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The Effect of 2,4,5-T on Embryogenic Tissue Formation in Rose

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ABSTRACT

2,4,5-trichlorophenoxyacetic acid (2,4,5-T) has recently been reported to induce somatic embryogenesis in *Rosa hybrida* L. 'HARwelcome' (Livin' Easy™). This study takes a closer look at the effect 2,4,5-T on embryogenic tissue and somatic embryo morphology in 'HARwelcome' (Livin' Easy™). Anatomical studies were conducted to gain insight into the types of tissue initiated by treatment with 2,4,5-T. The findings showed that only two out of 10 morphologically different presumptive embryogenic tissue (PET) types were embryogenic. The morphological traits of PET described in this study could be useful as early selection criteria for embryogenic tissue in rose. Somatic embryo morphology in 'HARwelcome' (Livin' Easy™) was heterogeneous in cotyledon number. There was no significant difference in germination rates amongst somatic embryos with varying numbers of cotyledons indicating that cotyledon number would not be a useful indicator of germination potential. A histological study showed that shoots arose from cotyledon axillary meristems or adventitiously from hypocotyl regions, thus suggesting that cotyledon development occurred despite a lack of shoot apical meristem formation. 2,4,5-T and 2,4-dichlorophenoxyacetic acid (2,4-D) were compared to gauge their effectiveness to induce embryogenic tissue in eight other rose cultivars. Five out of the eight cultivars produced embryogenic tissue when treated with 2,4,5-T compared to only two cultivars when treated with 2,4-D. The results support the premise that individual genotypes within a given species vary greatly in terms of the conditions required for embryogenic induction. These findings also demonstrate that 2,4,5-T may be useful for initiating embryogenic tissue in cultivars unresponsive to 2,4-D.

Keywords: cotyledon, embryogenesis, embryogenic callus, germination, presumptive embryogenic tissue
Abbreviations: **2,4,5-T**, 2,4,5-trichlorophenoxyacetic acid; **2,4-D**, 2,4-dichlorophenoxyacetic acid; **ET**, embryogenic tissue; **PET**, presumptive embryogenic tissue; **SE**, somatic embryogenesis; **se**, somatic embryo

INTRODUCTION

Roses, *Rosa hybrida* L, are economically important plants, valued for their use as garden shrubs and in the floral and perfume industries. Conventionally, roses have been asexually propagated using procedures such as rooted cuttings or grafting. However, these methods can be costly, labour intensive and time-consuming. Somatic embryogenesis (SE), a process first reported in carrot in 1958, is an alternative method for the clonal propagation of plants (Reinert 1958; Steward *et al*. 1958). SE is the process by which somatic cells from an elite plant are induced into an embryogenic state. The resulting embryogenic tissue ultimately gives rise to bipolar somatic embryos that are capable of germinating into plants genetically identical to the plant used as the source tissue.

Reports of SE in roses first emerged in the early 1990s (reviewed by Roberts *et al*. 1995). However, rose SE technology requires improvement before it can be implemented as a production system on a commercial scale. Several rose cultivars have shown to be unresponsive to routine methods employed to induce embryogenic tissue and SE (Murali *et al*. 1996; Kintzios *et al*. 1999) or have resulted in low embryogenic tissue initiation rates (Hsia and Korban 1996; Murali *et al*. 1996; Rout *et al*. 1999; Kim *et al*. 2003). Individual genotypes within a given species vary greatly in terms of embryogenic capability (von Arnold *et al*. 2002) thereby necessitating the need for cultivar-specific research.

In order to utilize SE methods for commercial scale propagation of rose cultivars, it is necessary to identify and multiply tissues which have the potential for regeneration. A greater emphasis on characterizing and defining the stages involved from dedifferentiation through to the transition of callus into an embryogenic state would help elucidate the processes involved in SE. Identifying characteristics, such as morphology, which indicate the probability of embryogenic competence, would enable specific selection for these cell types and enhance the overall efficiency of the process. This aspect was addressed in the present study through extensive histological examinations of calli and presumptive embryogenic tissue in 'HARwelcome' (Livin' Easy™) cultures treated with 2,4,5-T. Embryogenic and non-embryogenic tissue types were characterized.

Another critical aspect in the regeneration of roses via SE is the further development and germination of the somatic embryo (Dohm *et al*. 2001). Although large numbers of somatic embryos may be obtained rapidly through SE in some rose cultivars, the subsequent plants, which are often the desired commercial product, have sometimes been difficult to recover from these embryos. Stuart and colleagues (1985) cited morphological features to be identifiable markers of conversion-competent embryos. Morphological abnormalities in somatic embryos have been reported in roses (Murali *et al*. 1996; Visessuwan *et al*. 1997). A normal rose somatic embryo is bipolar with a distinct hypocotyl, radical and two cotyledons. Abnormal embryos consisting of cotyledons that are fused to form a cup-like structure have been observed (Murali *et al*. 1996; Visessuwan *et al*. 1997). These abnormal embryos reportedly fail to germinate. In addition to abnormal cotyledon shape, variation in the cotyledon number in somatic embryos has been reported (Arene *et al*. 1993; Visessuwan *et al*. 1997).

2,4-dichlorophenoxyacetic acid (2,4-D) is a synthetic auxin commonly used to induce SE in a number of plants, including rose (Roberts *et al*. 1990; Matthews *et al*. 1991; Noriega and Söndahl 1991; Marchant *et al*. 1996). Al-

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though 2,4-D is commonly used in rose SE studies, it has proven ineffective for several cultivars (Li *et al*. 2002). 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), like 2,4-D, is a synthetic analogue of the naturally occurring auxin indole-3-acetic acid, which plays a role in differentiation and elongation of plant cells (Venkov *et al*. 2000). 2,4-D is reported to have both cytotoxic and mutagenic effects while 2,4,5-T is reported to have cytotoxic effects but very weak, if any, mutagenic effects (Venkov *et al*. 2000). This difference in mutagenic effect is important since variation is often an undesired occurrence in clonal propagation systems. In the present work, 2,4,5-T and 2,4-D were assessed for their effectiveness to initiate embryogenic tissue in eight commercially-valuable rose cultivars. Histological examination was conducted in this study to characterize the morphological heterogeneity of 'HARwelcome' somatic embryos and assess the impact of this heterogeneity on the conversion of embryos into plantlets and subsequent plant development.

MATERIALS AND METHODS

Leaflet explants (5-7 mm in length) were aseptically excised from *in vitro* shoot cultures of 'HARwelcome'. Using a scalpel, the leaflets were scored five times across the top surface of the leaf to wound the tissue and help promote callus growth. The scored leaflets were placed flat onto callus induction media with the cut-side up. Murashige and Skoog (MS) basal medium, modified as per Owen and Miller (1992) and supplemented with 3% sucrose, was employed as the basal medium. The basal medium was supplemented with 10 or 25 µM 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (Sigma T-5785) (Estabrooks *et al*. 2007). The medium was adjusted to pH 5.7 using 1.0 N NaOH, solidified using 0.4% Phytagel™ (Sigma P8169), and autoclaved for 20 min at 121°C, 104 kPa. Forty-five leaflets were utilized for each treatment (nine explants per Petri dish, each plate a replicate). The 100 × 15 mm plastic Petri dishes were sealed with Parafilm® (American National Can Company, Norwalk, CT) and incubated at 24°C in the dark. The entire experiment was repeated twice over time.

Embryogenic tissue initiation and isolation

After four weeks, the explants were transferred to embryogenic tissue (ET) initiation media, which consisted of MS medium, modified as per Owen and Miller (1992), 3% sucrose and 40% the original concentration of auxin (i.e. 4 or 10 µM 2,4,5-T). After one week on ET initiation media, and again after two weeks, presumptive embryogenic tissue (PET) was isolated and placed onto medium of the same composition (referred to as ET proliferation media). Tissue was considered PET if it exhibited any of the following traits: mucilaginous, soft, yellow, white or cream in colour, and/or nodular in appearance (**Fig. 1**). The cultures were observed weekly using a dissecting scope for signs of embryogenic response such as the formation of nodular tissue or somatic embryos.

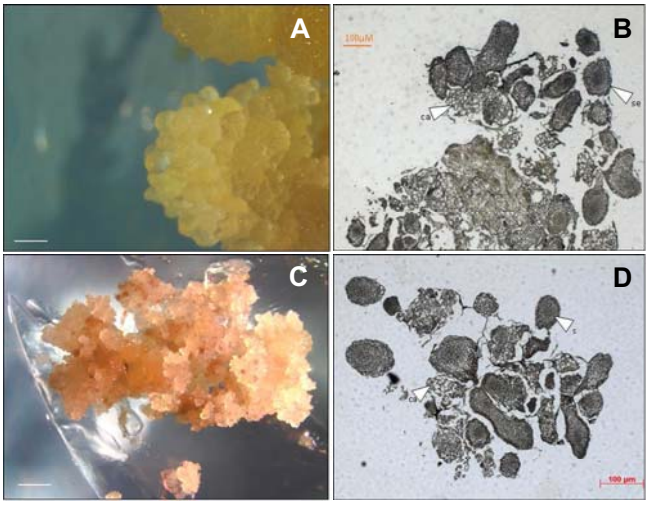

Fig. 1 'HARwelcome' (Livin' Easy™) presumptive embryogenic tissue with early stage somatic embryos interspersed with callus cells. (**A**) Type 1 presumptive embryogenic tissue (bar 1000 µm). (**B**) Anatomic structure of Type 1 presumptive embryogenic tissue. Note somatic embryos (se) intermixed with callus (ca). (**C**) Type 2 Presumptive embryogenic tissue (bar 1000 µm). (**D**) Anatomic structure of Type 2 presumptive embryogenic tissue. Note somatic embryos (se) intermixed with callus (ca).

Histological comparison of PET from 'HARwelcome'

After four weeks on ET proliferation media, PET from 'HARwelcome' was observed and photographed using a dissecting microscope equipped with a N2000 Nikon digital camera. The tissue was grouped into categories based on morphological features. Approximately 12-15 samples of tissue from each category were selected for histological analysis as described below.

Within a drop of 3% glutaraldehyde in 0.025 M potassium phosphate buffer, tissues were cut into pieces approximately 1-2 mm in length. The sectioned samples were fixed in a 3% glutaraldehyde solution in 0.025 M potassium phosphate buffer in a glass 25 mL vial equipped with a screw lid. The samples were stored at 4°C until histological processing. One drop of erythrosine B was added to each vial and stained for 30 min. The samples were centrifuged for approximately five seconds to remove the supernatant and then placed into an agar medium prior to solidification (0.2% agar in 0.025 M potassium phosphate buffer, pH 6.8). After the agar medium solidified, it was cut into small blocks using a fresh razor blade. Dehydration was carried out using 70% ethanol to 95% butanol series; ethanol was replaced with the secondary solvent butanol. Infiltratation was performed by placing the tissue into a 2.0 ml 25% paraffin butanol solution for 30 min. Every 30 min for 1.5 h, 1.0 ml of 100% paraffin was added to the vials containing the samples. After 1.5 h, the samples were stored with the lid off overnight at 57°C. The following morning, the paraffin was poured off and replaced with 100% paraffin. The samples were embedded in paraffin, sectioned into 7.0 µM sections using a microtome, stained with hematoxylin and mounted in permount. Serial sections of the tissue were studied and photographed using a compound microscope equipped with a digital camera.

Maturation

After eight weeks on ET proliferation media, 0.25 g of embryogenic tissue was placed into each of three 250 ml flasks containing 30 ml liquid modified MS plant growth regulator (PGR)-free medium supplemented with 3% sucrose (Owen and Miller 1992; Estabrooks *et al.* 2007). The cultures were shaken for 24 h at 110 rpm on a rotary shaker (G10 Gyrotory® Shaker, New Brunswick Scientific, N.J., U.S.A) and kept in the dark at 24°C. Three 10 ml aliquots from each flask were vacuum filtered onto Whatman #1 filter paper and placed onto a MS maturation medium containing 7.57 µM ±-*cis*, *trans*-abscisic acid (ABA) (Sigma A-1049). Medium was adjusted to pH 5.7 using 1.0N NaOH, solidified using 0.4% Phytagel™ (Sigma P8169) and autoclaved for 20 min at 121°C, 104 kPa.

Germination

After eight weeks on maturation medium, the cultures were placed into cold storage (4°C) in the dark for 8 weeks. After the cold period, the somatic embryos were observed for cotyledon number and other aspects of morphology such as colour and size. The embryos were classified into different categories based on cotyledon number. Twenty somatic embryos from each of the morphological types were randomly selected and fixed for histological analysis (following the same process as above). All other somatic embryos were individually transferred onto germination medium consisting of PGR-free modified MS medium (Owen and Miller 1992) supplemented with 2% sucrose. The somatic embryos were numbered and observations were made regarding colour and cotyledon number. After eight weeks, the plantlets were transferred to 98 cell trays (2″ deep) containing Pro-Mix HP (Premier Horticulture, Rivière-du-Loup, Québec). The growing medium was treated with the fungicide Benomyl®, at a concentration of 5 ml fungi-

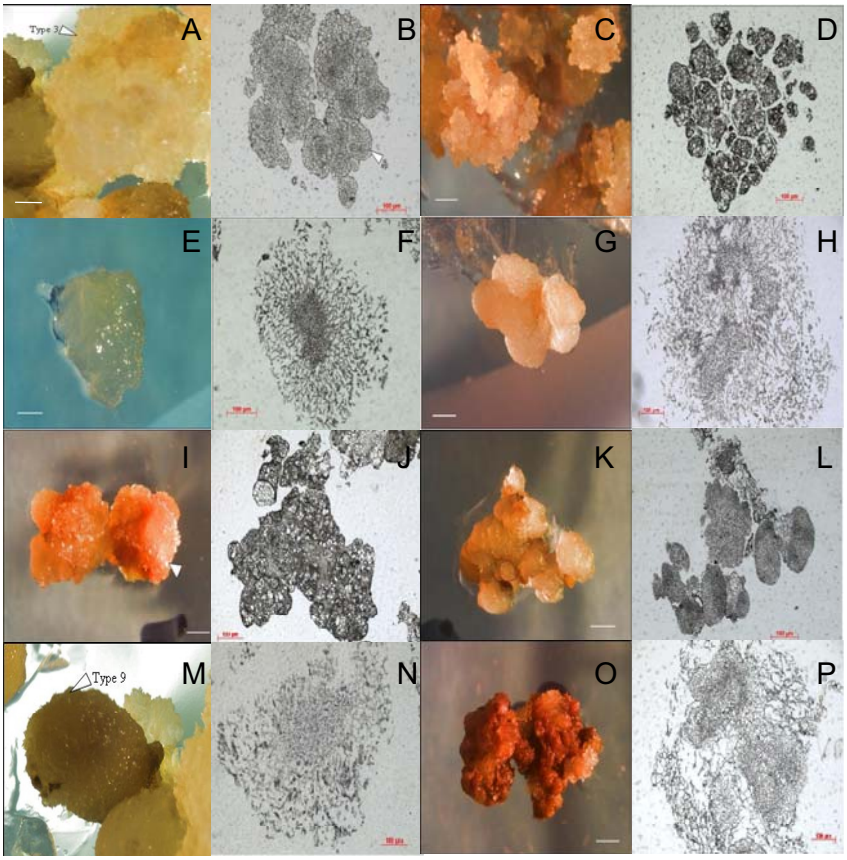

Fig. 2 'HARwelcome' (Livin' Easy™) presumptive embryogenic tissue Types 3 to 10. Note the lack of somatic embryo development. (**A**) Type 3 (bar 1000 µm). (**B**) Anatomic structure of Type 3. (**C**) Type 4 (bar 1000 µm). (**D**) Anatomic structure of Type 4. (**E**) Type 5 (bar 1000 µm). (**F**) Anatomic structure of Type 5. (**G**) Type 6 (bar 1000 µm). (**H**) Anatomic structure of Type 6. (**I**) Type 7 (bar 1000 µm). (**J**) Anatomic structure of Type 7. (**K**) Type 8 (bar 1000 µm). (**L**) Anatomic structure of Type 8. (**M**) Type 9 (bar 1000 µm). (**N**) Anatomic structure of Type 9. (**O**) Type 10 (bar 1000 µm). (**P**) Anatomic structure of Type 10.

cide/2 L of water, prior to transplanting by saturating the medium with the fungicide solution. The plantlets were transferred to the growing medium with care being taken not to damage the roots and misted frequently during the transplanting process to avoid desiccation. Each tray was covered with a clear plastic dome to retain humidity and secured with six clothes pins. The plantlets were kept in a growth room, at 24°C, with a 16 hour photoperiod of light at 38 µmol m^{-2} s^{-1}. They were misted daily and watered three times a week. Over the following six weeks, a clothes pin was removed from each tray each week to gradually acclimatize the plants to the *ex vitro* environment. Once all of the clothes pins were removed, the dome was removed completely. Survival rates were recorded monthly for three months after transplanting.

Multi-cultivar assessment

Leaflet explants (5-7 mm in length) were aseptically excised from *in vitro* shoot cultures of the roses: 'AUSmas' (Graham Thomas®), 'Betty Prior', 'HARflow' (Easy Going™), 'HARwelcome', 'John Franklin', 'MEIneble' (Red Meidiland™), 'Morden Sunrise', 'William Baffin' and a genotype of *R. rugosa* var. *alba* Rehder. The procedures were the same as those described earlier for 'HARwelcome' and based on the results of a previous study by Estabrooks and colleagues (2007).

Statistical analysis

All computations were performed using the MINITAB 15 statistical analysis package (Minitab Inc., State College PA). Normally distributed data were analysed using either ANOVA at the 5% probability level or the Two Sample T-test, as appropriate.

RESULTS AND DISCUSSION

Characterization of PET from 'HARwelcome'

After the white mucilaginous callus was isolated from 'HARwelcome', it was grown on ET proliferation medium consisting of medium containing 40% of the original concentration of auxin. The resulting presumptive embryogenic tissue (PET), after four weeks growth, could be characterized into 10 different groups based on the morphological traits of color and texture (**Table 1**). Anatomical studies showed that although most of these PET types had some cell differentiation and tissue organization, only Types 1 and 2 are real embryogenic tissues because of possessing early stage somatic embryos.

Table 1 Types of Presumptive Embryogenic Tissue resulting from white mucilaginous callus from the rose 'HARwelcome' (Livin' Easy™) after four weeks growth on embryogenic tissue initiation media.

Type #	Texture	Color	ET
1	defined nodular	yellow	+
2	defined nodular	white	+
3	slightly nodular	yellow	–
4	slightly nodular	white	–
5	soft	yellow	–
6	soft	white	–
7	crystal-like	yellow–brown	–
8	large round masses	orange/beige	–
9	soft	brown	–
10	hard	brown	–

Type 1 PET was yellow in color with distinct nodules (**Fig. 1A**). Type 2 was very similar to Type 1 with the distinguishing white color (**Fig. 1C**). Examination of the anatomic structure of these two PET types revealed the presence of early stage somatic embryos interspersed with callus cells (**Fig. 1B, 1D**). The somatic embryos consisted of groups of small, darker stained cells with very prominent nuclei. There was a well-defined epidermis surrounding each group of cells. The somatic embryos had darkly stained procambium, indicative of a high degree of organization and differentiation within the embryo. Dispersed among the somatic embryos were groups of callus cells. The callus cells were large in size and highly vacuolated with a large central vacuole occupying the majority of the cells interior.

Types 3 to 10 PET were identified and separated by

their surface morphology and colours as summarized in **Table 1** and **Fig. 2**. None of these PETs had procambium developed as found in an embryo. Although some tissues had small, densely stained cells with large nuclei dispersed throughout, the majority of cells in these PETs were highly vacuolated with a large central vacuole. Cell differentiation and specialization were observed sometimes without any sign of embryogenesis.

Somatic embryo morphology and germination

Variation in the cotyledon number was observed amongst the somatic embryos of 'HARwelcome' (**Fig. 3**). The cotyledon number varied from one to eight and somatic embryos from each cotyledonary group were capable of successful germination (**Table 2**). Root formation was observed and occurred by week one. The earliest formation of shoots was observed by week three. The shoots did not appear to be 'true epicotyls' in that they did not originate from a primary apical meristem. Shoots seemed to arise from cotyledon axillary meristems located at the junction where the hypocotyl meets the cotyledon. Shoots also formed adventitiously on the hypocotyls.

Grossly deformed germinants, defined as those embryos that were misshaped and without distinct hypocotyls or radicles (regardless of cotyledons number), were observed in 9.3 ± 2.1% of the plantlets. The occurrence of grossly deformed germinants did not differ significantly amongst the cotyledonary groups (P = 0.375, F = 1.09). Plantlets from each cotyledonary group were capable of survival after three months *ex vitro* (**Table 2**). The resulting plantlets grew normally, regardless of cotyledon number.

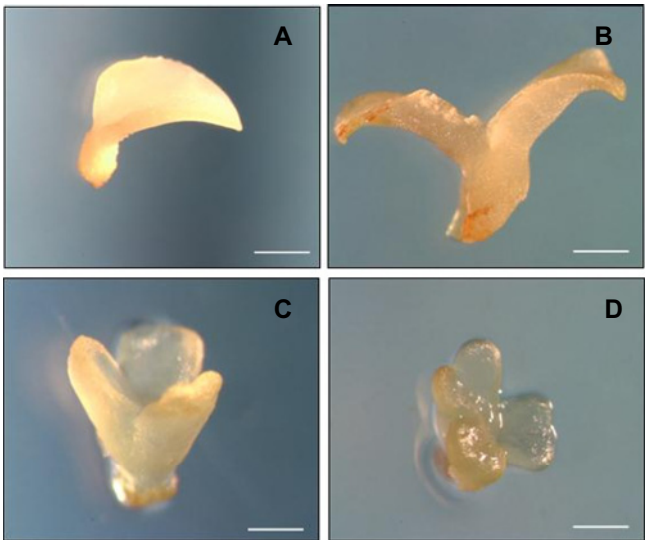

Fig. 3 Morphologically different somatic embryos of 'HARwelcome' (Livin' Easy™). (**A**) Somatic embryo with single cotyledon. (**B**) Somatic embryo with two cotyledons. (**C**) Somatic embryo with three cotyledons. (**D**) Somatic embryo with multiple cotyledons (bar 1000 μm).

Table 2 Germination percentage of somatic embryos, with varying cotyledon number, from the rose 'HARwelcome' (Livin' Easy™) after six weeks growth on Murashige and Skoog germination medium. Percentage of plantlets transplanted *ex vitro* and survival of transplanted plantlets after three months growth *ex vitro*.

2,4,5-T (μM)	Cotyledon #	Germination (Mean ± SD)	Transplanted (Mean ± SD)	Survival (Mean ± SD)
25	1	98.0 ± 3.4	44.3 ± 5.2	87.5 ± 11.7
25	2	95.0 ± 5.0	58.6 ± 14.6	78.1 ± 11.6
25	3	90.2 ± 8.6	63.9 ± 20.2	81.7 ± 8.2
25	4	100	38.9 ± 17.6	81.7 ± 2.4
25	5	100	0	na
10	1	93.5 ± 6.3	40.6 ± 15.4	62.1 ± 13.5
10	2	94.9 ± 8.9	37.3 ± 7.8	69.6 ± 6.4
10	3	95.3 ± 5.0	59.1 ± 15.7	69.2 ± 20.6
10	4	91.7 ± 14.4	36.0 ± 11.4	77.5 ± 3.54
10	5	97.8 ± 3.9	69.1 ± 17.0	55.6 ± 10.9
10	6	75.0 ± 15.4	75.0 ± 10.3	58.5 ± 12.0
10	7	100	50 ± 0.0	54.5 ± 3.7
10	8	100	100	100

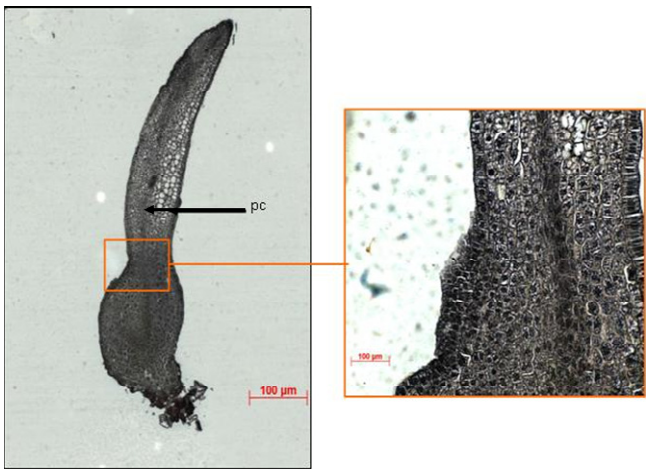

Fig. 4 Anatomic structure of 'HARwelcome' (Livin' Easy™) somatic embryo possessing a single cotyledon. Note the dense group of cells that are small and unspecialized in nature. Note the procambium development (pc).

Examination of serial histological sections revealed the somatic embryos had well defined cotyledons, albeit varying in number. Differentiation of the procambium tissue was evident in the central region of many of the somatic embryos. The procambium furcated into the cotyledons, even in somatic embryos possessing a number of cotyledons other than the 'normal' two. The radicle and hypocotyl were well developed, especially notable in somatic embryos possessing two cotyledons. Observation of the serial histological sections showed a lack of shoot apical meristems in all somatic embryos observed. There were groups of cells located at the base of cotyledons that appeared to retain meristematic activity; evident by their small size, abundant number and unspecialized nature (**Fig. 4**). Although the somatic embryos in the present study successfully germinated, they did not follow the normal path of zygotic embryogenesis. The initial epicotyledonary shoot observed upon germination of a zygotic embryo is formed from the apical meristem; a region located between the cotyledons in which cellular division actively occurs. In the present study, somatic embryos lacked shoot apical meristems and developed instead from axillary meristems or adventitiously. These findings support previous studies that reported the germination of rose somatic embryos lacking apical meristems (Visessuwan *et al*. 1997; Estabrooks *et al*. 2007). This is in contrast to other studies that reported difficulties in obtaining normal plantlets from rose somatic embryos due to a lack of apical meristem formation (Kunitake *et al*. 1993).

Multi-cultivar assessment

By week four on callus induction media, all of the explants had developed callus. The callus induction rates were similar in all treatments and all cultivars averaging 97 ± 4%. The calli produced in 'AUSmas', 'Morden Sunrise', 'HARflow', and 'MEIneble' (Red Meidiland™) were dark brown on media supplemented with 2,4-D and 2,4,5-T. The calli produced by 'William Baffin' was pale yellow on both media. 'Betty Prior' and 'John Franklin' both produced light beige mucilaginous callus. Although the quantity of callus was not measured, there was notably less callus per explant for 'John Franklin' than the other cultivars. Rhizogenesis, in the form of aerial roots, occurred on the callus of 'Betty Prior', 'HARflow', and 'Morden Sunrise'.

The differences observed in color and texture of callus derived from the various cultivars are similar to previous

Table 3 Embryogenic responses of various rose cultivars (*Rosa* sp.) to Murashige and Skoog medium containing either 10 µM 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) or 10 µM 2,4-dichlorophenoxyacetic acid (2,4-D). (se, somatic embryo).

Cultivar	Embryogenic tissue initiation	
	2,4,5-T	2,4-D
'AUSmas' (Graham Thomas®)	+[a]	–
'Betty Prior'	+	+
'HARflow' (Easy Going™)	–	–
'John Franklin'	+	–
'MEIneble' (Red Meidiland™)	–	–
'Morden Sunrise'	–	–
Rosa rugosa var. alba	+	–
'William Baffin'	+	+[a]

[a] Somatic embryos were present.

studies in which variation in callus morphology and color have been reported (review by Roberts *et al*. 1995). Callus morphology in rose differs with growth regulator treatments and with explant type (Kim *et al*. 2003). Although there are initial differences in callus morphology, a transition to pale, nodular, mucilaginous tissue seems to be necessary before somatic embryos develop (Roberts *et al*. 1995).

Five of the eight cultivars produced embryogenic tissue in response to the 2,4,5-T treatment whereas only two responded similarly to 2,4-D (**Table 3**). Two cultivars produced both embryogenic tissue and somatic embryos: 'AUSmas' on medium containing 2,4,5-T and 'William Baffin' on medium containing 2,4-D.

These findings support the premise that individual genotypes within a given species vary greatly in terms of the conditions required for embryogenic induction (de Wit *et al*. 1990; Hsia and Korban 1996; Marchant *et al*. 1996; Kintzios *et al*. 1999; von Arnold *et al*. 2002; Kim *et al*. 2003). The ET initiation rate in this study is high compared to studies using agents other than 2,4,5-T, which had rates of two out of twenty varieties (Murali *et al*. 1996) and one out of five (Kim *et al*. 2003). This suggests that 2,4,5-T may be a more effective auxin to use in rose ET induction.

The successful induction of embryogenic tissue with 2,4,5-T in 'AUSmas' also contrasts with a previous study in which SE could not be induced in this cultivar using a combination of 2,4-D and zeatin (Kim *et al*. 2003). The lack of embryogenic tissue production in 'HARflow', was somewhat unexpected, as this cultivar originated as a sport of 'HARwelcome'. Due to the genetic similarity between the two cultivars, it was anticipated 'HARflow', would respond to 2,4,5-T similarly to 'HARwelcome'. This finding needs further study but demonstrates the stringency of genotype specificity governing rose SE.

In conclusion, the findings of the present study demonstrate that 2,4,5-T may be useful for the initiation of embryogenic tissue in cultivars other than 'HARwelcome', including those unresponsive to 2,4-D. The findings have obvious benefits and applications for clonal propagation of roses, particularly 'AUSmas' and 'William Baffin'. Somatic embryos may also be utilized for the production of synthetic seeds. Other potential applications include the use of the SE systems as a means for genetic manipulations and transformation systems. The use of callus or embryogenic tissue is ideal since somatic embryogenesis arises from single cells and may be used to multiply transformed cells.

Germination was not tested for all cultivars in the present study so conversion rates would need further investigation. The findings also support the premise that individual genotypes within a given species vary greatly in terms of the conditions required for ET induction. Based on studies with 'HARwelcome', there appear to be morphological differences in PET and somatic embryos within a cultivar. The findings from studies with 'HARwelcome' support previous studies that cotyledon number would not be a suitable indicator of germination potential and that shoot development is capable despite a lack of an apical meristem.

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