Plant Cell Reports (2001) 20:175–182
DOI 10.1007/s002990000305

CELL BIOLOGY AND MORPHOGENESIS

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# Somatic embryogenesis on various potato tissues from a range of genotypes and ploidy levels

Received: 10 May 2000 / Revision received: 16 October 2000 / Accepted: 7 November 2000 / Published online: 8 February 2001


**Abstract** Somatic embryos (SEs) formed on in vitro-cultured stem internodes, leaves, microtubers and roots of 18 tetraploid potato (*Solanum tuberosum* L.) cultivars, diploid and monoploid germplasm and three wild *Solanum* species. A two-step protocol with 6-benzylaminopurine or thidiazuron in the first medium, and zeatin, indoleacetic acid and gibberellic acid in the second medium produced SEs within 14–28 days. SEs developed through the globular, heart and torpedo stages to produce thin-stemmed plantlets resembling potato seedlings. Plantlets transferred to the greenhouse produced greenhouse tubers. Secondary SEs were observed at the base of germinating torpedo-stage SEs in culture. SEs formed on stem internode sections, leaves and microtuber slices of in vitro-grown plants. Genotypic differences in regenerative capacity were clearly evident.

**Keywords** Regeneration · Tissue culture · Embryoids · *Solanum tuberosum*

**Abbreviations** *BAP* 6-Benzylaminopurine · $GA_3$ Gibberellic acid · *IAA* Indoleacetic acid · *SE* Somatic embryogenesis · *TDZ* Thidiazuron

## Introduction

Regeneration of somatic embryos (SEs) from plant tissues has been reported for many plant species (see reviews: Raghavan 1986; Bajaj 1995a; Brown et al. 1995). SEs reportedly originate from single cells and thereby avoid the problem of producing plants with chimeric tissues, which can occur when regeneration has originated from shoots (Nagmani et al. 1987; Nuti Ronchi and Giorgetti 1995). The large-scale production of plants (Denchev and Atanassov 1995) using bioreactors (Nishimura et al. 1993; Bajaj 1995a) is more efficient when SEs are used as propagules because SEs lend themselves to automation and to sorting techniques such as digital image analysis (Smith 1995). The rapid multiplication of plants via synthetic seed (Gray and Purohit 1991a; Redenbaugh et al. 1991; Redenbaugh 1993; Gray et al. 1995), in which SEs can be encapsulated for direct seeding of crops, will require regeneration over a large range of genotypes and ploidy levels. SEs can be used with cryopreservation techniques (Bajaj 1995b; Kunitake and Mii 1995; Nuutila et al. 1995) to alleviate rapid multiplication production difficulties and are a valuable tool for industry. Furthermore, flexibility is available if a propagation protocol has the capability of regenerating viable propagules from several explant sources such as leaves, roots, tuber sections and stem sections.

There are reports of SEs on potato tissues in vitro (Bragdø-Aas 1977; Lam 1977; Upadhya and Chandra 1977, 1978; Pretová and Dedičová 1990; García and Martínez 1995). SEs have also been described on several explant tissues – leaf callus (Upadhya and Chandra 1977; Chandra et al. 1983), tuber discs (Bragdø-Aas 1977; Lam 1977), stem nodal sections from in vitro-grown plants (García and Martínez 1995), stem segments from in vitro-grown plantlets (Upadhya and Chandra 1977, 1978) and zygotic embryos (Pretová and Dedičová 1990). Most SE regeneration protocols use tetraploid plants (Bragdø-Aas 1977; Lam 1977; Upadhya and Chandra 1978; Pretová and Dedičová 1990; García and Martínez 1995). However, regeneration from diploid or dihaploid material has been reported (Chandra et al. 1983). None of the published protocols noted above effectively regenerate SEs on an extensive range of tetraploid potato cultivars as well as diploid and monoploid tissues and wild *Solanum* species.

Communicated by S. Gleddie

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**Table 1** Somatic embryo (*SE*) formation on stem internode explants of potato (*Solanum tuberosum* L.) in vitro

| Potato cultivars | Number of explants | Number of explants with SEs | Time to initiate SE formation (weeks) | Total time for explants to produce SEs (weeks) |
|---|---|---|---|---|
| AC Novachip | 140 | 139 | 2 | 5 |
| Atlantic | 119 | 119 | 2 | 4 |
| Brador | 120 | 120 | 3 | 5 |
| Caribe | 109 | 41 | 3 | 7 |
| Désirée | 40 | 40 | 3 | 4 |
| F80054 | 110 | 110 | 3 | 6 |
| F83065 | 123 | 123 | 3 | 6 |
| Katahdin | 147 | 134 | 2 | 5 |
| Kennebec | 160 | 156 | 2 | 5 |
| Royal Gold | 30 | 24 | 3 | 4 |
| Ruby Gold | 32 | 3 | 4 | 2 |
| Russet Burbank | 72 | 72 | 3 | 5 |
| Russet Norkotah | 127 | 111 | 3 | 5 |
| Saginaw Gold | 113 | 102 | 3 | 6 |
| Shepody | 120 | 20 | 3 | 6 |
| Superior | 121 | 121 | 2 | 3 |
| Temagami | 30 | 5 | 5 | 5 |
| Yukon Gold | 114 | 58 | 2 | 5 |

## Materials and methods

### In vitro stock plantlets

Stock tissue culture plantlets of potato cultivars propagated by single-node cuttings were established from shoot tips excised from potato sprouts that had been disease-tested (Seabrook and Douglass 1994). The basal culture medium (medium 1) for plantlet propagation was a Murashige and Skoog (1962) salt solution, vitamins, 30 g/l sucrose, no growth regulators and solidified with 6.5 g/l agar (A 1296, Sigma, St. Louis, Mo.). All media used in this study were autoclaved at 121°C for 15 min. Microtubers were produced by placing single node cuttings from in vitro-grown potato plantlets in a medium containing 90 g/l sucrose.

### Culture conditions

Stock plantlet cultures of 16 potato cultivars and two advanced breeding lines and diploid and monoploid clones (Table 1) were maintained in test tubes (150×25 mm) containing 10 ml of medium 1. The cultures were grown in a tissue culture growth room maintained at 19°±1°C under a 16 h (day)/8 h (night) photoperiod with light provided by cool-white fluorescent lamps (CWF VHO F48T12) at an irradiation intensity of 100 µmol $m^{-2}$ $s^{-1}$. Plantlet cultures were dissected into nodal sections and transferred to fresh medium every 4 weeks. Care was taken to keep the stock cultures vigorous by regular transfers to fresh medium and by selecting only healthy-looking plantlets for explant material. Explants from cultured plantlets were maintained in a Conviron T16 controlled environment cabinet at 19°±1.0°C under a 16 h (day)/8 h (night) photoperiod with light provided by cool-white fluorescent lamps (F40T12/CW/EG) at an irradiation of 120 µmol $m^{-2}$ $s^{-1}$.

### Induction of nodular callus and SEs

The following explants from in vitro-grown potato plantlets were tested for morphogenic ability: stem internode sections, 0.5–1.5 cm in length, placed in a horizontal position; leaves, roots and transverse slices (approx. 1.0 mm thick) from microtubers. Medium 2 for the induction of nodular callus on explants was the same as basal medium 1 except for the addition of 19 µ*M* IAA and 0.15 µ*M* TDZ or 0.15 µ*M* BAP. Auxin can be omitted from medium 2.

**Table 2** Number of potato somatic embryos (*SEs*) per stem internode explant after 1 week on medium 2 and 9 weeks on medium 3. See Table 1 for the number of explants per cultivar (*SEM* standard error of the mean)

| Cultivar | Mean number of SEs per stem explant (±SEM) |
|---|---|
| AC Novachip | 13.5±8.6 |
| Atlantic | 15.9±8.3 |
| Brador | 23.1±11.2 |
| Caribe | 6.5±7.8 |
| Désirée | 36.1±20.7 |
| F80054 | 25.1±18.7 |
| F83065 | 44.5±23.5 |
| Katahdin | 14.3±9.8 |
| Kennebec | 10.2±6.7 |
| Royal Gold | 17.4±12.2 |
| Ruby Gold | 3.0±2.2 |
| Russet Burbank | 28.4±17.0 |
| Russet Norkotah | 10.5±8.8 |
| Saginaw Gold | 8.5±6.2 |
| Shepody | 3.9±4.0 |
| Superior | 10.7±5.9 |
| Temagami | 4.1±3.2 |
| Yukon Gold | 2.2±1.8 |

After 7–14 days of growth explants were transferred to medium 3 consisting of medium 1 with 12 µ*M* zeatin, 50 n*M* IAA and 550 n*M* $GA_3$. No further transfers to fresh medium were required. The media components $GA_3$, IAA and TDZ were filter-sterilized prior to being added to cooled autoclaved media. The cultures on medium 3 were observed once every week, and data on the number of explants which produced SEs and the number of SEs formed were recorded for each clone (Table 2). SEs at the torpedo stage of development were excised when they were approximately 0.5 cm in length and placed on medium 1. Observations on growth characteristics and morphology of the resulting emblings were recorded.

### Explant sources

Stem internodes, leaves and microtubers from in vitro-grown plantlets were used as explants. True seeds of *Solanum caps-*

*icibaccatum*, *S. polyadenium* and *S. trifidum* were surface-sterilized and germinated in vitro (Singh et al. 1988). Stem internode sections were dissected from the seedlings. Care was taken not to use the node region of stem explants so that tissue from axillary buds was not part of the stem internode explant. Both young leaves from the apical region of the plantlet and older leaves from the distal portion of the plantlet were cultured. Microtubers, 1–2 cm in diameter, which had been produced in vitro, were sectioned transversely into discs 1–3 mm thick. Again, care was taken to remove any buds from tuber eyes.

### Scanning electron microscopy

A Joel model JSM6400 (Joel USA, Peabody, Mass.) scanning electron microscope at an accelerating voltage of 5 kV, equipped with a Emscope SP-2000 cryostage (Emitech, Ashford, Kent, UK), was used to observe specimens that had been sputter-coated with gold. The temperature inside the microscope was –1,500°C.

## Results and discussion

### Stock plantlets

Frequent re-culturing and maintenance of plant vigour appeared to influence the time to form SEs and the number of SEs formed on potato explants. All explants (microtubers, leaves, stem internodes and roots) were excised from in vitro-grown potato plantlets. Quraishi and John (1985) noted that the rejuvenation of potato tissues associated with in vitro culture influenced the amount of callus and shoots formed.

### Orientation of explant in medium

Upon transfer to media 2 and 3, stem internode explants were placed in a horizontal position. In this orientation the distal end of the stem internode section formed more nodular callus than the proximal end of the stem explant (Fig. 1a). Placing stem internodes in a vertical position, with a acropetal or basipetal orientation, resulted in only a few SEs. Explant orientation has been shown to be an important factor controlling somatic embryogenesis in many plants (Leshem et al. 1982; McClelland and Smith 1990; Santarem et al. 1997).

### Development of nodular callus on potato tissues

On medium 2 a pale-yellow, nodular callus formed at the cut surface of the basal end of stem internode explants (Fig. 1a, b). A similar callus formed in smaller quantities on the proximal end of stem internode sections and on cut surfaces of leaf, root and tuber slice explants. Nodular callus usually formed 1–2 weeks after the transfer onto medium 2 for stem internode explants and approximately 1 week later for other explants. After the transfer to medium 2, tissues increased in size fivefold within 2 weeks.

### Regeneration of SEs

SEs formed within 2–3 weeks after explants were transferred to medium 3 (Table 1) and were observed first on the perimeter of the nodular callus; they subsequently formed over all of the callused cut surface. Over 80% of explants from cvs. AC Novachip, Atlantic, Brador, Désirée, Katahdin, Kennebec, Superior and F80054 formed SEs within 5 weeks (Table 2). Cultivars Caribe, F83065, Ruby Gold, Shepody and Temagami formed fewer than 14% SEs within 5 weeks (Table 1). After 8 weeks in culture more than 80% of stem internode explants from 13 cultivars had produced SEs (Table 2). SEs were more prevalent on the distal surface of stem internode explants, but this was genotype-dependent (Fig. 1a). Occasionally, SEs formed on the lateral surface of the stem explants. Wounding tissues appeared to stimulate embryogenesis, confirming reports of similar findings by Sidorov et al. (1999) and Santarem et al. (1997) on potato and soybean, respectively. When auxin was present in the medium few SEs were formed on Royal Gold, Ruby Gold and Temagami explants (Table 1); if auxin was omitted from medium 3 more SEs were produced. Sensitivity to auxin and wounding probably share common features (Dudits et al. 1995). Komamine et al. (1992) noted that auxin is only necessary for the formation of meristematic cell clusters and is inhibitory to subsequent developmental phases of SE.

In vitro-grown tissues of stem internode (Tables 1, 3), leaf blades, roots and microtuber slices (Table 4) formed SEs. This is a more extensive range of explants than previously reported (Bragdø-Aas 1977; Lam 1977; Upadhaya and Chandra 1977; Chandra et al. 1983; Pretová and Dedičová 1990; García and Martínez 1995). The SEs (Fig. 1) observed conformed to the expected morphology (Yeung 1995) and were similar to zygotic embryos of potato (Seabrook and Douglass 1990).

SEs have been reported in cultures of potato (Bragdø-Aas 1977; Lam 1977; Upadhya and Chandra 1977, 1978; Chandra et al. 1983; Pretová and Dedičová 1990; García and Martínez 1995). García and Martínez (1995), in the most recent report on SEs in vitro, used a stock plantlet medium containing $GA_3$, which may have pre-disposed the tissues for regeneration. In the study reported here, the vigour of plantlets was maintained by frequent transfers, the selection of only healthy material for explants and omission of $GA_3$ from medium 1. Furthermore, García and Martínez (1995) report that they used nodal explants, while we report here that stem internodes, leaves, tuber slices and roots can form SEs. The nodal explants cultured by García and Martínez (1995) produced a friable, green callus, whereas the explants reported here produced a light-green/yellow, nodular and compact callus. The use of cytokinins in the media 2 and 3 reported here is a major difference from the protocols of García and Martínez (1995) in which no cytokinin was used. SEs formed in 150 days for García and Martínez (1995) versus the 14–35 days reported here (Table 1). The range of tissues (Tables 1, 3) and genotypes (Table 1) on which

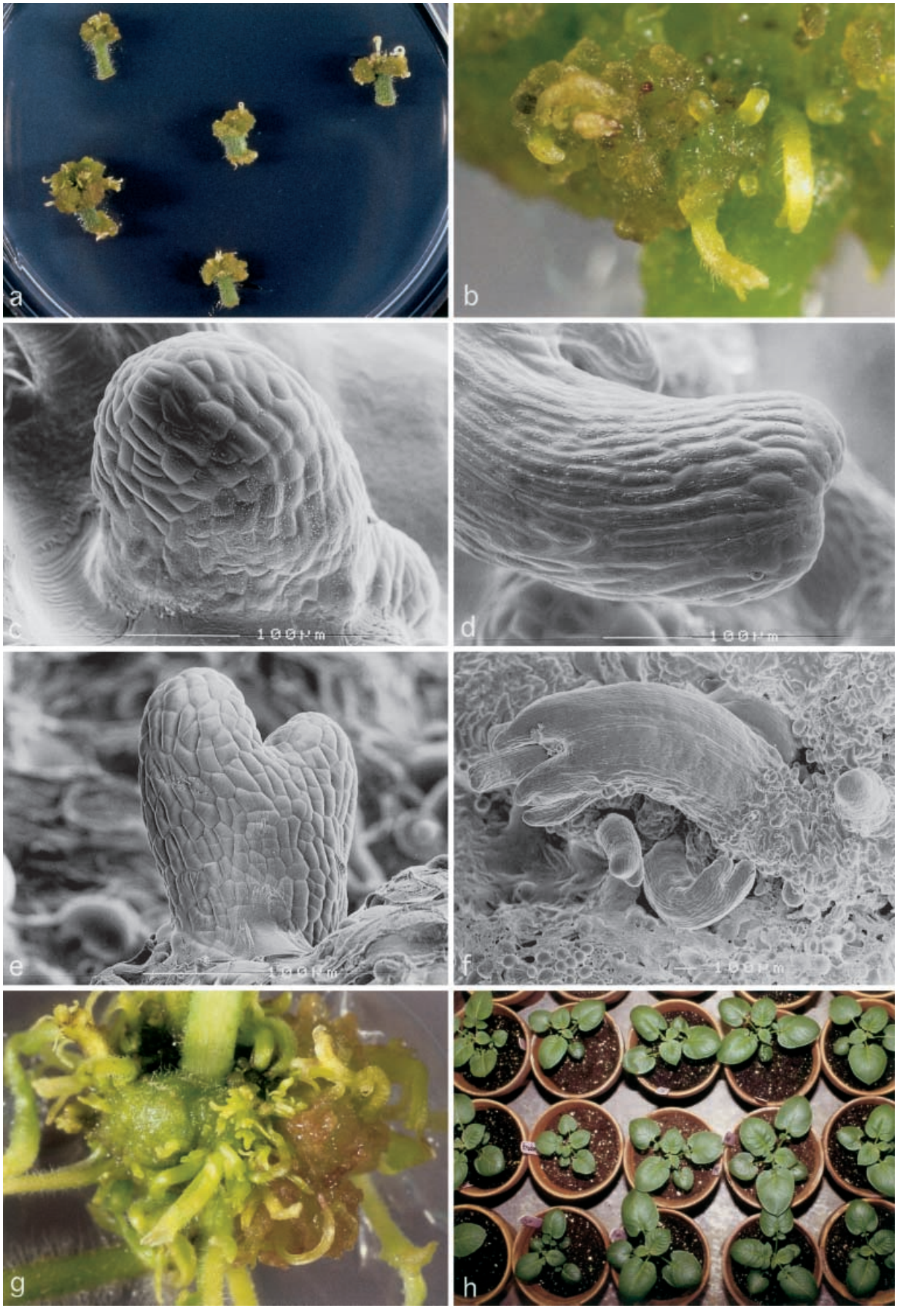
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**Table 3** Somatic embryo (*SE*) production on stem internode explants in vitro of diploid *Solanum* species, diploid potato breeding lines and monoploids derived from anther culture of a diploid breeding line[a] of 50% tbr, 50% chc and wild Argentinian species

| Solanum species | Number of explants | Number of explants with SEs | Time to initiate SE formation (weeks) | Total weeks in SE production |
|---|---|---|---|---|
| *S. capsicibaccatum* | 30 | 17 | 4 | 6 |
| *S. polyadenium* | 27 | 13 | 2 | 7 |
| *S. trifidum* | 30 | 26 | 3 | 5 |
| Diploid breeding lines | | | | |
| 590012.11 | 14 | 14 | 3 | 3 |
| 590001.7 | 35 | 15 | 3 | 5 |
| 590015.1 | 15 | 2 | 4 | 4 |
| 590016.7 | 11 | 6 | 3 | 5 |
| 591128.43 | 30 | 30 | 3 | 3 |
| Monoploids from anther culture of 2n 9507.04 | | | | |
| 9507.04 | 24 | 9 | 5 | 4 |
| 9507.04 | 36 | 35 | 4 | 4 |

[a] The diploid breeding line was a hybrid of *Solanum tuberosum* (tbr), *S. chacoense* (chc) and wild Argentinian species

**Table 4** Somatic embryo (*SE*) production on microtuber and leaf explants of tetraploid potato cultivars in vitro

| Explant | Cultivar | Number of explants | Number of explants producing SEs | Time to initiate SEs (weeks) | Weeks in SE production |
|---|---|---|---|---|---|
| Microtuber slices | F83065 | 99 | 53 | 3 | 4 |
| Microtuber slices | Russet Burbank | 80 | 71 | 2 | 7 |
| Leaves | Shepody | 14 | 5 | 5 | 4 |
| Leaves | Royal Gold | 4 | 1 | 5 | 4 |

SEs formed in the present investigation is extensive, whereas García and Martínez (1995) used only stem tissue from cv. Désirée. SEs were produced on every potato clone we tested, including 18 tetraploid cultivars (Table 1), several diploid clones, monoploids and three species (Table 2), whereas García and Martínez (1995) report only that cv. Désirée was regenerative. In addition to regenerating SEs on 18 tetraploid potato cultivars, we also observed SEs on stem internode tissues of three wild *Solanum* species, five diploid breeding lines and two monoploid clones (Table 3).

Apical dominance was apparent in many of the cultures. Clusters of proembryos and globular- and heart-shaped embryos frequently formed at the base of germinating SEs. If a dominant SE was not removed from the explant when approximately 0.5 cm in size, the growth and development of other SEs in the cluster did not continue.

## SEs on leaf and tuber explants

SEs were regenerated from leaf sections and microtuber slices from in vitro-grown plantlets (Table 4). Sections from young leaves produced SEs, but older leaves did not respond. The techniques reported here are therefore suitable for regeneration from several different tissues.

◀ **Fig. 1a–h** Somatic embryos (*SEs*) forming on potato (*Solanum tuberosum* L.) tissues. **a** Callus and SE formation on the distal end of stem internode explants of cv. Russet Burbank (×1). **b** Developing SEs on explants as in **a** (×10). **c**–**f** SEM of SEs, cv. Russet Burbank: **c** Globular-stage SE. **d** Heart-stage SE, **e** Late-heart/early-torpedo-stage SE showing typical unequal growth of coltyledons, **f** Torpedo-stage SEs, **g** SEs of cv. Russet Burbank in various stages of development (×10). **h** Cultivar Brador plantlets derived from SEs growing in the greenhouse (×0.2)

## Secondary SEs

Secondary embryos did not readily form in our cultures of potato. SEs were observed forming at the base of germinating embryos and, on occasion, several were noted in various stages of development (Fig. 1f). However, a scanning electron microscope study of the embryo-forming cultures revealed that this was fairly uncommon (Fig. 1c–f). While embryogenic callus could not be subcultured continuously to produce potato SEs, and fresh stem internode explants had to be cultured to produce more embryos, re-culturing SEs at the late-torpedo stage or germinating SEs on media 2 and 3 produced nodular callus which was highly regenerative. The regeneration of new SEs occurred at the basipetal end of the re-cultured SEs after nodular callus formation. Stem internode sections from germinating SEs regenerated new SEs after 7 weeks of culture compared with the 3–5 weeks that SEs take to form from cultured potato plantlet stem internode explants.

## Conversion and germination of SEs to form regenerated plantlets

The development of SEs from the early-torpedo stage to regenerated plantlets took approximately 2–4 weeks.

There was no discernible halt in development except when a large, vigorous SE became dominant. Potato SEs at the late-torpedo stage were easily dissected from the explant tissue and "converted" (Nickle and Yeung 1994) readily into plantlets. These plantlets resembled seedlings from true potato seeds; that is, they had a vine-like growth habit with thin stems, and small leaves. SEs at the early-torpedo stage developed and germinated to become plantlets within approximately 2–4 weeks. Thus, no quiescence or dormancy (Gray and Purohit 1991b; Nickle and Yeung 1994) was observed in the potato SE system reported here, although no attempt was made to apply the stress of desiccation to the material. Gray and Purohit (1991b) note that SEs rarely become dormant.

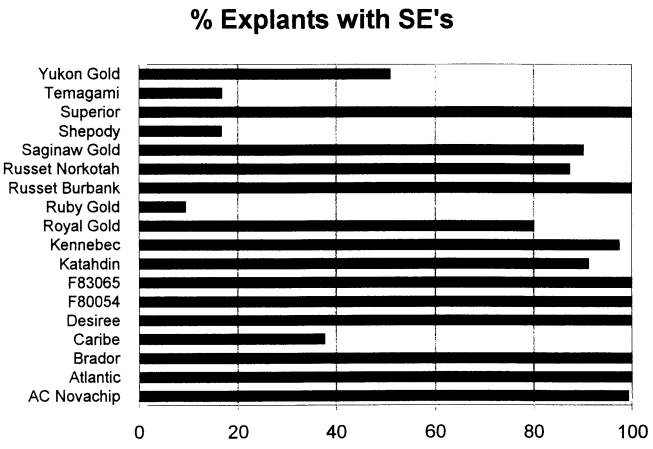


**Fig. 2** Percentage of potato stem internode explants with SEs after 1 week on medium 2 and 9 weeks on medium 3

## Greenhouse plants from SEs

Germinating SEs were acclimatized to greenhouse conditions and resembled true potato seedlings in growth habit. Minitubers were harvested after 3 months. Some off-types were observed, and this appeared to be influenced by genotype. Off-types have been reported from tissue culture regimes of potato (Harding 1994, 1995).

## SEs on potato material of various ploidy levels

To the best of our knowledge the induction of SEs on potato germplasm at the tetraploid, diploid and monoploid levels has never been reported. We succeeded in regenerating SEs on tissues of five diploid breeding lines, two monoploid lines generated via anther culture and three wild *Solanum* species: *S. capsicibaccatum*, *S. polyadenium* and *S. trifidum* (Table 2).

## Regeneration of SEs – genotypic differences

The productivity of the SE technology is clearly under genetic control (Tables 1, 2, 3). The time to form SEs (Table 1), the number of explants responding to induction techniques (Table 2) and the number of SEs per explant (Table 2) all vary with the cultivar being tested. Royal Gold, Ruby Gold and Temagami had a low percentage of explants producing SEs (Table 1) and are siblings from the same breeding programme (Ali-Khan et al. 1996a, b). A strong genotypic component in the ability of tissues to form SEs is therefore evident. Gleddie et al.(1983) reported that although SEs formed on leaf explants of all seven *S. melongena* (eggplant) cultivars tested, there were significant differences in the number of SEs formed. Tables 1 and 2 indicate that this is also the case for *S. tuberosum*.

Clonal differences in the number of stem internodes which produced SEs embryos were evident (Tables 1, 2, 3, 4). Most cultivars produced SEs on all explants, but potato cultivars Caribe (28%), Ruby Gold (9%), Shepody (17%), Temagami (17%) and Yukon Gold (51%) only produced SEs on a portion of the cultured explants (Fig. 2). Changes in media formulation were required to produce SEs on some potato cultivars. For instance, Ruby Gold and Temagami would only form SEs when placed on medium 2 with auxin omitted. While the presence of auxin is mandatory for the induction of SEs in most plant systems (Raghavan 1986), we determined that exogenous auxin is not necessary in medium 2 although it does appear to be required in medium 3.

Potato clones varied in the time required to produce SEs (Table 1). The cultivars which readily formed SEs in culture – AC Novachip, Atlantic, Brador, Désirée, F80054, Kennebec, Russet Norkotah, Russet Burbank, Saginaw Gold and Superior – generally produced SEs on all of the explants within 3–4 weeks on medium 3. Notable exceptions were Ruby Gold and Temagami: Ruby Gold took 5 weeks to produce only 1 explant out of the 32 SEs cultured, and Temagani required 6 weeks to produce 2 explants out of the 30 SEs cultured.

The mean number of SEs produced per explant was also under genotypic control (Table 2). Rankings for the cultivars tested are expressed as mean number of SEs per explant: F83065 (45), Désirée (36), Russet Burbank (28), F80054 (25), Brador (23), Royal Gold (17), Atlantic (16), Katahdin (14), AC Novachip (14), Superior (11), Russet Norkotah (11), Kennebec (10), Saginaw Gold (9), Caribe (7), Temagami (4), Ruby Gold (3) and Yukon Gold (2).

SEs were produced on 18 cultivars of potato (*Solanum tuberosum* L.), three ploidy levels and three wild species in vitro. This protocol can be used for clonal multiplication of selected potato genotypes, regeneration after gene transformation or bombardment and the large-scale production of SEs for artificial seed production. SEs can be induced to form on various somatic tissues of the potato – stem internodes, roots, leaves, microtuber slices. Adjustments in the availability of auxin is necessary for the regeneration of SEs on tissues of

some cultivars of potato. Genotypic differences in SE production require further investigation, and we are currently pursuing this by testing the regenerative capacity of individuals of reciprocal crosses between potato lines.

**Acknowledgements** The technical assistance of Ms. Eve Brown is gratefully acknowledged. We thank Mr. Gerry Bance, University of New Brunswick (UNB) Electron Microscope Unit (EMU), for the operation of the SEM and Ms. Susan Belfry of EMU, UNB and Mr. Roger Smith of the Biology Department, UNB for the preparation of photographs.

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