GENETIC VARIATION IN SOMATIC EMBRYOGENESIS

OF *ROSA HYBRIDA* L.

A Thesis

by

ANNA MILDRED BURRELL

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2003

Major Subject: Horticulture

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Approved as to style and content by:

David H. Byrne (Chair of Committee)	R. Daniel Lineberger (Member)
Keerti Rathore (Member)	Tim Davis (Head of Department)

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ABSTRACT

Genetic Variation in Somatic Embryogenesis

of *Rosa hybrida* L . (December 2003)

Anna Mildred Burrell, B.A., Duke University

Chair of Advisory Committee: Dr. David H. Byrne

An in vitro technique was adapted for screening the ability of *Rosa hybrida* L. genotypes to form embryogenic callus to elucidate the inheritance of this ability. Filament and leaf petiole explants of modern rose cultivars 'Tournament of Roses' and 'Baby Love' were cultured on somatic embryogenesis induction media and evaluated for the ability to produce embryogenic callus. Cultures of 'Tournament of Roses' produced somatic embryos at a much higher frequency versus 'Baby Love' that produced no embryos. Subsequently, filament explants of eleven 'Tournament of Roses' x 'Baby Love' progeny genotypes were cultured on somatic embryogenesis induction media and evaluated for the ability to undergo somatic embryogenesis. The progeny genotypes produced somatic embryos at varied frequencies. The results obtained indicated that the ability to undergo embryogenesis in *Rosa hybrida* L. is heritable in an additive fashion with the involvement of more than one gene.

DEDICATION

To my parents, Mary and Kenneth Burrell,
who always encourage me to pursue all my dreams
and believe without a doubt that I will fulfill them.

ACKNOWLEDGMENTS

I could have never completed this project without the help of so many generous people. Words can never thank them enough. To my mentor and friend, David Byrne, thank you for teaching me to conduct research and find meaning even in what appears to be failure. To Dan Lineberger, thank you for all the assistance in recording images of my cultures and especially for your enthusiasm to do so. To Keerti Rathore, thank you for your cooperation in this project and taking the time to be part of this project. To Roberta Smith, thank you for recognizing what kind of study needed to be conducted.

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INTRODUCTION AND LITERATURE REVIEW

Modern Rose Breeding

In an era when genetic transformation of plant material occurs routinely via the insertion of foreign molecular constructs, such techniques can be applied to *Rosa hybrida* L., one of the most important nursery and florist crops in the world. Among the thousands of rose genotypes, potential for great improvement exists in disease resistance, vase life, color range, fragrance and form. The modern commercial rose cultivars, most of which are complex tetraploid and triploid hybrids, are derived from eight to ten wild diploid and tetraploid species. These modern roses are typically highly heterozygous, vegetatively-propagated cultivars with the desired combinations of growth, production, color, form, fragrance and post harvest characteristics. Due to the risk of inbreeding depression, commercial breeders of roses tend to release F_1 hybrids as new varieties. For example, one of the most widely sold cultivars throughout the world, 'Mister Lincoln', is a 'Chrysler Imperial' x 'Charles Mallerin' seedling. In other cases, new varieties are selected from a single back cross or F_2 progeny. However, despite the tremendous effort expended on rose cultivar development, all the existing cultivars have at least one improvement that could be made by altering just one trait.

Disease and insect infestations in commercial roses are generally controlled with pesticides at a substantial expense to the grower. Approximately $3.6 million was spent

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This thesis follows the style and format of Plant Cell, Tissue and Organ Culture.

in the United States in 1987 to control powdery mildew on cut rose crops alone (U.S. Department of Commerce, 1989). Commercial growers of roses apply fungicidal sprays 20 times during an average growing season to control powdery mildew on rose. This number increases for greenhouse-grown roses (Guillino and Garibaldi, 1995). In East Texas, approximately 30% of the production costs of field grown plants for the landscape trade is consumed in plant protection costs (verbal communication by Dr. Brent Pemberton, Texas Agricultural Experimental Station, Overton, Texas). Thus, disease resistance has been one of the primary breeding objectives in the Basye Rose Breeding and Genetics Program at Texas A&M University. Looking to many of the often diploid, wild species for sources of disease resistance to the blackspot fungus (*Diplocarpon rosae* [Lib.] Wolf) and powdery mildew *Sphaerotheca pannosa* (Wallr. [ex Fr.] Lev), this program has faced hurdles of incompatible ploidy levels in progeny parents in addition to multiple generations of backcrosses to impart the disease resistance of the wild rose into a showy modern tetraploid rose. This process is further hampered by the poor germination of rose seed (Kumar, et al. 1985). The use of biotechnology to insert single genes into sporophytic tissue that is not subject to undergo any genomic alterations due to crossing over during meiosis promises advances in a significantly reduced time.

The preferred tissue for transformation is embryogenic callus at the single cell stage, from which a clonal somatic embryo will develop. Chimeras are often the final result in transformation systems utilizing multicellular shoot apical meristems.

However, if transformation is accomplished at the single cell stage, the risk of a resulting chimeric plant is decreased (Marchant, et al. 1996).

The overall objective of this research project was to optimize the regneration protocol for roses to facilitate studies in transformation of rose via *Agrobacterium*-mediated gene insertion. The first step was to screen a set of diverse genotypes for their capacity to produce embryogenic callus. Using petiole tissue and filament tissue, 15 diverse genotypes utilized in the Basye Rose Breeding and Genetics Program were screened on two modified somatic embryogenesis protocols previously suggested for rose (Hsia and Korban, 1996 and Noriega and Sondahl, 1991).

Focus was turned to 'Tournament of Roses', a medium pink, moderately blackspot susceptible modern Hybrid Tea cultivar, because the genotype produced the greatest amount of embryogenic callus regardless of basal salts, carbohydrate source or explant type. On the other hand, 'Baby Love', a lemon yellow, moderately blackspot resistant miniature shrub rose, consistently produced no embryogenic callus on any medium. The stark difference in the two genotypes' responses led to interest in testing 11 of their progeny rather than trying to optimize somatic embryo production in only a few cultivars, following the example of previous reports. The goal of this evaluation was to elucidate the genetic component that controls somatic embryogenesis.

Somatic Embryogenesis in Rose

Somatic embryogenesis in rose has been obtained. However, the numerous reports consistently are plagued with inefficient embryogenic callus production and difficulty in regeneration for a minute sampling of genotypes. Reports have been made for approximately 25 of the approximately 20,000 genotypes that exist. An early attempt in the literature reported success using in vitro-derived leaf tissue of *Rosa hybrida* cvs. 'Domingo' and 'Vicky Brown' (De Wit, et al. 1990). In this study, both somatic embryogenesis and regeneration of plantlets were observed. The following year, leaf tissue was used in *Rosa hybrida* L. cv. 'Landora' to produce somatic embryos, which reportedly did not undergo regeneration due to germination failure (Rout, et al. 1991). Noriega and Sondahl (1991) reported somatic embryogenesis and regeneration of *Rosa hybrida* L. cv. 'Royalty' using filaments as explants. In the following years to the present, further studies have been published reporting somatic embryogenesis from a variety of genotypes (primarily cultivated modern roses) and explant sources, including seed calli, petals, nodes, shoot apices, petioles, in vitro-derived leaf tissue and even mature leaf tissue (Kunitake, et al. 1993, Arene, et al. 1993, Matthews, et al. 1994, Marchant, et al. 1996, Hsia and Korban, 1996, Kintzios, et al. 1999, Uzunova, 2000, Sarasan, et al. 2001, Castillon and Kamo, 2002, and Li, et al. 2002.) Despite these successes an efficient system to generate the most ideal target for transformation, embryogenic callus, remains elusive. Some studies reflect wide scale screenings of genotypes, some of which can be induced into embryogenesis and many others in which embryogenesis appears impossible (DeWit, et al. 1990, Rout, et al. 1991, Hsia and

Korban, 1996). Numerous researchers have remarked on the genotypic differences in embryogenic capacity that appear unrelated to concentration of medium components or incubation conditions (Hsia and Korban, 1996, Marchant, et al. 1996, Kintzios, et al. 1999, Sarasan, et al. 2001, Castillon and Kamo, 2002, and Li, et al. 2002).

Genetic Studies of Somatic Embryogenesis

Genetic studies of embryogenic capability exist among many different crops, primarily agronomic, but have not been reported in rose. Previous studies of the genetic basis of embryogenic capability among agronomic crops have been motivated by the desire for synthetic seed production in significant numbers (Seabrook, et al. 2001). As a rule, these studies have been designed utilizing one genotype known for adequate regeneration from callus crossed with a genotype known for its inability to reproduce itself through callus. In many cases, observations of F_1, F_2 and F_1BC_1 are provided leading to inferences regarding inheritance. Studies have been conducted in the following crops: alfalfa (Hernandez-Fernandez and Christie, 1989), barley (Komatsuda, et al. 1989), rice (Takeuchi, et al. 1997), grape (Mozsar and Viczian, 1996), red clover (McLean and Nowak, 1998), corn (Bronsema, et al. 1997), cotton (Kumar, et al. 1998), soybean (DiMauro, et al. 2001) and potato (Seabrook, et al. 2001). It has been suggested that regenerative ability via somatic embryogenesis is under the control of two complementary genes that have additive effects in alfalfa (Hernandez-Fernandez and Christie, 1989), barley (Komatsuda, et al. 1989), and soybean (DiMauro, et al. 2001). Some have suggested complete dominance in rice (Takeuchi, et al. 1997) while others claim regenerative ability through somatic embryogenesis is not a dominant character in

cotton (Kumar, et al. 1998). In many cases, however, no system of inheritance has been suggested. It should be noted that inheritance may be different in different crops. Additionally, screening and scoring protocols for these previous studies have been conducted with a variety of methodologies. Rose is a woody dicotyledonous perennial whereas many of the existing studies have used herbaceous annual crops.

This research had two objectives:

1. To screen petiole and filament tissue from 15 diverse genotypes utilized in the Basye Rose Breeding and Genetics Program under two modified somatic embryogenesis protocols (Hsia and Korban, 1996 and Noriega and Sondahl, 1991). This screen was conducted in order to determine which genotypes would produce embryogenic callus that could be utilized for efficient transformation studies.
2. To examine the ability of 'Tournament of Roses' (high regenerative ability), 'Baby Love' (poor regenerative ability), and 11 of their progeny to assess the genetic component of the genotypic ability to be regenerated through somatic embryogenesis.

MATERIALS AND METHODS

Somatic Embryogenesis in 15 Diverse Tetraploid *Rosa hybrida* L. Genotypes

Genotypes Utilized

The *Rosa hybrida* L. genotypes screened for their ability to undergo somatic embryogenesis included 13 cultivars (‘Tournament of Roses’, ‘Baby Love’, ‘Graham Thomas’, ‘Prominent’, ‘Crimson Glory’, ‘Iceberg’, ‘Red Meidiland’, ‘Ingrid Bergman’, ‘Carefree Beauty’, ‘All That Jazz’, ‘Perfume Delight’, ‘Dortmund’, ‘Sunflare’) and two seedling progeny from the Basye Rose Breeding and Genetics Program, SF83-2 and 90-202. These genotypes were chosen due to their usage as parents in the Basye Rose Breeding and Genetics Program. Research emphasis is placed on parental genotypes proven to exhibit superior resistance to the fungal pathogens that cause blackspot and powdery mildew diseases. All genotypes employed in this study were tetraploid and illustrate diversity in growth habit, bloom color and parentage (Table 1).

Table 1. Characterization of *Rosa hybrida* L. genotypes screened for their ability to produce somatic embryogenic callus.

Genotype	Classification	Color	Parentage
All That Jazz	Shrub	Orange Pink	Gitte' x unreported seedling
Baby Love	Miniature	Dark Yellow	Sweet Magic x miniature seedling
Carefree Beauty	Shrub	Medium Pink	Seedling x Prairie Princess
Crimson Glory	Hybrid Tea	Dark Red	Catherine Kordes seedling x W.E. Chaplin
Dortmund	Kordesii	Medium Red	Seedling x *R. kordesii*
Graham Thomas	Shrub	Dark Yellow	Seedling x (Charles Austin x Iceberg seedling)
Iceberg	Floribunda	White	Robin Hood x Virgo
Ingrid Bergman	Hybrid Tea	Dark Red	Unreported seedling x unreported seedling
Perfume Delight	Hybrid Tea	Medium Pink	Peace x ((Happiness x Chrysler Imperial) x El Capitan)
Prominent	Grandiflora	Orange	Colour Wonder x Zorina
Red Meidiland	Shrub	Red Blend	Seafoam x (Picasso x Eyepaint)
Sunflare	Floribunda	Yellow	Sunsprite x seedling
Tournament of Roses	Grandiflora	Medium Pink	Impatient x seedling
SF 83-2	Shrub	Pink Blend	Sunflare x (*R. rugosa* x *R. wichuriana* tetraploid)
90-202	Shrub	Light pink	Basye seedling 74-193 x Basye seedling 65-626

Maintenance of Plants

With the exception of ‘Baby Love’, SF83-2 and 90-202 which were own-rooted plants, all the tissue for explantation was obtained from two year old, t-budded rose plants. All study plants were field-grown in 5-gallon nursery containers and therefore subjected to the fluctuating temperature and humidity conditions of the field. Annual weather conditions in Brazos County, Texas, the site of this experiment, are characterized by 39.1 inches annual rainfall, an average minimum temperature of 39 degrees F (January), and an average July maximum temperature of 94 degrees F (Texas Cooperative Extension, 2003). Regular irrigation with RO water was supplied. These

specimens were fertilized twice monthly with Peters 20-20-20 water-soluble fertilizer and not treated with any pesticides.

Culture Methods

Leaf petioles and flower buds were collected one day prior to anthesis from mature field grown plants and surface-sterilized with a 20% sodium hypochlorite solution, containing 3 drops L^{-1} Tween-80 for 20 minutes. Following this treatment, explants were rinsed with autoclaved deionized, distilled water 3 times. In the laminar air flow hood, filaments were excised from the flower buds. Leaf petioles were sliced into 1 mm segments. Each explant type from the fifteen genotypes was subjected to ten replications in each of four media, distinguished as: MS plus glucose (20 g l^{-1}), MS plus sucrose (30 g l^{-1}), B5 plus glucose (20 g l^{-1}), B5 plus sucrose (30 g l^{-1}). The B5 medium (Noriega, 1991) consisted of B5 salts (Gamborg, 1968), ammonium sulfate (329 mg l^{-1}), thiamine HCl (5.0 mg l^{-1}), myo-inositol (100 mg l^{-1}), pyridoxine (1.5 mg l^{-1}), nicotinic acid (1.5 mg l^{-1}), glycine (2.0 mg l^{-1}), 2,4-D (2, 4-dichlorophenoxyacetic acid, 2.0 mg l^{-1}), zeatin (1.5 mg l^{-1}), MS vitamins (Murashige, 1962), carbohydrate treatment and gel-rite (2.4 g l^{-1}). The pH of the each treatment was adjusted to 5.6. The MS medium consisted of one-half MS salts (Murashige, 1962), 2, 4-D (2.0 mg l^{-1}), zeatin (1.5 mg l^{-1}), MS vitamins (Murashige, 1962), carbohydrate treatment and Scott Lab Gel-rite (2.4 g l^{-1}). All media treatments were autoclaved for 20 minutes at 121 °C 15 psi then poured into 100 x 15 mm Petri dishes at a volume of 20 ml per dish. The Petri dishes containing the cultures of 10 filaments per plate were wrapped with parafilm and incubated in the

dark for five weeks at ambient temperatures (24 ± 3 °C). The progress of the callus cultures was observed weekly under the stereoscope.

All experimental units were subcultured after five weeks and incubated for an additional eight weeks. At thirteen weeks, experimental units were observed for embryogenic regions. Results were recorded through photographic images.

Data Analysis

The following scale was employed to quantify the genotypic response to the culture media. The percentage describes the proportion of embryogenic callus formed with respect to the total amount of callus produced by the explant. Visual observations were conducted via a stereoscope.

1 – Crystalline callus only
2 – Less than 10% of experimental unit embryogenic
3 – 10 to 19% of experimental unit embryogenic
4 – 20 to 29% of experimental unit embryogenic
5 – More than 30% of experimental unit embryogenic

The data were subjected to the Shapiro-Wilcoxon test for normality then Friedman's non-parametric analysis of variance and Tukey's Studentized Range Test, using SAS computer software (SAS Institute, 1989).

Genetic Study in Somatic Embryogenesis of 11 *Rosa hybrida* L. 'Tournament of Roses' x 'Baby Love' Progeny Genotypes

Genotypes Utilized

Eleven progeny genotypes of a 1998 cross of 'Tournament of Roses' x 'Baby Love' in addition to the parental genotypes were used. The genotypes have been designated with and will be subsequently referred to by the following names: 98-00391, 98-00392, 98-00394, 98-00395, 98-00396, 98-00397, 98-00399-01, 98-00399-02, 98-00399-03, 98-00399-04, and 98-00399-05. The genotypes vary widely in growth habit, petal color, fragrance, bloom shape, bloom size and resistance to the blackspot fungus (Table 2). All progeny plants used for this study were rooted from original seedlings (graciously provided by Dr. Keith Zary of Jackson and Perkins in Somis, California).

Table 2. Characterization of genotypes evaluated for their ability to undergo somatic embryogenesis

Genotype	Color	Petal #	Bloom Diam.	Growth Form	BS Res.
Baby Love	Medium yellow	5	3 cm	Shrub	3
98-00391	Light yellow	5	3 cm	Shrub	5
98-00392	Medium yellow	5	5 cm	Shrub	5
98-00394	Medium yellow	5	4 cm	Shrub	5
98-00395	Medium yellow	10	4 cm	Shrub	6
98-00396	Light yellow	10	5 cm	Hybrid tea form	5
98-00397	Apricot	5	6 cm	Upright shrub	7
98-00399-01	Light apricot	5	5 cm	Shrub	5
98-00399-02	Light pink	35	5 cm	Hybrid tea form	1
98-00399-03	Yellow/Apricot Blend	15	4 cm	Upright shrub	8
98-00399-04	Pink/Apricot Blend	10	5 cm	Upright shrub	7
98-00399-05	Light yellow	10	5 cm	Upright shrub	8
Tournament of Roses	Light pink	35	6 cm	Hybrid tea form	4

*BS Res. = Blackspot Disease Resistance.
Scale of 0 to 9, 0 least lesions, 9 most lesions.

Maintenance of Plants

The tissue for explantation was maintained in a greenhouse. The plants were irrigated with RO water and fertilized with water-soluble fertilizer (Peter's 20-20-20) bimonthly and supplemented with a slow release fertilizer (Osmocote). Pesticides were applied as needed to prevent infestation of thrips, aphids and red spider mites. The temperature of the greenhouse fluctuated between 18 and 29 degrees C. In the months of July and August, the temperature was observed to exceed 29 degrees C due to equipment failure. The explants were obtained in the month of June.

Culture Methods

Callus Induction Phase

Sporophytic filament tissue was chosen as the explant material due to the sterile nature of the flower bud interior. The sterile environment inside the unopened flower bud greatly reduced the risk of contamination, thereby substantially decreasing the risk of missing data points during data analysis. All flower buds used for this study were harvested one day prior to anthesis so that the filaments were fully expanded and the flower bud was not yet exposed to insect or microbe pests. Flower buds were removed from plants in the greenhouse then surface-sterilized in a 20% sodium hypochlorite solution including 0.1% Tween-20 for 20 minutes then rinsed with autoclaved deionized, distilled water. In the laminar air flow hood, filaments were removed from the floral bud. Anthers were excised and discarded. Ten filaments were placed in a Fisher 100 x 15 mm Petri dish containing 20 ml callus induction medium, consisting of B5 salts

(Gamborg, 1968), ammonium sulfate (329 mg l^{-1}), thiamine HCl (5.0 mg l^{-1}), myo-inositol (100 mg l^{-1}), pyridoxine (1.5 mg l^{-1}), nicotinic acid (1.5 mg l^{-1}), glycine (2.0 mg l^{-1}), 2,4-D (2, 4-dichlorophenoxyacetic acid, 2.0 mg l^{-1}), zeatin (1.5 mg l^{-1}), sucrose (30 g $l^{-1)}$ and gel-rite (2.4 g l^{-1}). The pH was adjusted to 5.6 (Noriega, 1991.) One modification was made to this medium in the addition of 1% caffeic acid per liter. The addition of caffeic acid was made to reduce phenolization of the explant tissue (verbal communication by Dr. Roberta H. Smith). The medium was autoclaved for 20 minutes at 121 °C and 15 psi. Heat labile KM-8P (1X) vitamins were filter-sterilized then added to the medium following autoclave sterilization. Twenty ml of media was poured into 100 x 15 mm Petri dishes (Noriega and Sondahl, 1991). Following explantation into media, the Petri dishes were wrapped with parafilm and incubated in the dark. After 13 weeks incubation in dark conditions at 25ºC ± 3 with a subculture of the explants at 5 weeks, observations were made of embryogenic callus clusters. At this time, digital and film images of the plates were taken. Also at this time, non-embryogenic cultures were noted. Prior to the transfer of the cultures to the subsequent embryogenic tissue medium, the following scale was employed to quantify the genotypic response to the culture medium. Visual observations were conducted via a stereoscope.

1 – Crystalline callus only
2 – Less than 10% of experimental unit embryogenic
3 – 10 to 19% of experimental unit embryogenic
4 – 20 to 29% of experimental unit embryogenic
5 – More than 30% of experimental unit embryogenic

Data ratings were assigned the title, Embryogenic Callus Ratings then subjected to the Shapiro-Wilcoxon test for normality then Friedman's non-parametric analysis of variance, using SAS computer software (SAS Institute, 1989).

Embryogenic Tissue Phase

After 13 weeks, cultures were transferred to an embryogenic tissue medium, consisting of B5 salts (Gamborg, 1968), ammonium sulfate (329 mgl^{-1}), thiamine HCl (5.0 mg l^{-1}), myo-inositol (100 mg l^{-1}), pyridoxine (1.5 mg l^{-1}), nicotinic acid (1.5 mg l^{-1}), glycine (2.0 mg l^{-1}), zeatin (1.5 mg l^{-1}), GA_3 (gibberellic acid, 1.0 mg l^{-1}), NAA (-napthaleneacetic acid, 0.25 mg l^{-1}), caffeic acid (1 mg l^{-1}), sucrose (20 g l^{-1}), and gel-rite (2.4 g l^{-1}). The pH was adjusted to 5.7. The medium was autoclaved for 20 minutes at 121 °C 15 psi. Heat labile KM-8P (1X) vitamins were filter sterilized then added to the medium following autoclave sterilization. Twenty ml of the medium was poured into 100 x 15 mm Petri dishes (Noriega, 1991). Cultures were incubated in dark conditions at 25ºC ± 3 for 4 weeks.

Differentiation Phase

At the end of this period, friable embryogenic tissue was detectable and subcultured onto differentiation medium, containing MS Salts, thiamine HCl (5.0 mg l^{-1}), myo-inositol (200 mg l^{-1}), pyridoxine (1.5 mg l^{-1}), nicotinic acid (1.5 mg l^{-1}), glycine (2.0 mg mg l^{-1}), GA_3 (gibberellic acid, 1.0 mg l^{-1}), ABA (abscisic acid, 2.0 mg l^{-1}), caffeic acid (1 mg l^{-1}), sucrose (20 g l^{-1}), and gel-rite (2.4 g l^{-1}). The pH was adjusted to 5.5. The medium was autoclaved for 20 minutes at 121 °C 15 psi. Heat labile KM-8P (1X) vitamins were filter-sterilized then added to the medium following autoclave

sterilization. 20 ml of the medium was poured into 100 x 15 mm Petri dishes (Noriega, 1991). After 8 weeks on this medium under the aforementioned conditions, the cultures were transferred onto a maturation medium.

Maturation Phase

Starch accumulation was initiated when the embryogenic cultures were transferred to the maturation medium containing MS Salts, thiamine HCl (5.0 mg l^{-1}), myo-inositol (100 mg l^{-1}), pyridoxine (1.5 mg l^{-1}), nicotinic acid (1.5 mg l^{-1}), glycine (2.0 mg l^{-1}), GA3 (gibberellic acid, 1.0 mg l^{-1}), ABA (abscisic acid, 0.2 mg l^{-1}), caffeic acid (1 mg l^{-1}), coconut water (10% v/v), sucrose (20 g l^{-1}), and gel-rite (2.4 g l^{-1}). The pH was adjusted to 5.5. The medium was autoclaved for 20 minutes at 121 °C 15 psi. Heat labile KM-8P (1X) vitamins were filter-sterilized then added to the medium following autoclave sterilization. The medium was poured into 100 x 15 mm Petri dishes at a volume of 20 ml per dish. (Noriega, 1991) Cultures were incubated in dark conditions at 25ºC ± 3 for 8 weeks.

After 14 weeks on the maturation medium with a subculture at 8 weeks, cultures were rated visually under the stereoscope for somatic embryo production according to the following scale:

1 – Callus friable but lacking embryos
2 – Less than 5% of experimental unit containing embryos
3 – 5 to 9% of experimental unit containing embryos
4 – 10 to 15% containing embryos
5 – 15 to 20% containing embryos
6 – more than 20% containing embryos

The tissue culture protocol indicated that 8 weeks was a sufficient period for embryo maturation (Noriega, 1991). However, in this study, due to protracted starch accumulation, these cultures were allotted an additional 6 weeks on this medium prior to ratings. Data ratings were assigned the title, Somatic Embryo Ratings data. At this time, cultures were photographed.

Data Analysis

Both Embryogenic Callus Ratings and Somatic Embryo Ratings data sets were subjected to the Shapiro-Wilcoxon test for normality then Friedman's non-parametric analysis of variance, using SAS computer software (SAS Institute, 1989).

RESULTS

Somatic Embryogenesis in 15 Diverse Tetraploid *Rosa hybrida* L. Genotypes

Analysis of Variance (ANOVA)

The objective of this experiment was to determine which genotypes would undergo somatic embryogenesis in an efficient manner so that these genotypes could be used in subsequent *Agrobacterium*-mediated gene transfer studies. Eight media treatments were employed for each of 15 genotypes (Tables 3, 4).

The ANOVA of the embryogenic callus ratings indicated that two main effects (genotype and salt) and several of the interactions (genotype*salt, genotype*carbohydrate) were significant (Tables 4, 5). Although all were statistically significant, the genotype effect explained the most variation (74.9% vs. 1.28-0.57%).

The Shapiro-Wilcoxon Test for Normality ($W=0.92$, $Pr < W = < 0.0001$) indicated a lack of normality. When various transformations (log, log + 1, square root and arcsin) were employed, none of these attempts were successful to establish a Gaussian distribution of the data. Six of the genotypes tested demonstrated no variation among the treatments causing this non-Gaussian distribution, violating the parametric assumption that scores from different populations should have the same variability (Table 4).

Table 3. Means of embryogenic callus ratings after 11weeks of culture.

Genotype	MS G F*	MS G P	MS S F	MS S P	B5 G F	B5 G P	B5 S F	B5 S P
All That Jazz	2.70**	2.80	2.73	2.80	2.80	3.00	2.80	2.80
Baby Love	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Carefree Beauty	2.10	2.30	2.82	2.90	2.20	2.20	2.82	2.90
Crimson Glory	1.30	1.40	1.65	1.30	1.40	1.40	1.85	1.70
Dortmund	1.30	1.60	1.36	1.30	1.30	1.30	1.49	1.80
Graham Thomas	1.20	1.20	1.27	1.20	1.30	1.10	1.58	1.20
Iceberg	1.80	2.50	1.84	2.50	1.70	1.60	1.85	1.80
Ingrid Bergman	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Perfume Delight	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Prominent	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Red Meidiland	1.30	1.40	1.84	1.90	1.40	1.60	2.04	1.90
Sunflare	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Tournament of Roses	4.80	4.30	4.80	4.80	4.70	4.80	4.90	4.80
SF 83-2	1.20	1.30	1.27	1.20	1.30	1.30	1.77	1.60
90-202	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

*MS G F = MS salts + glucose, filament explant B5 G F = B5 salts + glucose, filament explant
MS G P = MS salts + glucose, petiole explant B5 G P = B5 salts + glucose, petiole explant
MS S F = MS salts + sucrose, filament explant B5 S F = B5 salts + sucrose, filament explant
MS S P = MS salts + sucrose, petiole explant B5 S P = B5 salts + sucrose, petiole explant

** Rating Scale

1 – Crystalline callus only, 2 – Less than 10% of experimental unit embryogenic, 3 – 10-19% or more of experimental unit embryogenic, 4 – 20-29% or more of experimental unit embryogenic, 5 – 30% or more of experimental unit embryogenic

Table 4. Analysis of variance for 15 individual *Rosa hybrida* L. genotypes evaluated for the ability to undergo somatic embryogenesis.

Genotype	Sum of Squares	Mean Square	F Value	Pr > F
All That Jazz	0.600	0.086	0.51	0.8272
Baby Love	0	0	n/a	n/a
Carefree Beauty	8.75	1.25	8.04	< 0.0001
Crimson Glory	3.188	0.455	1.96	0.072
Dortmund	2.488	0.356	1.51	0.1763
Graham Thomas	0.988	0.141	0.75	0.6287
Iceberg	8.79	1.26	5.68	< 0.0001
Ingrid Bergman	0	0	n/a	n/a
Perfume Delight	0	0	n/a	n/a
Prominent	0	0	n/a	n/a
Red Meidiland	5.188	0.741	4.20	0.0006
Sunflare	0	0	n/a	n/a
Tournament of Roses	2.388	0.341	1.87	0.0863
SF 83-2	2.600	0.371	1.71	0.1192
90-202	0	0	n/a	n/a

Rating Scale
1 – Crystalline callus only, 2 – Less than 10% of experimental unit embryogenic, 3 – 10-19% or more of experimental unit embryogenic, 4 – 20-29% or more of experimental unit embryogenic, 5 – 30% or more of experimental unit embryogenic

Interaction Effects

In three of the genotypes evaluated, significant differences in the observed treatment means were determined. Due to the varied response among treatments, further analysis was conducted to determine if interaction existed in the three varied components of each treatment. Significant two-way interaction was observed between the genotype and the salt and carbohydrate source respectively, explaining 1.28 and 0.90 percent of the variation (Table 5).

Table 5. Analysis of variance for 15 tetraploid *Rosa hybrida* L. genotypes to form embryogenic callus on 8 individual *in vitro* medium treatments.

Source*	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	119	87409042.3	734529.8	34.682	<.0001
Error	1080	22876682.7	21182.1		
Corrected Total	1199	110285725.0			
R-Square	Coeff Var	Root MSE	RATING Mean		
0.792	24.236	145.540	600.50		
Source	DF	Anova SS	Mean Square	F Value	Pr > F
genotype	14	82556533.73	5896895.27	278.39	<.0001
carb	1	92945.60	92945.60	4.39	0.036
salt	1	635260.08	635260.08	29.99	<.0001
explant	1	19691.10	19691.10	0.93	0.3352
genotype*salt	14	1414050.29	101003.59	4.77	<.0001
genotype*carb	14	997580.86	71255.78	3.36	<.0001
genotype*explant	14	488083.81	34863.13	2.39	0.0036
carb*salt	1	120701.02	120701.02	5.70	0.017
salt*explant	1	11963.77	11963.77	0.56	0.45
carb*explant	1	37274.45	37274.45	1.76	0.18
genotype*carb*salt	14	426387.94	30456.28	1.44	0.13
genotyp*salt*explant	14	68917.79	4922.70	0.23	0.99
genotyp*carb*explant	14	342618.17	24472.73	1.16	0.30
geno*carb*salt*expla	15	197033.68	13135.58	0.62	0.8603

Rating Scale
1 – Crystalline callus only, 2 – Less than 10% of experimental unit embryogenic
3 – 10-19% or more of experimental unit embryogenic, 4 – 20-29% or more of experimental unit embryogenic, 5 – 30% or more of experimental unit embryogenic

The interaction effect of the genotypes with salt*carb*explant appears to be due to the response of 3 rose genotypes: Carefree Beauty, Iceberg and Red Meidiland (Table 4). For both Carefree Beauty and Red Meidiland, sucrose encouraged greater

development of embryogenic callus than did glucose (Tables 6-9) whereas with Iceberg, the petiole cultures on MS media elicited greater embryogenesis (Tables 10-11). Given that these effects explained only a small amount of the experimental variation (approximately 2.5%) versus that explained by the main genotype effect (74.9%), it is unlikely that the genotypic differences were unduly obscured by the significant interaction effects.

The minor amount of variation explained by interaction was an aid in choosing the treatment for the subsequent genetic study.

Table 6. Factorial model based on Analysis of variance for *Rosa hybrida* L. cv. 'Carefree Beauty' to undergo somatic embryogenesis.

Source	DF	ANOVA SS	F Value	Pr > F
salt	1	0.00	0.00	1.00
carb	1	8.45	54.32	<.0001
explant	1	0.20	1.29	0.26
salt*carb	1	0.00	0.00	1.00
salt*explant	1	0.05	0.32	0.57
carb*explant	1	0.00	0.00	1.00
salt*carb*explant	1	0.05	0.32	0.57

Data based on rating scale:
1 – Crystalline callus only, 2 – Less than 10% of experimental unit embryogenic, 3 – 10-19% or more of experimental unit embryogenic, 4 – 20-29% or more of experimental unit embryogenic, 5 – 30% or more of experimental unit embryogenic

Table 7. Carbohydrate effect on the embryogenic callus ratings based on ANOVA and Tukey's Studentized Range (HSD) Test for *Rosa hybrida* L. cv. 'Carefree Beauty'.

Treatment	N	Mean	Tukey Grouping
Sucrose	40	2.85	AB
Glucose	40	2.2	C

*Alpha	0.05
Error Degrees of Freedom	72
Error Mean Square	0.156
Critical Value HSD	4.414
Minimum Significant Diffference	0.551

Data based on rating scale:
1 – Crystalline callus only
2 – Less than 10% of experimental unit embryogenic
3 – 10-19% or more of experimental unit embryogenic
4 – 20-29% or more of experimental unit embryogenic
5 – 30% or more of experimental unit embryogenic

Table 8. Factorial model based on Analysis of variance for *Rosa hybrida* L. cv. 'Red Meidiland' for embryogenic callus ratings.

Source	DF	Anova SS	Mean Square	F Value	Pr > F
salt	1	0.3125	0.3125	1.77	0.1874
carb	1	4.5125	4.5125	25.58	<.0001
explant	1	0.1125	0.1125	0.64	0.4271
salt*carb	1	0.0125	0.0125	0.07	0.7908
salt*explant	1	0.0125	0.0125	0.07	0.7908
carb*explant	1	0.1125	0.1125	0.64	0.4271
salt*carb*explant	1	0.1125	0.1125	0.64	0.4271

Data based on rating scale:
1 – Crystalline callus only, 2 – Less than 10% of experimental unit embryogenic
3 – 10-19% or more of experimental unit embryogenic, 4 – 20-29% or more of experimental unit embryogenic, 5 – 30% or more of experimental unit embryogenic

Table 9. Carbohydrate effect on the embryogenic callus ratings based on ANOVA and Tukey's Studentized Range (HSD) Test for *Rosa hybrida* cv. 'Red Meidiland'.

Treatment	N	Mean	Tukey Grouping
Sucrose	40	1.9	AB
Glucose	40	1.43	BC

Alpha	0.05
Error Degrees of Freedom	72
Error Mean Square	0.18
Critical Value HSD	4.42
Minimum Significant Difference	0.59

Data based on rating scale:
1 – Crystalline callus only
2 – Less than 10% of experimental unit embryogenic
3 – 10-19% or more of experimental unit embryogenic
4 – 20-29% or more of experimental unit embryogenic
5 – 30% or more of experimental unit embryogenic

Contrary to the carbohydrate effect observed in 'Carefree Beauty' and 'Red Meidiland,' basal salt and explant source were shown to have a significant effect on the response of 'Iceberg'(Tables 10, 11).

Table 10. Factorial model based on Analysis of variance for *Rosa hybrida* L. cv. 'Iceberg' for embryogenic callus ratings

Source	DF	Anova SS	F Value	Pr > F
salt	1	3.613	16.36	0.0001
carb	1	0.113	0.51	0.478
explant	1	2.113	9.57	0.003
salt*carb	1	0.113	0.51	0.478
salt*explant	1	2.813	12.74	0.0006
carb*explant	1	0.013	0.06	0.8126
salt*carb*explant	1	0.013	0.06	0.8126

Data based on rating scale:
1 – Crystalline callus only
2 – Less than 10% of experimental unit embryogenic
3 – 10-19% or more of experimental unit embryogenic
4 – 20-29% or more of experimental unit embryogenic
5 – 30% or more of experimental unit embryogenic

Table 11. Salt and Explant effect on the embryogenic callus ratings based on ANOVA and Tukey's Studentized Range (HSD) Test for *Rosa hybrida* cv. 'Iceberg'.

Treatment	N	Mean	Tukey Group
MSP	20	2.5	A
MSF	20	1.8	B
B5F	20	1.75	B
B5P	20	1.7	B

*Alpha	0.05
Error Degrees of Freedom	72
Error Mean Square	0.221
Critical Value HSD	4.415
Minimum Significant Diffference	0.656

Data based on rating scale:
1 – Crystalline callus only
2 – Less than 10% of experimental unit embryogenic
3 – 10-19% or more of experimental unit embryogenic
4 – 20-29% or more of experimental unit embryogenic
5 – 30% or more of experimental unit embryogenic

Main Effects

Accounting for 74.9% of observed variation, the genotype effect observed in this experiment supports previous studies that suggest the ability to undergo somatic embryogenesis in rose is genotype specific (Hsia and Korban, 1996, Marchant, et al. 1996, Kintzios, et al. 1999, Sarasan, et al. 2001, Castillon and Kamo, 2002, and Li, et al. 2002). The minor variation due to type of basal salt (0.57%), carbohydrate (0.08%) and explant (0.01%) utilized gives further credence to this idea. The superior ability to initiate somatic embryogenesis of 'Tournament of Roses' in comparison to the other experimental genotypes made it a parent of interest for an inheritance study (Table 3).

In all treatments, 'Tournament of Roses' produced the most embryogenic callus (Table 3). Its callus was observed to be friable, characterized by small, round cells with a smooth almost wet appearance. The color was best described as ecru (Figure 1).

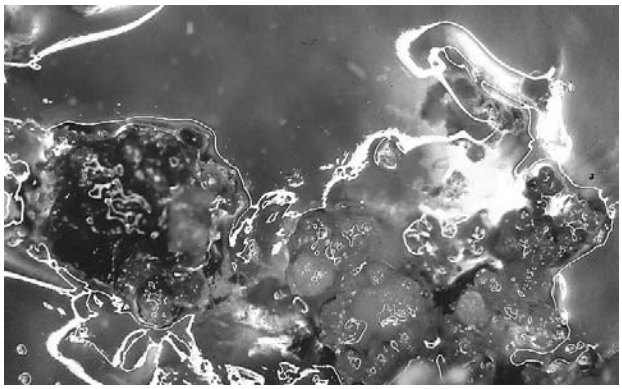

Figure 1. Callus produced by *Rosa hybrida* L. cv. 'Tournament of Roses' after 8 weeks.

The amount and quality of embryogenic callus produced by ‘Tournament of Roses’ far surpassed all other genotypes tested (Figure 1). ‘All that Jazz’ and ‘Carefree Beauty’ consistently initiated embryogenesis in all experimental units although at a reduced frequency in comparison to ‘Tournament of Roses’ (Table 3). The other genotypes tested produced hard, crystalline non-embryogenic callus (Figure 2). Any embryogenic regions were anomalous in the experimental units, typically smaller than 10% of the unit and occurring at a low frequency.

In all treatments, ‘Baby Love’ produced a somewhat translucent crystalline callus containing no embryogenic clusters (Figure 2). The cells were visually larger and more loosely aggregated than the callus produced by ‘Tournament of Roses.’

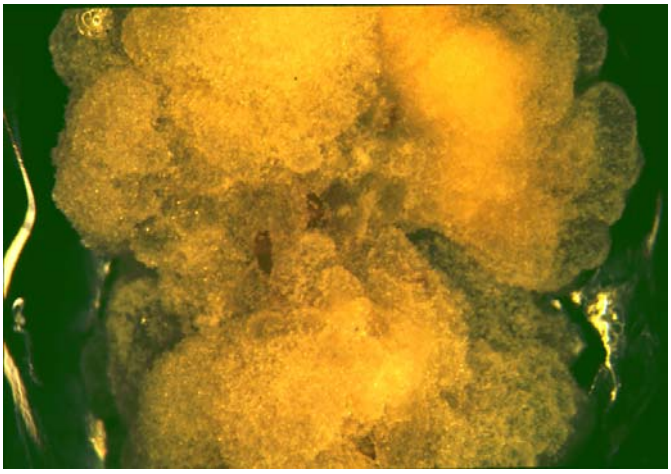

Figure 2. Crystalline callus produced by genotype ‘Baby Love’ after approximately 11 weeks.

Petiole cultures tended to be plagued with greater occurrences of contamination than cultures of filament explants. Both fungal and bacterial pathogens proliferated and caused the cultures to be unusable (Figures 3 and 4).

Figure 3. Bacterial contamination

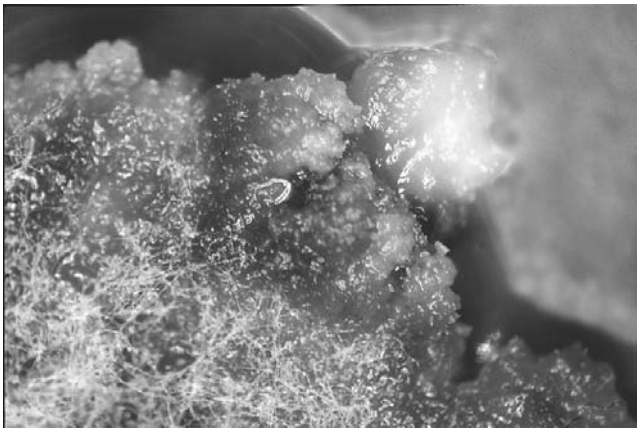

Figure 4. Fungal contamination

The presence of phenolic compounds released into the culture medium was noted in experimental units of ‘Red Meidland’, ‘Iceberg’ and ‘Prominent’ (Figure 5). Phenolic compounds appear as dark spots in callus clusters.

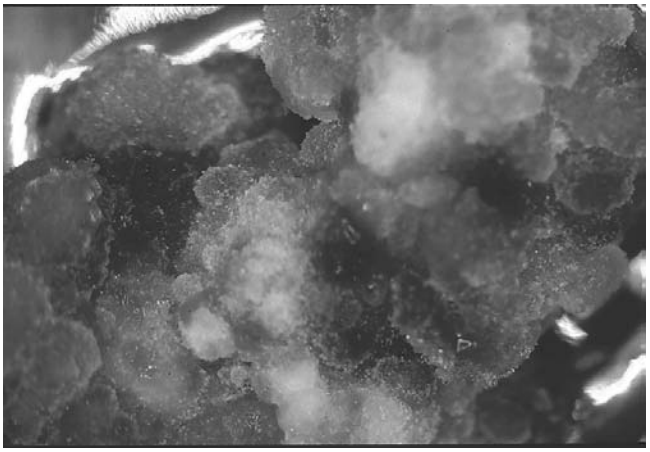

Figure 5. Phenolic compounds evident in callus produced by *Rosa hybrida* L. cv. ‘Iceberg’.

The B5 basal salt was noted to elicit superior embryogenesis. The reduced form of nitrogen contained in this mixture has been suggested to be more readily assimilated by explants initiating embryogenesis (Trigiano, et al. 1992).

Genetic Study in Somatic Embryogenesis of 11 *Rosa hybrida* L. 'Tournament of Roses' x 'Baby Love' Progeny Genotypes

Callus Proliferation

The level of phenolic compounds released into the culture medium was greatly reduced by the addition of caffeic acid to the protocol. While contamination was observed, the frequency of fungal and bacterial pathogens was reduced in comparison to the frequency in "Somatic Embryogenesis in 15 Diverse Tetraploid *Rosa hybrida* L. Genotypes". This was likely the result of using sterile filaments as the explant material.

Each progeny genotype produced callus during culture in the 2, 4-D supplemented medium, which has been reported to promote callus formation (Gamborg, et al. 1976). Callus became visible without magnification at approximately three weeks (Figure 6).

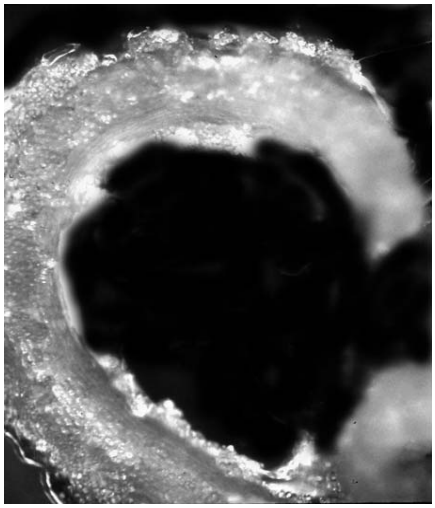

Figure 6. Callus formation in 98-00391 after approximately 3 weeks following explantation.

Genotypes 98-00394 and 98-00396 were observed to produce approximately twice the volume of callus in comparison to the other 9 genotypes. This response was noted in parent genotype ‘Baby Love’. In the cultures of ‘Baby Love’ and the two aforementioned progeny genotypes, the texture of the callus bore morphological similarities in that the callus produced tended to be whitish in color. When manipulated, some of this callus was noted to be hard and resistant to cutting with a no. 10 scalpel blade. This type of callus was observed in the cultures of all the tested progeny genotypes although at varying frequencies.

Initially, the cultures of 98-00396 consistently produced no regions of anomalous embryogenic callus whereas many cultures of 98-00394 did. Although the frequencies in which 98-00396 produced embryogenic callus were not great enough to be allocated to a significantly different population than ‘Baby Love’, the observation of embryogenesis in any degree separates the two genotypes in their ability to initiate somatic embryogenesis (Table 12, 13). The ability of 98-00396 to undergo somatic embryogenesis, albeit limited, suggests that all the F_1 genotypes of a ‘Tournament of Roses’ x ‘Baby Love’ cross would be capable of undergoing somatic embryogenesis.

Table 12. Analysis of variance of *Rosa hybrida* L. cvs. 'Tournament of Roses' x 'Baby Love' progeny for embryogenic callus ratings.

Source	Df	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	1211857.63	100988.14	140.73	<.0001
Error	247	177249.38	717.609		
Corrected Total	259	1389107.00			
R-Square	Coeff Var	Root MSE	Rating Mean		
0.87	20.53	26.79	2.596154		

Statistical Analysis included 'Tournament of Roses' and 'Baby Love' data.

Rating Scale: 1 – Crystalline callus only, 2 – Less than 10% of experimental unit embryogenic, 3 – 10-19% of experimental unit embryogenic, 4 – 20-29% embryogenic, 5 – More than 30% embryogenic

While in the same statistical grouping as 'Baby Love', 98-00394, 98-00396 and 98-00399-03 produced embryogenic callus no matter how meager and demonstrated a qualitative difference from the parental behavior of 'Baby Love' (Table 13).

Genotypes 98-00392, 98-00397 and 98-00399-02 demonstrated embryogenic callus in quantities intermediate between 'Baby Love' and 'Tournament of Roses'. It should be noted that embryogenesis in rose rarely occurs in quantities exceeding more than 30% of the experimental unit (Noriega and Sondahl, 1991). The difference between the previously discussed population and the one to which 98-00392, 98-00397 and 98-00399-02 were assigned exists in the consistent occurrence of embryogenic callus among the experimental units although not exceeding 10% of the total callus produced within the plate (Figure 7).

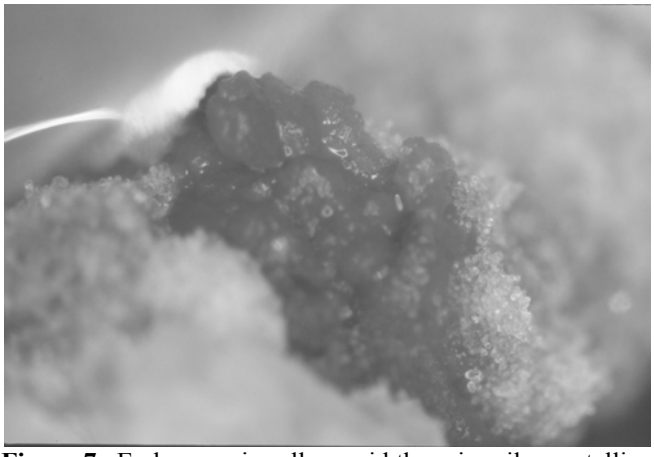

Figure 7. Embryogenic callus amid the primarily crystalline callus of 98-00399-02.

Genotypes 98-00391, 98-00399-01 and 98-00399-04 (mean = 3.00) demonstrated between 10 to 19 % embryogenic regions among the experimental units. The embryogenic callus was typically friable and easily separated from the crystalline regions of callus.

Two genotypes, 98-00395 and 98-00399-05 resembled ‘Tournament of Roses’ in callus color, friability and mean production of embryogenic callus.

Table 13. *Tukey's Studentized Range (HSD) Test based on analysis of variance for callus ratings at 11 weeks.

Tukey Group	Mean	N	Genotype	Standard Deviation
A	4.75	20	Tournament of Roses	0.444
A	4.60	20	98-00399-05	0.470
A	4.30	20	98-00395	0.571
B	3.20	20	98-00391	0.523
B	3.05	20	98-00399-01	0.510
B	2.75	20	98-00399-04	0.444
C	2.05	20	98-00397	0.394
C	2.05	20	98-00399-02	0.605
C	2.05	20	98-00392	0.224
D	1.45	20	98-00399-03	0.605
DE	1.40	20	98-00394	0.598
DE	1.10	20	98-00396	0.308
E	1.00	20	Baby Love	0.000

*Alpha	0.05
Error Degrees of Freedom	247
Error Mean Square	0.223
Critical Value HSD	4.731
Minimum Significant Diffference	0.499

Rating Scale
1 – Crystalline callus only
2 – Less than 10% embryogenic
3 – 10-19% embryogenic
4 – 20-29% embryogenic
5 – More than 30% embryogenic

Maturation Phase

Opacity in regions of embryogenic callus signals evidence of starch accumulation. Starch accumulation confirms that the embryogenic callus is maturing into somatic embryos, a crucial step in regeneration (Raghavan, 1997).

Overall, starch accumulation occurred at a significantly slower rate than reported for *Rosa hybrida* L. cultivar 'Royalty' in the study after which this experimental protocol was modeled (Noriega and Sondahl, 1991). The addition of caffeic acid,

omitted in the Noriega study, was the sole difference in the experimental protocols. It is possible the caffeic acid caused the delay in starch accumulation. An additional period of 6 weeks on the Maturation medium was required for the majority of genotypes to elicit sufficient opacity for rating purposes.

An analysis of variance of the eleven progeny and two parental genotypes for their ability to form somatic embryos showed a significant difference among the genotypes in the 72.79 F value (Table 15). Tukey's Studentized Range Test separated the genotypes into 5 statistically different populations (Table 15). The midparental value based on the means of 'Tournament of Roses' and 'Baby Love' data was 2.40, while the progeny mean derived from mean ratings was 2.15. Included in the same segment of the rating scale (2, indicating less than 5% of the experimental unit containing embryos), these similar values suggest an additive gene effect.

Despite the absence of auxin in the maturation medium, root formation was observed in many of the experimental units of genotypes 98-00391 and 98-00399-01 (Figure 8).

Table 14. Analysis of variance for 11 progeny genotypes of *Rosa hybrida* L. cvs. 'Tournament of Roses' x 'Baby Love' to form somatic embryos.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	*12	1050887.40	87573.95	72.79	<.0001
Error	247	297168.60	1203.11		
Corrected Total	259	1348056.00			
R-Square	Coeff Var	Root MSE	Rating Mean		
0.78	26.58	34.69	2.192		
Source	DF	Anova SS	Mean Square	F Value	Pr > F
Genotype	*12	1050887.40	87573.95	72.79	<.0001

* Statistical Analysis included 'Tournament of Roses' and 'Baby Love' data.
Rating Scale: 1 – Callus friable but lacking embryos, 2 – Less than 5% of experimental unit containing embryos, 3 – 5 to 9% of experimental unit containing embryos, 4 – 10 to 15% containing embryos, 5 – 16 to 20% containing embryos, 6 – more than 20% containing embryos

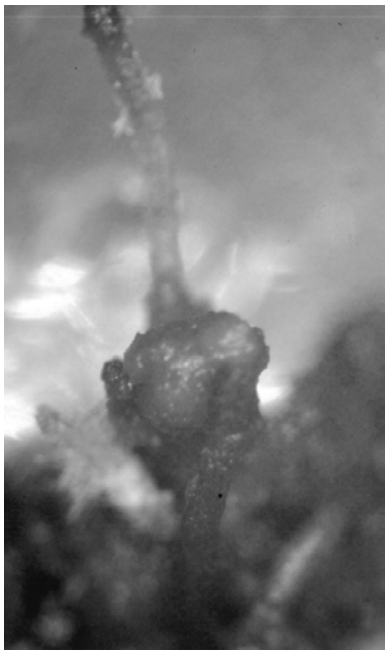

Figure 8. Example of root formation in 98-00391 after 13 weeks.

As anticipated, genotypes 98-00394, 98-00396 and 98-00399-03 produced few embryos primarily due to the fact that these genotypes had initiated insubstantial and inconsistent amounts of embryogenic callus. However, upon maturation, the embryos produced by these genotypes were of equal quality to those produced by the other genotypes evaluated.

The embryogenic regions of 98-00394 were particularly slow at accumulating starch, initiating the process 5 weeks after starch accumulation was observed in genotype 98-00399-02.

Starch accumulation began first in genotype 98-00399-02 in the prescribed period of the experimental protocol (Noriega and Sondahl, 1991). While experimental units of genotypes 98-00392 and 98-00397 typically contained similar quantities of embryos, the earlier maturation of 98-00399-02 demonstrated a noteworthy qualitative difference.

Due to its unexpected maturation pattern, genotype 98-00395 showed a mean rating of 1.40 (Table 15). Following the substantial initiation of embryogenic callus, 65% of the experimental units demonstrated a tendency to desiccate that was not observed in any of the other genotypes evaluated. This desiccation was characterized by a browning of the callus and a cessation of cell expansion. This likely was the result of an inability to take up water from the medium possibly due to phenolization (Barkosky and Einhellig, 2003). Thirty-five percent of the experimental units of 98-00395 demonstrated slow starch accumulation and a tendency toward similar desiccation, although sporadic regions of somatic embryos continued to develop. Thus the ability of

this genotype to undergo embryogenesis was illustrated. However, its habit of desiccating following callus initiation skewed the observation data resulting in 65% of the experimental units ranked as containing no embryos and 35% at a lower ranking than the initial embryogenic callus would have suggested. Possible remedies for the tendency to desiccate might include more frequent subcultures involving excision of the callus layer in direct contact with the medium, alteration of growth regulators in the medium and a change in the medium pH (Ramage and Williams, 2002).

Genotypes 98-00391, 98-00399-01 and 98-00399-04 demonstrated timely starch accumulation in anticipated quantities. However, genotype 98-00399-05 consistently produced the greatest number of embryos of the 11 genotypes tested. Although the most efficient of the 11 progeny genotypes, the ability of 98-00399-05 to undergo embryogenesis remained visibly but not statistically inferior to that of parental genotype 'Tournament of Roses'.

With the exception of genotype 98-00395, the ability to produce embryogenic callus is linked to subsequent production of somatic embryos. Only embryogenic callus developed into somatic embryos.

Table 15. Mean somatic embryo ratings of 'Tournament of Roses' x 'Baby Love' progeny and parents.

Tukey	Group	Mean	N	Genotype
	A	3.80	20	Tournament of Roses
	A	3.70	20	98-00399-05
	A	3.45	20	98-00399-01
	A	3.30	20	98-00391
	B	2.35	20	98-00399-04
C	B	2.00	20	98-00397
C	B	1.95	20	98-00399-02
C	D	1.75	20	98-00392
E	D	1.40	20	98-00394
E	D	1.40	20	98-00395
E	D	1.35	20	98-00399-03
E		1.05	20	98-00396
E		1.00	20	Baby Love

*Alpha = 0.05
Error df = 247
Error Mean Square = 0.255
Critical Value of HSD = 4.731
Minimum Significant Difference = 0.534
**Means with the same letter are not significantly different.
1 – Callus friable but lacking embryos
2 – Less than 5% of experimental unit containing embryos
3 – 5 to 9% of experimental unit containing embryos
4 – 10 to 19% containing embryos
5 – More than 20% containing embryos

DISCUSSION AND CONCLUSION

The vast differences of embryogenic ability observed among the 15 unrelated rose genotypes supports the claim that the ability to undergo somatic embryogenesis is under genotypic control (Loiseau, et al. 1996). This concept is further illustrated by the spectrum of somatic embryogenesis observed in the eleven 'Tournament of Roses' x 'Baby Love' progeny genotypes.

The data of Experiment: Genetic Study in Somatic Embryogenesis of 11 *Rosa hybrida* L. 'Tournament of Roses' x 'Baby Love' F_1 Progeny suggests that a single gene with complete dominance does not control somatic embryogenesis in *Rosa hybrida* L. The marked array of somatic embryogenesis displayed by the genotypes alludes to a more complex heredity with more than one gene involved. The notable similarities in the midparent value and progeny means for both data sets alludes to an additive effect.

Further investigation into the embryogenic ability of backcrosses and F_2 hybrids could provide additional insight into the inheritance of this trait and under what genetic control it lies. By understanding the gene(s) controlling somatic embryogenesis, the hurdles of regeneration could be overcome by breeding strategies designed to incorporate the regenerative trait into progeny.

In order to accomplish this, the following breeding strategy is suggested:

1. Adopt an efficient screening technique for potential parents. Within a 4-week to 8-week period, embryogenic regions in callus cultures are generally evident under this experimental protocol. An increase in

efficiency might be obtained by using an alternative auxin source and incubating cultures at lower temperatures.

2. Choose parents with other desirable traits such as disease resistance, flower form, vase life and growth habit to undergo screening procedure.
3. Cross genotypes with good regenerative ability. Substantial inbreeding must be strictly avoided in rose. Thus the F_1, F_2 and BC_1 progeny will likely be the genotypes with the most economically desirable traits. As evidenced in the F_1 progeny of 'Tournament of Roses' and 'Baby Love', some degree of regenerability should be transferred into subsequent generations if highly regenerative parents are utilized. It should be considered that even a genotype with limited ability to regenerate itself through somatic embryogenesis could be useful in transformation. By establishing a stock plant, further increase can be obtained efficiently through subsequent asexual propagation by budding following regeneration.

Understanding the genetic basis for somatic embryogenesis is a link to creating new progenies that can be further improved through transformation. Conventional breeding and biotechnology are both crucial vehicles to crop improvement. Used effectively, these methods can yield advancements in disease and pest resistance, vase life, color range, fragrance and form to offer the public healthier, longer-lasting, beautiful roses and, most importantly, reduce the amount of harmful pesticides entering the environment.

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VITA

Candidate: Anna Mildred (Millie) Burrell

Degree: M.S., Texas A&M University, Horticulture, 2003

Address: 10307 Shady River, Houston, TX. 77042

Education: B.A., Duke University, 1992, Latin

Professional Experience: Texas A&M University

Teaching Assistant for HORT 605 (Internet Technology), 2003.

Lab Instructor for HORT 308 (Landscape Plant Materials) 2002-2003

Teaching Assistant for HORT 319 (Fruit and Nut Production), 2000-2003

Teaching Assistant for HORT 423 (Tropical Horticulture), 2003

Lab Instructor for HORT 326 (Plant Propagation), 2001

Lab Instructor for HORT 431 (Nursery Production and Management), 2001

Web Page Designer (http://aggie-horticulture.tamu.edu/rose/rose.html) 2001-2003