).

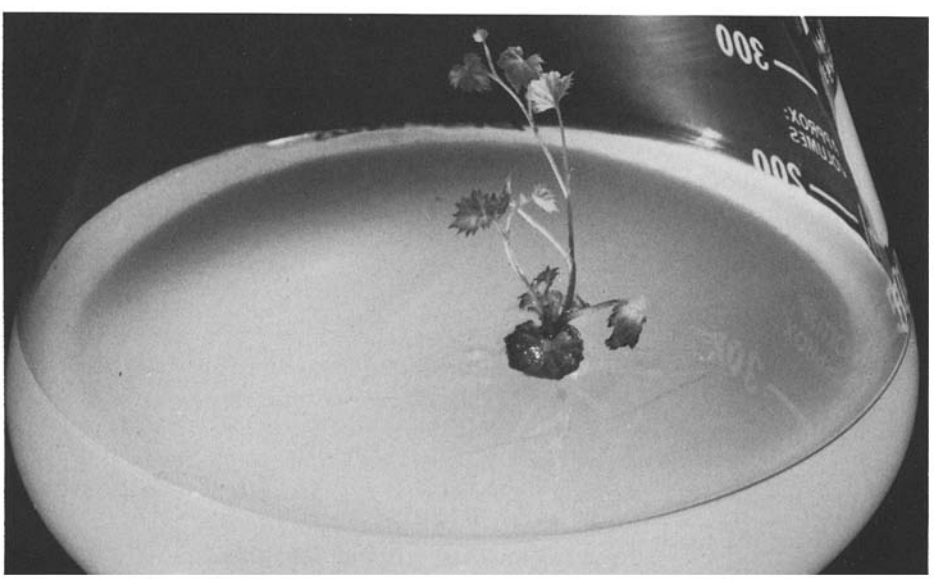

Fig. 2. A rooted adventitious shoot of 'Thornless Loganberry' which has developed from meristem-derived callus.

Fig. 3. A pure thornless (non-chimeral) 'Loganberry' that has flowered and been used for hybridisation.

seeds were pricked out and transferred to individual pots. When large enough, they were evaluated for the thorned or thornless condition.

RESULTS

Most media supported TL callus proliferation, but only the basic medium supplemented with GA_3 (5.0 mg/litre) and BAP (0.05 mg/litre), developed rooted shoots (Figure 1 and 2). A total of 3 shoots developed. Two of these died when transferred to soil. The remaining plant, which was totally thornless, survived, grew well, and eventually flowered (Fig. 3). This tissue culture-derived plant (TL_{tc}) was fully male and female fertile and indistinguishable from TL (Fig. 3). No adventitious shoots were obtained from the stratified root cuttings.

Three hundred selfed seed were treated for germination; 100 of these germinated and 100 grew enough to be evaluated. Among the seedlings both thorny and completely thornless offspring (63%) were found (Fig. 4).

Fig. 4. A thornless (right) and thorny (left) seedling derived by self pollinating a pure thornless (non-chimeral) 'Loganberry'.

DISCUSSION

'Loganberry' has been used extensively as a parent for hybridberry improvement because of its superior fruit qualities and general combining ability (personal communication, F. J. Lawrence, USDA, Corvallis, Oregon, USA; DARROW, 1973). Unfortunately, TL normally produces only spriny offspring. The one case of a thornless seedling from TL arose from a cross of TL × Eldorado (8×) (HULL, 1958). Since this plant produced thornless root suckers, Hull suggested that the seedling was an apomict. However, it is unlikely that this was the case because the thornless character of TL normally is epidermal, not internal, and sexual organs, including the nucellus, are of internal origin (DERMAN, 1947). The thornless apomict could only have arisen from a region of sectoral, not periclinal, chimeral arragement. However, sectoral chimeras could arise unobserved in TL, since both chimeral and pure epidermal tissues can be fully fruitful and morphologically indistinguishable. In contrast, the epidermal-derived shoots of the similar chimeral cultivar, 'Thornless Evergreen', are relatively sterile (MCPHEETERS, 1985) and morphologically distinct (MCPHEETERS & SKIRVIN, 1983). No references to Hull's plant being used for breeding have been published and it is presumed to have died.

When TL_{tc} was selfed it produced both thornless and thorny seedlings (Fig. 4). The relatively low germination percentage (36.7%) was not atypical for *Rubus* (KE et al., 1985). The percentage of thornless offspring was high enough (63%) to suggest dominant inheritance, however, the genetics of 'Loganberry' thornlessness require further investigation.

Thornlessness is of particular value for hand harvesting brambles; pickers harvest fruit faster and more thoroughly when they expect no damage from thorns. Thornlessness is also important for mechanical harvesting. While thorns do not damage machinery, the vigorous activity of mechanical harvesting can dislodge thorny plant parts

which contaminate harvested fruits and/or processed products (personal communications, various Oregon caneberry growers and processors). In addition, thorn-punctured fruits result from mechanical harvesting. Punctured drupelets, which are found on both harvested and unharvested fruits, bleach, leak juice, and decay rapidly. In the future, processors are expected to make increased demands for thornless polyploid hybridberry cultivars. 'Loganberry' thornlessness genes may help geneticists supply these thornless cultivars.

In conclusion, the isolation of a pure thornless 'Loganberry' (TL_{tc}) demonstrates the usefulness of tissue culture for the improvement of standard fruit cultivars. Because TL_{tc} is fruitful, it may have direct use as a new thornless 'Loganberry' cultivar in regions with severe winters. Since TL_{tc} passes its thornless character through the sexual cycle, the thornlessness of TL can now be used to produce thornless hybridberries.

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