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Separation of Thornless Rose Chimeras into Their (*Rosa* sp.) Consistent Genotypes *in vitro*

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Abstract: To isolate a pure thornless rose, a thornless sport of *Rosa multiflora* ('Fairmount 1') was established in tissue culture. To determine whether the sports were chimeral and consisted of both thorny and thornless tissue, the regenerants obtained in the tissue culture studies were assessed for the thorny or thornless condition both *in vitro* and *ex vitro*. Chimeral plants were expected to yield both thorny and thornless regenerants. Among these regenerants we expected to obtain a pure thornless rose. All tissue culture experiments with 'FM1' yielded both thorny and thornless regenerants, clearly demonstrating that 'FM1' is chimeral. TDZ significantly affected segregation both *in vitro* and *ex vitro*. The percentage of thorny plants was highest at 14.4 μM TDZ and lowest with control (0 μM TDZ). The linear relationship between increasing TDZ concentrations and percentage of thorny plants *ex vitro* was significant. These results show rose chimeras can be separated into their component genotypes by growing them on media with high concentrations of TDZ (3.6, 5.4 7.2 and 14.5 μM TDZ). Also among 240 plants from the TDZ experiments, 21 plants were classified as thornless after a seven months in the greenhouse.

Key words: Thornless, rose, TDZ, *in vitro*, chimera

INTRODUCTION

Roses (*Rosa* sp.) are one of the most important flower crops in the world (Short and Roberts, 1991). Roses grow as shrubs, bushes and climbing plants. Most have 'thorns'. The term "thorn" is commonly used to refer to the hard multicelled epidermal appendages found on stems and leaves of roses. Since they have no vascular tissue and develop epidermally they are technically known as "prickles" (Nobbs, 1984; Rosu *et al.,* 1995). Thorns make roses difficult to grow and handle; retailers commonly "dethorn" roses prior to sale. Furthermore, in the industrial production of oils, essence and other products for the pharmaceutical and cosmetic trade, thorns add to the problems of harvest. Therefore, a thornless rose would be preferred by many growers, merchandisers, retailers and by the public (Nobbs, 1984).

Researchers have used natural mutations, conventional breeding methods and chance seedlings to breed thornless roses. Spontaneous thornless mutants of rose have been described (Morey, 1969; Nobbs, 1984; Oliver, 1986; Druitt and Shoup, 1991 and Canli, 1997); unfortunately, the best thornless mutants are sterile and/or have reverted to the thorny condition following temperature shock, freezing, or other environmental extremes (Nobbs, 1984). This instability suggests that many thornless roses are chimeras and consist of mutant thornless tissue that grows together with normal thorny tissue (Rosu *et al.,* 1995). The instability could also be due to transposable elements. The genetic basis of thornlessness in roses remains largely unknown because most thornless roses are infertile making genetic investigations and thornless cultivar improvement difficult (Morey, 1969). A tissue culture method to distinguish pure thornless and chimeral plants, would enable us to distinguish between these possibilities.

There are a number of methods that have been used to either separate chimeras into pure forms or rearrange their histogenic layers. All of these methods basically involve the selection of desired types among a population of individuals. In some cases, the mutant may segregate spontaneously, while in other cases separation occurs following wounding or mutagenesis. Segregation of mutant types from chimeras can occur in several ways: a) the result of changes in the histogenic layer composition b) selective growth of cells from one histogenic layer to produce an individual of pure type; c) differentiation of a single cell to form an entire organized shoot or plantlet of pure type, or d) mutation (McPheeters and Skirvin, 1983). Induction of rapid multiplication in shoot tips and

adventitious shoot formation *in vitro* are the most common methods used to sperate chimeras into their consistent genotypes.

Hall *et al.* (1986) succeeded in obtaining a pure 'Thornless Loganberry' (*Rubus loganococcus*) from a periclinal parent type using meristem tips *in vitro*. McPheeters and Skirvin (1983; 1989) succeeded in obtaining pure thornless blackberries (a close relative of roses) from chimeral thornless plants using tissue culture. We believe that thornlessness in roses is analogous to thornlessness in blackberries and they too could be forced to yield pure thornless forms.

Rosu *et al.* (1995) modified the procedures used earlier by McPheeters and Skirvin (1983, 1989) for chimeral blackberries to obtain putative pure thornless *R. multiflora* thunb ex. J. Murr. rose. Rosu *et al.* (1995) studied the development of putative adventitious shoots from a chimeral thornless rose and then developed a regeneration system for *R. multiflora*. According to the authors, most of the parental plant's stems were thornless, but some lateral branches had recurved thorns. Since no thorns were observed on the stem of regenerants and three of the regenerants were classified as almost smooth, even on the petioles, these authors assumed they had succeeded in obtaining a pure thornless rose by separating the chimeral tissues. Because the chimeral status of the parental plant was unknown, the authors tried to force adventitious shoots (suckers) from the roots of the putative chimeral plant without success (Rosu *et al.*, 1995). The authors assumed that a chimeral thornless rose that possessed a thornless epidermis (LI) that overlayed a core of thorny cells (LII and LIII) should produce thorny, not thornless, root suckers.

If our thornless mutant plants are chimeras, tissue culture could allow us to separate the pure thornless genotype from the thorny tissue growing with it. In this way pure thornless forms of rose cultivars could be tested on their own merits. In addition, we assume that pure thornless roses will pass the thornless character through a sexual cycle allowing geneticists access to new genes for thornlessness. These can be used in traditional breeding cycles of crosses followed by selection or through genetic engineering efforts where the thornless gene could be introduced directly into outstanding thorny cultivars to yield thornless plants.

MATERIALS AND METHODS

Plant material: 'Fairmount 1' ('FM1'), a chimeral *R. multiflora* rose, was found by Dr. Philip Dziuk on his farm in Fairmount, Illinois. He found a thornless shoot growing from the base of a thorny plant. This clone grows very vigorously and erect. Most fruits of this plant contained empty seeds suggesting infertility. However, there were some normal seeds. Since 'FM1' arose as a sport on a thorny parent, we assumed that it is a chimera with a thornless epidermis that surrounds a core of thorny genotype cells (thornless, thorny, thorny).

Sterilization, establishment of cultures and culture room conditions: Shoots were harvested from actively growing thornless sections of 'FM1'. 'FM1 thorny' shoots were harvested as a control. These shoots were collected from parental plants growing on Dr. Philip Dziuk's farm in Fairmount, Illinois, in early spring and brought to the laboratory in plastic bags. Shoots were cut into four to five cm sections and all leaves were removed except those at the tips of the shoots. These were placed in bottles of detergent water and washed for ten minutes on a shaker (100 rpm). They were rinsed with tap water, dipped into 70% alcohol for two minutes, again rinsed with tap water. Then, the explants were surface sterilized with 15% bleach (0.787% sodium hypochlorite) for 15 minutes and rinsed with Sterile Distilled Water (SDW) for 10 minutes. Explants were again sterilized with 15% bleach for 20 minutes and rinsed with SDW for 15 minutes on a shaker.

Then shoots of cultivars were cut into 1.5 to 2 cm sections containing one or two buds and explanted on Skirvin and Chu (1979) modification of Murashige and Skoogs (MS, 1962) proliferation medium containing, BA (8.8 μM), NAA (0.54 μM), 30 sucrose and 7.5 g l^{-1} agar. The pH of the medium was adjusted to 5.4-5.6 with HCl or KOH prior to autoclaving. Then the medium was dispensed into 25 x 150 mm culture tubes (10 mls per tube) and autoclaved at 121 °C at 18 atm for 20 minutes.

Culture tubes were placed on racks in a culture room maintained at 24±1°C with a 16 h photoperiod supplied by cool-white fluorescent (131 μMm^{-2} s^{-1} as measured with a Li-cor, Inc.integrating quantum/ radiometer/ photometer, model LI-188B), at about 40% relative humidity.

Experimental media, explanting and evaluation of shoots for thornlessness: All media described in these experiments were Murashige and Skoog (MS, 1962) high mineral salts combined with Staba vitamins (Staba, 1969), myo-inositol (100 mg l^{-1}), Na_2EDTA (37.25 mg l^{-1}), $FeSO_4.7H_2O$ (27.85 mg l^{-1}), sucrose (30 g l^{-1}) and agar (7.5 g l^{-1}). The pH of the media were adjusted to 5.4-5.6 before autoclaving.

Shoots were harvested from 6-week-old tissue culture plants growing on SMS proliferation medium in jars under culture room conditions. Shoots were cut into 1 cm stem

segments with one node and put onto the experimental medium in jars and placed on shelves in a completely randomized experimental design in a culture room maintained at 20±2 °C under cool white fluorescent light with 16 h photoperiod. *In vitro* data were collected seven weeks later. Individual plants were then evaluated for the presence or absence of thorns (*in vitro* evaluation).

All plants were moved to a greenhouse where they were acclimated under a periodic mist. These plants were again evaluated for the presence or absence of thorns five to six weeks later (acclimatization evaluation). The thorny plants were discharged and some of the thornless plants were potted and moved to a greenhouse. They were evaluated again for the presence and absence of thorns after three to four months later (first greenhouse evaluation) and again seven to twelve months later (second greenhouse evaluation).

All data were analyzed using ANOVA. Means were subjected to LSD tests at the 5% level using the SAS.

Effects of TDZ on chimeral segregation of 'FM 1' rose: To study the effects of TDZ on segregation of the putative 'FM1' chimera, shoots were harvested from 6-week-old plants of growing on subculturing medium (described earlier). Shoots were cut into 1 cm stem segments with one node and placed on experimental medium supplemented with NAA (0.54 μM) and TDZ (0, 0.9,1.8, 3.6, 5.4, 7.2, 14.5 and 29 μM). Each jar contained five explants. Each concentration was replicated six times. Jars were placed on shelves in a completely randomized experimental design in the culture room. Data were collected as described earlier.

RESULTS

In *in vitro* results, TDZ significantly affected the percentage of thorny plants. The percentage of thorny plants was highest at 14.5 μM TDZ (100 %) and lowest at 0 μM TDZ (23%) concentrations (Table 1). TDZ significantly affected shoot length, shoot number, rooting percentage, root number, root length, callus formation and callus diameter (Table 1).

The largest number of shoots per explant was obtained at 1.8 μM TDZ (Table 1). The tallest shoots were observed at the lowest level of TDZ (0.9 μM) (Table 1). The percentage of rooted explants was highest for concentrations of TDZ between control (0 μM TDZ) and 5.4 μM TDZ. The longest roots were observed media with low concentrations of TDZ (0.9, 1.8, 3.6 μM) (Table 1). The average root number per explant was highest for control and decreased as the TDZ concentration increased (Table 1). All explants formed callus, however, the average callus diameter was higher in the presence of TDZ than for control (Table 1).

Table 1: Effects of different TDZ concentrations on stability and segregation of 'FM1' rose shoots *in vitro* (n=30)

TDZ (μM)	No. of shoots/explant	Shoot length (cm)	Thorny shoots %	rooted shoots %	Length of longest root (cm)	No. of primary roots	Callus diameter (cm)	Callus forming shoots[z] %
0.0	1.62abc	2.95bc	23.33c	100.0a	3.9bc	12.50a	0.15d	26c
0.9	2.04abc	7.60a	54.15bc	95.83a	7.82a	9.64b	0.81b	100a
1.8	2.80a	4.32b	48.58bc	82.77a	7.91a	5.47c	1.0ab	78b
3.6	2.06abc	4.09b	88.86ab	91.67a	6.7ab	4.85c	0.93b	100a
5.4	2.58ab	2.30cd	66.6abc	94.43a	3.0cd	3.3cd	1.10a	100a
7.2	1.65abc	1.20de	75.0abc	47.26b	1.4cd	1.7de	1.19a	100a
14.5	0.90c	0.6de	100.0a	23.33c	0.36d	0.56e	0.97ab	100a
29.0	1.45bc	0.20e	83.3ab	0.00c	0.00d	0.00e	0.53c	100a
Sign:	*	*	*	*	*	*	*	*

*, NS significant or not significant by ANOVA at 5%. Mean separation by LSD (Least Significant Difference); numbers within columns followed by different letters are significantly different.

[y] All data are presented as treatment means, [Z] Callus diameter was measured at the stem base

Table 2: Effects of different TDZ concentrations on stability and segregation of 'FM1' rose shoots

Treatment TDZ (μM)	Total no. of shoots	Thorny shoots No.	Thorny shoots %
0.0	17	4	23.5
0.9	14	8	57.1
1.8	13	3	23.0
3.6	15	8	53.3
5.4	12	9	75.0
"7.2 and above" [z]	9	8	88.0
Significance:	*, $r^2 = 0.74$		

NS,* Nonsignificant or significant at P< 0.05, respectively. Linear regression ($y = \beta_0 + \beta_1 x + \epsilon$).

[z] Since most of the shoots died at acclimatization in this particular experiment at 7.2, 14.5 and 29 μM TDZ concentrations, the surviving shoots from these treatments were combined and named as '7.2 μM and above'

Table 3: Reversion rate in thornless 'FM1' rose shoots classified as thornless at acclimatization stage of TDZ experiments

TDZ (μM)	n	Thorny shoot	Thornless shoots	Approximate location of first thorn[z]
0.0	-	-	-	-
0.9	4	0	4	-
1.8	7	0	7	-
3.6	8	1	7	tip section
5.4	3	0	3	-

[z] Approximate location of first thorn presented as base, middle and tip section of a shoot

In acclimatization results, TDZ significantly affected the percentage of thorny plants that survived to acclimization. There was a significant linear relationship between increasing TDZ concentrations and percentage of thorny plants. Higher ("7.2 and above") TDZ concentrations yielded more thorny shoots than control or lower concentrations of TDZ (Table 2).

In greenhouse evaluation, among 22 greenhouse plants, only one plant recurved thorns at the tips of its shoots (Table 3).

DISCUSSION

TDZ significantly affected segregation both *in vitro* (Table 1) and *ex vitro* (Table 2). The percentage of thorny plants was highest at 14.4 μM TDZ and lowest at 0 μM TDZ (Table 1). *Ex vitro* results also showed a significant linear relationship (r^2= 0.74) between increasing TDZ concentrations and percentage of thorny plants (Table 2).

Based on my data, scientists interested in forcing chimeral rose types to segregate pure thornless forms should use relatively high levels of TDZ (3.6 μM or higher). A portion of the thornless regenerants will be chimeral, another portion will be pure thornless. Unfortunately, it is difficult to distinguish these two plant types by tactile or visual means alone. However, when they flower, plants with a thornless LII should produce thornless seedlings in a segregating population.

Our results agrees with the results of McPheeters and Skirvin's (1989). McPheeters and Skirvin studied the periclinal structure of the 'Thornless Evergreen' ('TE') blackberry which is a close relative of *Rosa* sp. They reported that 'TE' (*Rubus Lacinatus* Willd.) is a periclinal chimera in which the epidermis has mutated to a thornless state (designated 't') that encloses internal genetically thorny portions (tTT). In shoot tip cultures, new shoots arise from axillary buds. Therefore it is expected that all propagules derived from these tips to be true-to-type. However, the thornless suckers of some tissue culture derived plants are thornless while others are thorny like the parent. The plants with thornless suckers were found to be no longer chimeral (ttt) and they probably arose adventitiously from epidermal tissue (McPheeters and Skirvin, 1989).

Hall *et al.* (1986) reported separating chimeral 'Thornless Loganberry', another periclinal chimeral blackberry, into its component parts by using meristem tips *in vitro.* This gene also passed as a dominant (S_{TL}).

McPheeters and Skirvin (1989) evaluated 900 plants from *in vitro* shoot tip cultures of 'TE'. They obtained thornless plants. The pure thornless ones produced thornless root suckers, the chimeral plants made thorny suckers. They assumed that the pure thornless types developed adventitiously from the epidermis (LI) while the chimeral types arosed from axillary buds and remained chimeral.

Rosu *et al.* (1995) modified the procedures used by McPheeters and Skirvin (1983, 1989) to obtain a putative pure thornless rose. (*R. multiflora* thunb ex. J. Murr.). According to the authors most of the parental plant's stems were thornless, but some lateral branches had recurved thorns. Since no thorns were observed on the stem of regenerants and three of the regenerants were classified as almost smooth even on the petioles, these authors assumed they had succeeded in obtaining a pure thornless rose by separating the chimeral tissues. The authors also stated that a chimeral thornless rose that possessed a thornless epidermis (LI) that overlayed a core of thorny cells (LII and LIII) should produce thorny, not thornless, root suckers. To test their hypothesis the authors tried to force adventitious shoots (suckers) from the roots of the putative chimeral plants without success (Rosu *et al.,* 1995).

The major hypothesis of this research was that 'FM1' was a chimera consisting of thorny and thornless tissues growing together. All my tissue culture experiments yielded both thorny and thornless regenerants suggesting that 'FM1' is chimeral.

Among 240 plants from TDZ experiments, 21 plants were classified as thornless after a seven months *ex vitro*.

Another hypothesis was that cytokinins induce chimeral segregation *in vitro*. A significant linear relationship was observed between TDZ and the percentage of thorny plants (Table 2). These results suggest that researchers interested in separating chimeras into their component genotypes should grow their plants in media with relatively high concentrations of cytokinins.

REFERENCES

Canli, F.A., 1997. Separation of Rose Chimeras Into Their (*Rosa* sp.) Consistent Genotypes *in vitro* Master thesis. University of Illinois at Urbana-Champaing, USA.

Druitt, L. and M. Shoup, 1991. Thornless Roses. Horticulture, 69: 78-82.

Hall, H.K., H. Quazi and R.M. Skirvin, 1986. Isolation of a pure thornless 'Loganberry' by meristem tip culture. Euphytica, 35: 1039-1044.

McPheeters, K.D. and R.M. Skirvin, 1983. Histogenic layer manipulation in chimeral 'Thornless Evergreen' trailing blackberry. Euphytica, 32: 351-360.

McPheeters, K.D. and R.M. Skirvin, 1989. Somaclonal variation among *ex vitro* 'Thornless Evergreen' trailing blackberries. Euphytica, 42: 155-162.

Morey, D., 1969. Selection criteria for breeding, 278-290. In: Roses. (Mastalerz, J.W. and Langhans, R.W. Pennsylvania flower growers. New York State flower grower association Inc.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Phsio. Plant., 15: 473-97.

Nobbs, K.J., 1984. Breeding thornless roses. American Rose Annual 1984. The American Rose Society, Shreveport, LA., pp: 37-43.

Oliver, W.G., 1986. A precis of thornless development. The rose. The Royal National Rose Society, 80. (3).

Rosu, A., R.M. Skirvin, A. Bein, M.A. Norton and M. Kushad, 1995. The development of putative adventitious shoots from a chimeral thornless rose (*Rosa multiflora Thunb*. ex J. Murr.) *in vitro*. J. Hort. Sci., 70: 901-907.

Short, K.C. and A.V. Roberts, 1991. Rosa spp. (roses): *In vitro* culture, Micropropagation and production of secondary products: 376-97. In: Bajaj, Y.P. S., (Eds.). Biotechnology in Agriculture and Forestry, Vol. 15, Medical and Aromatic Plants. Springer-Vevlag, Berlin.

Skirvin, R.M. and M.C. Chu, 1979. *In vitro* culture of ‘Forever yours’ rose. Hort. Sci., 14: 608-610.

Skirvin, R.M., M.C. Chu and H.J. Young, 1990. Rose: 716-743. In: Ammirato, P.V., D.A. Evans, W.R. Sharp and Y.P.S. Bajaj (Eds.). Handbook of plant cell culture. Volume 5. Ornamental Species. McGraw Hill Publishing Co., New York.

Skirvin, R.M., M.C. Chu and H.J. Young, 1990. Rose: 716-743. In: Ammirato, P.V.,D.A. Evans, W.R. Sharp and Y.P.S. Bajaj (Eds.). Handbook of plant cell culture. Volume 5. Ornamental Species. McGraw Hill Publishing Co., New York.

Skirvin, R.M., K.D. McPheeters and M. Norton, 1994. Source and frequency of somaclonal variation. Hort. Sci., 29: 1232-1236.

Staba, J.E., 1969. Plant tissue culture as a technique for the phytochemist. Recent Adv. in Phytochem., 2: 80.