In Vitro Propagation of Modern Roses

Hao-Ching Wang and Nancy A. Reichert
Department of Horticulture, Mississippi State University, Mississippi State, Mississippi 39762

Modern roses (*Rosa* spp.) comprise the major share of rose propagation for cut-flower sales and landscape use (Wolf, 1983). With a steady to increasing market demand (Federal-State News, 1989), more efficient propagation strategies would be economically advantageous. Modern roses are primarily propagated by T-budding, partly due to difficulties encountered in attempting to root cuttings. T-budding propagation takes approximately 16 months (Davies, 1980; Khosh-Khui,1982). In vitro propagation of modern roses may be a viable alternative for commercial rose production in the future (Queralt, 1991a; Shirvin, 1990). Goals could include, disease elimination and cultivar improvement as well as faster production.

In vitro regeneration of modern roses from explants other than preformed meristematic buds has been difficult. Successful shoot multiplication in vitro has been achieved on a few modern rose cultivars using shoot tips and axillary buds (Bressan, 1982; Douglas, 1989; Hasegawa, 1980). However, in vitro rooting of shoots was a common problem (Alderson, 1988; Bressan, 1982; Douglas, 1989). To make in vitro propagation of modern roses applicable to commercial propagation, reliable rooting methods must be developed.

Two main objectives were outlined that are adaptable for use on a number of cultivars. In order to achieve this we needed to: 1) define a medium or media that will induce rhizogenesis on internodal stem explants of modern roses and, 2) adapt the rooting protocol for shoots derived from axillary buds on six modern rose cultivars.

MATERIALS AND METHODS

Internodal-Stem Explant Preparation. Young vegetative shoots (10 to 15 cm in length) of ' Mister Lincoln' roses were collected from the Disease Research Rose Garden at Mississippi State University. Shoots (minus leaves) were disinfested by immersion in 20% Clorox plus 0.5% sodium dodecyl sulfate (SDS) for 15 min, then rinsed four times with sterile distilled water. Stems were cut into internodal sections, 7 mm long, and split longitudinally. Ten internodal sections were placed cut surface down onto each of 16 different media containing four concentrations each of NAA and TDZ (Table 1). The basal medium was of Murashige and Skoog basal salts (Murashige, 1962); vitamins (per liter: 100 mg myo-inositol, 1.0 mg thiamine-HCl, 0.5 mg nicotinic acid, 0.5 mg pyridoxine-HC1); 30 g/l sucrose; and 8 g/l phytagar (Gibco). The pH was adjusted to 5.8, then steam sterilized. Filter-sterilized plant growth regulators were added to media after steam sterilization. All tissue cultures were placed in growth chambers with a 16-h photoperiod, 4.1 Klux with cool white fluorescent bulbs, and 25/21°C day/night temperatures.

Nodal-Stem Explant Preparation. Nodal stem explants of 'Mister Lincoln' roses were obtained under the conditions stated above, except for the surface disinfestation time (25 min) and explant length (7 to 10 mm). Five explants were

placed per plate. All nodal sections were initially placed onto MS medium containing 0.15 mg/l NAA and 3.0 mg/l BAP (6-benzylaminopurine) to stimulate axillary bud break and shoot multiplication. As shoots reached 10 mm in height (approximately 3 weeks), they were excised from the node and transferred (5 shoots per plate) onto each of the 6 rooting media determined the best from the internodal study (Table 2).

Table 1. Effects of NAA and TDZ on in vitro rhizogenesis of'Mister Lincoln' stem explants[1]

NAA (mg/l)	TDZ (mg/l)	Mean no. roots per plate	Mean no. stem rooted per plate
0.0	0.005	0.0 a[2]	0.0 a
	0.01	0.0 a	0.0 a
	0.02	0.0 a	0.0 a
	0.04	0.0 a	0.0 a
0.2	0.005	2.5 a	1.5 a
	0.01	0.0 a	0.0 a
	0.02	0.5 a	0.5 a
	0.04	0.0 a	0.0 a
0.4	0.005	13.0 d	6.5 d
	0.01	11.0 d	5.5 d
	0.02	4.0 b	2.5 a
	0.04	0.5 a	0.5 a
0.8	0.005	10.0 bc	6.5 d
	0.01	13.5 d	7.0 d
	0.02	10.0 bc	6.0 cd
	0.04	7.0 b	4.0 c
NAA × TDZ		**	**

[1] Ten stem explants per plate with two replications.
[2] Means within column not followed by the common letter differ at $P < 0.05$.
** = interaction significant at $p < 0.001$.

Table 2. Root initiation on 'Mister Lincoln' shoots in vitro: comparison of six media[1]

NAA (mg/l)	TDZ (mg/l)	Mean no. roots per plate	Mean no. shoots rooted per plate
0.4	0.005	11.5 ab[2]	4.0 ab
0.4	0.01	6.5 bc	3.5 b
0.8	0.005	14.5 a	5.0 a
0.8	0.01	13.0 a	4.5 ab
0.8	0.02	6.5 bc	4.0 ab
0.8	0.04	4.5 c	4.5 ab

[1] Five shoots per plate with two replications.
[2] Means within column not followed by the common letter differ at $P < 0.05$.

Three media of the 6 tested induced the best rooting response on 'Mister Lincoln' shoots. Shoots from 5 other cultivars ('Canadian White Star', 'Double Delight', 'Lady X', 'Queen Elizabeth', and 'Tiffany') were placed on the 3 media for determination of overall rooting ability. All cultivars were hybrid teas except 'Queen Elizabeth' (grandiflora).

The experimental design was completely randomized. Responses of tissues were observed at weekly intervals. Data were analyzed using the SAS (SAS, 1991) program in ANOVA or general linear model (GLM).

RESULTS AND DISCUSSION

Rhizogenesis on Internodal-Stem Explants. Approximately 14 to 21 days after initial culture, adventitious rhizogenesis was observed on 'Mister Lincoln' internodal-stem sections in 10 of 16 treatments (Table 1). Greater numbers of roots were obtained on media with reduced TDZ but higher NAA supplements. Rooting percentages greater than 59% were obtained on media containing 0.4/0.01, 0.8/0.005, 0.8/0.01, and 0.8/0.02 mg/l NAA/TDZ. The highest numbers of roots per plate (13.0 and 13.5) occurred on 2 media, NAA/TDZ at 0.4/0.005 and 0.8/0.01 mg/l. The highest concentration of TDZ (0.04 mg/l) seemed to suppress the ability of stems to produce roots.

Rhizogenesis on Shoots Derived From Nodal Explants. Differences were observed among 6 media regarding overall rooting ability of 'Mister Lincoln' shoots (Table 2). Greater numbers of roots and higher percentages of shoots that rooted were obtained on 3 media (0.4/0.005, 0.8/0.005, and 0.8/0.01 mg/l NAA/TDZ). Results were consistent with those obtained on internodal-stem explants. As with internodal sections on the media, the least response was obtained on media containing 0.8 mg/l NAA and 0.04 mg/l TDZ.

Table 3. Root initiation on modern roses in vitro: cultivar and media effects[1].

	NAA/TDZ (mg/1)					
	0.4/0.005		0.8/0.005		0.8/0.01	
Cultivar	NR[X]	SR[Y]	NR	SR	NR	SR
Mister Lincoln	10.8 Aa	4.0 Aa	12.5 Aa	4.3 Aa	9.3 Aa	3.8 Aa
Tiffany	3.0 Abc	2.0 Aab	5.5 Ab	4.0 Aab	8.5 Aab	4.0 Aa
Lady X	4.0 Abc	3.0 Aab	2.5 Ab	2.0 Ab	2.5 Abc	2.0 Aab
Double Delight	5.5 Ab	3.5 Aab	5.0 Ab	3.0 Ab	6.0 Aabc	4.0 Aa
Queen Elizabeth	4.0 Abc	4.0 Ab	6.5 Aabc	3.0 Aab	6.5 Aabc	4.0 Aa
CanadianWhite Star	2.0 Ac	1.5 Ab	3.5 Ab	2.5 Aab	1.5 Ac	1.0 Ab

[1] Five shoots per plate with three replications
A, a = Means within rows (NR or SR) and columns, respectively those not followed by a common letter differ at $p < 0.05$.
NR^X = mean number of roots
SR^y = mean number of shoots rooted

Adventitious roots formed on shoots of all 6 cultivars tested (Table 3). Within each rose cultivar, rooting responses on the 3 media were the same. However, the number of roots (NR) and number of shoots rooted (SR) varied markedly from one cultivar to another. 'Mister Lincoln' performed the best overall with a high shoots rooted value and the greatest number of roots produced. 'Tiffany', 'Double Delight', and 'Queen Elizabeth' all displayed an average of 4 (out of 5 total) shoots rooted (80%) on media containing 0.8 mg/l NAA and 0.01 mg/l TDZ (Table 3). 'Canadian White Star' and 'Lady X' responded poorly. This was expected since various explants from these two cultivars customarily respond poorly to all culture conditions tested (data not shown).

SUMMARY

Roots were obtained on all six cultivars tested, which included five hybrid teas and one grandiflora. Based on our results, rose internodal-stem responses to rooting treatments were reliable indicators of shoot responses to those same treatments. Split internodal explants generate twice the number of explants initially as buds, and bud explants take up to 3 weeks to develop before the shoots can be tested. Therefore, obtaining similar treatment responses from the 2 different explants is advantageous.

This is the first report regarding TDZ effects on roses in vitro. TDZ has been used as a substitute for adenine-based cytokinins in many woody plant cultures (Fiola, 1990; Mok, 1987). In bioassays, TDZ behaved like a cytokinin (Mok, 1987) and has been estimated to be 10,000 times more active than other widely-used cytokinins (Pierik, 1987). However, instead of obtaining a shoot-forming response, rooting of rose shoots occurred at lower concentrations of TDZ when NAA was present.

NAA in combination with other cytokinins did not induce root production on stem or leaf explants (data not shown). The ability to root rose shoots and stem explants in vitro, using the same medium should make it possible to maintain stock plants under aseptic conditions without continually culturing explants for shoot development and multiplication. Also through tissue culture, as much as 24 months production time might be saved (Queralt, 1991b). Unresolved, yet very important, questions relate to plantlet viability after transfer to soil, and acceptable growth with own-root systems.

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