# Asparagus somatic embryogenesis: detection of somaclonal variation using molecular and cytological analyses

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**Dedicated to:**

My parents - Rudolph and Shirley Hollingsworth

*".....because you love me....."*

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*In pursuit of excellence !!!!!!*

## Abstract

The embryogenic potential for six asparagus cultivars (Aspiring, Karapiro, Pacifica, Turoa, Syn4, and UC157), and the genetic stability of the somatic embryogenic system were investigated. Experiments 1 to 3 investigated the embryogenic potential of select cultivars, whereas experiments 4 to 7 analysed the genetic stability of embryogenic cells and plantlets. In experiment 8, morphological, anatomical, cytological and molecular techniques were used to characterise different types of calli identified during the study.

For all cultivars, embryogenic callus was promoted on Murashige and Skoog (MS) media containing 3% sucrose, 1% agar and one of the following plant growth regulator (PGR) concentrations: 0.3, 1, 3, and 10 µM 2,4-D and 1.0 µM NAA/ 0.1 µM Kinetin. Plant genotype, PGR concentration and length of time in culture significantly influenced both the number of explants producing calli and the type of calli developing from explants.

The following sequence was found to be most effective in producing complete plantlets from embryogenic calli: callus induction (CI) on Murashige and Skoog (MS) media containing 3% sucrose, 1% agar and either of 1.0, 3.0 and 10 µM 2,4-D, followed by transfer onto liquid embryo induction media (EI) containing MS + 6% sucrose and finally regeneration on regeneration media (Rg4) containing MS + 0.2 g/l glutamine + 3% sucrose + 1% agar. Treatment of 'Pacifica' globular embryos at -15 $^{0}$C for 3 hr produced the highest percent converted plantlets (34 and 26% for 6-month-old embryogenic calli and 1 year-old embryogenic suspension cells respectively).

The number of *in vitro*-regenerated asparagus plantlets surviving acclimatisation was increased by acclimatising plantlets with minicrowns that contain 2-5 storage roots, and by removal of *in vitro*-formed cladophylls prior to acclimatisation.

Random amplified polymorphic DNA (RAPD) markers distinguished among asparagus cultivars, and revealed differences within seed-raised commercial cultivars. The RAPD

technique also detected changes in genomic DNA structure induced during culture of embryogenic cells. No change in genomic structure of plantlets regenerated from somatic embryos was detected.

Cytological analysis, using chromosome counts and DNA content analysis, were used to determine the genetic stability of embryogenic calli, suspension cells, and plantlets regenerated through somatic embryogenesis. The basic chromosome number of 20 (2n = 20) remained unchanged for all samples. The DNA content of explants and plantlets was similar, indicating that plantlets were diploid. The experiment was unable to detect somaclonal variation, revealed by altered ploidy level indicating that cytological analysis is not as sensitive as RAPD analysis for detecting somaclonal variation.

Extracellular protein profiles generated for embryogenic cells grown in suspension culture were influenced by PGR concentration and length of time in culture, and were therefore not suitable for monitoring somaclonal variation.

Overall, individual cultivars produced between 6 to 8 different calli types for all PGR treatments. Plant genotype and PGR treatment influenced the phenotype of calli developed for each cultivar. The results indicate that, for the six asparagus cultivars investigated in this study, nodular calli or nodular mucilaginous calli have more embryogenic potential than other calli types. These calli were also noted to produce embryogenic cells in suspension, and could, therefore, be used to successfully inoculate liquid cultures either for small or large-scale production of asparagus somatic embryos.

**Keywords**

*Asparagus officinalis* L., *in vitro*, plant growth regulator, somatic embryos, embryogenic calli, maturation, regeneration, plantlet acclimatisation, random amplified polymorphic DNA (RAPD), chromosome count, DNA content, extracellular protein

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| | |
|---|---|
| µg | microgram (s) |
| µl | microlitre (s) |
| µm | micrometer (s) |
| µM | micromolar (s) |
| µmol | micromole (s) |
| 2,4-D | 2,4-dichlorophenoxyacetic acid |
| 2C | nuclear DNA content of unreplicated diploid chromosome complement |
| ABA | Abscisic acid |
| AFLP | Amplified fragment length polymorphism |
| ASP | Asparagus cultivar Aspiring |
| BA (BAP) | Benzylamino purine |
| CFLP | Cleavase fragment length polymorphism |
| Chl- | Chlorophyll deficient embryos |
| Chl+ | Globular embryos containing chlorophyll |
| CI | Callus induction |
| CRD | Complete random design |
| DNA | Deoxyribonucleic acid |
| EI | Embryo induction |
| g | grams |
| $GA_3$ | Gibberellic acid |
| hr | hour (s) |
| IAA | 3-indole acetic acid |
| IEDC | Induced embryogenic determined cell |
| kD | kilodalton |
| kg | kilogram (s) |
| Kn | Kinetin |
| KP | Asparagus cultivar Karapiro |
| l or L | litres |
| LEA | Late embryogenesis protein |
| mg | milligram (s) |
| min | minute (s) |
| mm | millimetre (s) |
| MS | Murashige and Skoog |
| MW | molecular weight |
| NAA | α-Napthaleneacetic acid |
| °C | degrees Celsius |
| PC | Asparagus cultivar Pacifica |
| PCR | Polymerase chain reaction |
| PEDCs | Pre-embryogenic determined cell |
| PEG | Polyethylene glycol |
| PEMs | Proembryogenic masses |
| PGR | Plant growth regulator (s) |
| PI | Propidium iodide |
| Pur | Globular embryos with purple pigment |
| RAPD | Random amplified polymorphic DNA |

| | |
|---|---|
| RFLP | Restriction fragment length polymorphism |
| Rg | Regeneration |
| SEM | Standard error of mean |
| SN | Asparagus cultivar Syn4 |
| SSCP | Single stranded conformation polymorphism |
| TU | Asparagus cultivar Turoa |
| UC | Asparagus cultivar UC157 |

# SECTION 1

## ASPARAGUS PRODUCTION