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Biotechnology of *Schlumbergera* and *Rhipsalidopsis*

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ABSTRACT

Biotechnological tools have been successfully used with several plant species for improving existing cultivars and creating new ones. However, the application of such tools has been limited with ornamental plant species such as cactus. Cacti are known to possess a very slow growth rate and many of them are recalcitrant compared to other cultivated plant species thus creating problems in culturing or improving them. Some early work showed promise in establishing *in vitro* cultures with limited multiplication rates, but research findings recently indicate that *Schlumbergera* (Christmas cactus) and *Rhipsalidopsis* (Easter cactus) can be multiplied more rapidly via both axillary and adventitious shoots, and also via callus and somatic embryogenesis. These studies have discovered key factors that could enhance an understanding of morphogenesis in cacti. Studies were made on endogenous phytohormones and enzymes to elucidate the differences between the recalcitrant cultures and those with increased regenerative capacity of these plants. Recent studies have also shown that *Agrobacterium*-mediated genetic transformation is possible and a transformation efficiency of about 23% in *Rhipsalidopsis* is reported. This transformation efficiency was based on transgenic shoots expressing the activity of the reporter *β-glucuronidase* gene (GUS) indicated by blue staining of the regenerated shoots. Transgenic shoots regenerated in these studies through callus phase. Transformation was confirmed in the selected transgenic callus lines by GUS staining (for *uid*A gene), ELISA analysis and Southern blot hybridization (for *npt*II gene). The stability of transgenic calli was confirmed through their continuous sub-culturing on media containing the selectable marker kanamycin followed by testing for kanamycin resistance, GUS analysis and Southern hybridization. The regeneration system adopted for transformation was reliable as shown by the stable regeneration of calli and shoots growing on media both on selection and control media. However, due to the slow growth rate of these plants, no attempts were made to verify transformation in the greenhouse-grown plants.

Keywords: adventitious shoots, *Agrobacterium tumefaciens*, Christmas cactus, Easter cactus, genetic transformation, multiple cotyledons, organogenesis

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; **BA**, 6-benzyladenine; **CaMV 35S**, Cauliflower mosaic virus promoter; **CKX**, cytokinin oxidase/dehydrogenase; **ELISA**, Enzyme-linked immunosorbent assay; ***etr1-1***, ethylene mutant receptor gene; ***fbp1***, flower specific promoter; **GA_3**, Gibberellic acid; **GUS**, β-Glucuronidase; **IAA**, Indole-3-acetic acid; **IBA**, indole-3-butyric acid; **MS**, Murashige and Skoog (1962) medium; **NAA**, α-naphthaleneacetic acid; ***npt*II**, neomycin phosphotransferase gene; **POX**, total peroxidase; **SH**, Schenk and Hildebrandt (1972) medium; **TDZ**, Thidiazuron; ***uid*A**, coding region of the β-glucuronidase gene; ***vir***, Virulence gene; **Wm^{-2}**, photosynthetically active radiation

CONTENTS

INTRODUCTION

The global demand for flowering plants is increasing along with the demand for novelty in floriculture. Classical breeding has been used for creating new genotypes, but more recently, biotechnological methods have been used to create novelty in several plants (Chandler and Lu 2005; Tanaka *et al*. 2005). Increasing numbers of genes from wild types and the genes from unrelated species are being used for such creation.

Biotechnological methods have been invented for several floricultural plants for practices such as rapid propagation, virus indexing and genetic engineering. Protocols for mass propagation are also needed because of the frequent appearance of new cultivars and hybrids. Genetic engineering has produced novel plants such as purple carnation which is being marketed (Lu *et al*. 2003). Likewise, it should be possible to improve existing cultivars or to create new cultivars of cactus if appropriate biotechnological methods are established (several advances in Teixeira da Silva 2006).

Cacti have long been grown as ornamentals, and cer-

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Invited Mini-Review

tainly, the demand for them as potted plants is on the increase. *Schlumbergera*, popularly known as 'Christmas cactus' or 'Thanksgiving cactus', and *Rhipsalidopsis*, also known as 'Easter cactus', are epiphytes which are native to the forests in Brazil (Hammer 1992; Sriskandarajah and Serek 2004). The main economic importance of these cactus species lies in world-wide horticultural trade as ornamental plants. Christmas and Easter cactus are popular plants sold during the fall and spring times. These cacti are grown as flowering potted plants (Boyle 1997) and they make colourful potted plants which adapt well in the home and continue to re-flower with modest care (Dole and Wilkins 1999). The major countries for production of ornamental cacti include Denmark, USA, Japan, Great Britain, Germany and The Netherlands. A total of 8 and 2.8 million plants of *Schlumbergera* and *Rhipsalidopsis*, respectively, are produced annually in Denmark (GASA [Danish Growers Sale Organisation], Jørgen Andersen, pers. comm.). This constitutes about 70-80% of the production of the European market of these cacti (GASA [Danish Growers Sale Organisation], Jørgen Andersen, pers. comm.).

Cacti have members vulnerable to extinction because of over-collection and loss of habitat. Efficient propagation techniques are urgently required to help stop the disappearance of those endangered species (Rubluo *et al*. 1993). Plant species differ widely in their regenerative capacity. Cacti are known to be recalcitrant because of their slow seed germination, growth or lateral branching (Hubstenberger *et al*. 1992; Sriskandarajah *et al*. 2006). Recalcitrance can also be a major limiting factor for the *in vitro* regeneration. Some genera of cactus such as *Mammillaria* have been studied more extensively when compared to *Shlumbergera* and *Rhipsalisdopsis* (Papafotiov *et al*. 2001). *Opuntia* is another genus which has been worked on, and in this genus BA induced shoots while GA_3 induced spikes (Manuseth and Halperin 1975). Splitting or wounding also appeared to have had a positive effect on organogenesis. George (1996) has summarized the *in vitro* work on cacti and succulent plants. Indirect organogenesis in cactus callus has been reported by using high level of cytokinin (e.g. 10 mg/l 2iP) with a small amount of auxin (Johnson and Emino 1979). There has been advancement in the recent research findings on some species of cactus. This review will summarize the *in vitro* culture work of some economically important cultivars of *Rhipsalidopsis* (hybrids between *R. gaertneri* and *R. rosea*) and *Schlumbergera* (hybrids between *S. russeliana* and *S. truncata*).

MICROPROPAGATION

As the cacti exhibit slow growth (7 weeks for one phylloclade to develop), low seed production and germination, *in vitro* propagation could be a potential alternate method for propagation of these plants. Moreover, there is a need for rapid mass propagation protocols as new cultivars are continuously produced, and *in vitro* clonal propagation will also help to maintain the traits of the respective cultivars (Hubstenberger *et al*. 1992). Areole activation through breaking apical dominance is the most effective way to attain micropropagation in cacti. This was found in many cactus species by using cytokinins rather than auxins (Hubstenberger *et al*. 1992; Rubluo 1997). Dabekaussen *et al*. (1991) reported on some factors for areole activation in explants of *Sulcorebutia alba* Rausch. They found that inclusion of cytokinin BA followed by kinetin yielded best results and this was an essential prerequisite for areole activation. Optimal physical growth conditions for areole activation were a temperature at 27ºC and irradiance at 2.3-5 W m^{-2}. Some early work by Johnson *et al*. (1976) and Perez *et al*. (1999) has shown that *Schlumbergera* and *Rhipsalidopsis* could be propagated *in vitro*, but the multiplication rate obtained was not considered adequate. It took a minimum of 8 weeks to produce about 9 shoots per explant in a rotated liquid LS (Linsmaier and Skoog 1965) medium substituted with 10 mg/l kinetin. It should be noted here that liquid medium is more risky and cumbersome to use when compared to solid medium. More recently, Sriskandarajah and Serek (2004) reported on rapid propagation methods via both axillary and adventitious shoot regeneration systems in some economically important cultivars of both *Schlumbergera* and *Rhipsalidopsis*.

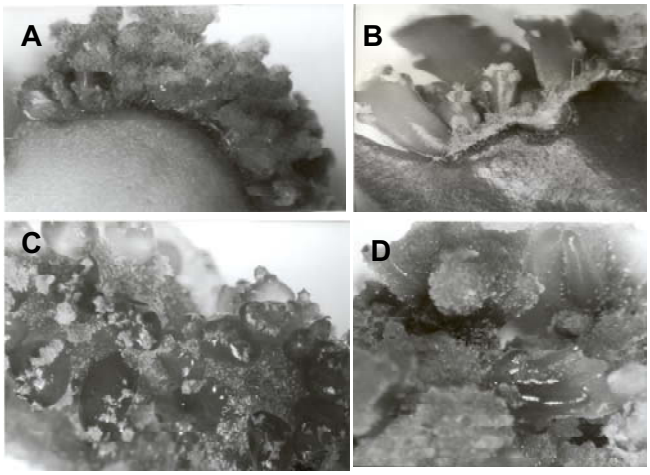

Fig. 1 Shoot regeneration in cultures of *Schlumbergera* and *Rhipsalidopsis*. (**A**) Apical phylloclade explants of cv. 'CB4' producing axillary shoots. (**B**) Apical phylloclade explants of Thor-Olga producing axillary shoots. (**C**) Adventitious shoots forming in cv. 'CB4' phylloclade explants from third subculture generation of shoot culture. (**D**) Adventitious shoots forming in callus clumps of Thor-Olga. Bars = 0.2 cm. (From **Sriskandarajah, Serek** (2004) *Plant Cell, Tissue and Organ Culture* **78**, 75-81, with kind permission Springer Science & Business Media.)

PROPAGATION VIA AXILLARY AND ADVENTITIOUS SHOOTS

Axillary shoot regeneration

Schlumbergera and *Rhipsalidopsis* could be propagated via axillary shoot regeneration by using phylloclade tips as explants. As indicated earlier, Johnson *et al*. (1976) were able to grow axillary shoots with limited multiplication rate using a liquid medium. In a more recent work, shoot numbers were increased to an average of 15 shoots of *Rhipsalidopsis* (cv. 'CB4', hybrid between *R. gaertneri* and *R. rosea*) when apical explants where grown on gelrite-based MS medium (Murashige and Skoog 1962) supplemented with 3.5 µM BA and 2.5 µM IBA in 4 weeks (Sriskandarajah and Serek 2004). Under these conditions, shoot initiation and growth rate in *Rhipsalidopsis* were 2-3 times greater than that in *Schlumbergera* (Sriskandarajah and Serek 2004; **Fig. 1A, 1B**). The latter methods were much more efficient and safe than those reported by Johnson *et al*. (1976) as there is more risk involved in contaminating liquid cultures than the solid cultures. Similarly, in the cactus *Mammillaria san-angelensis*, plant regeneration through *de novo* meristem formation and areole activation were observed (Rubluo *et al*. 2002). Sections of long-term subcultured shoots of *M. san-angelensis* responded to the addition of auxins by indirect organogenesis and the activation of the pre-existing axillary meristem (Rubluo *et al*. 2002).

Adventitious shoot regeneration

The first report on adventitious shoot production from phylloclade explants and callus cultures of *Rhipsalidopsis* and *Schlumbergera* was published by Sriskandarajah and Serek (2004). In this study, a pronounced improvement in adventitious shoot formation was reported in cultures by repeated subculturing of axillary shoots which originated from phylloclade explants. After the third subculture, 87% explants of *Rhipsalidopsis* cv. 'CB4' produced adventitious shoots at a frequency of about 12 shoots per explant. This occurred

on a solid MS medium containing 27 µM BA, 27 µM TDZ and 27 µM zeatin (Sriskandarajah and Serek 2004; **Fig. 1C**). Cultures from *Schlumbergera* failed to give such a response, however, adventitious shoots were produced from callus cultures (**Fig. 1D**).

Clumps of prolific callus were produced easily along the cut surfaces of phylloclade explants of *Rhipsalidopsis* and *Schlumbergera* (Sriskandarajah and Serek 2004; Al-Ramamneh *et al*. 2006a). Both species responded well to the callus induction medium based on MS salts, Staba vitamins (Staba 1969), 22.5 µM TDZ and 1.3 µM NAA. Cv. 'CB4' was more regenerative compared to the *Schlumbergera* cultivars, producing as many as 10 adventitious shoots per square cm of callus. These cacti also showed the ability to form somatic embryos as described elsewhere in this review. Subsequently, studies were made to improve the regeneration frequency by testing both solid and liquid media, and an efficient regeneration system was developed for *Schlumbergera* cv. 'Alex' and *Rhipsalidopsis* cv. 'CB5' (Al-Ramamneh 2006). Phylloclade explants were grown on MS-based solid medium containing 22.7 µM TDZ and 1.3 µM NAA to initiate callus. Callus developed on explants was sub-cultured on the same medium every two months over a period of nine to twelve months. Callus was then grown for 50 days for cv. 'Alex', or 30 days for cv. 'CB5', in liquid MS-based medium containing 7.0 µM kinetin or 4.7 µM kinetin for cv. 'Alex' and cv. 'CB5', respectively. The highest number of shoots in cv. 'Alex' (40 shoots per 1.0 g callus) and cv. 'CB5' (495 shoots per 0.5 g callus) were obtained when callus cultures were grown finally on solid MS-based medium devoid of plant growth regulators (Al-Ramamneh 2006). It appeared that a form of starvation from the hormones, in the last stage, had helped to regenerate shoots from these callus cultures.

Somatic embryogenesis

Induction of somatic embryogenesis has been reported in some cactus plants including *Turbinicarpus pseudomacrochele* (Torres-Muñoz and Rodríguez-Garay 1996), *Mammillaria san-angelensis* (Marín-Hernández *et al*. 1998), *Opuntia ficus-indica* (da Costa *et al*. 2001), *Ariocarpus kotschoubeyanus* (Moebius-Goldammer *et al*. 2003) and other succulent plants such as *Agave victoria-reginae* (Rodríguez-Garay *et al*. 1996). Recently, somatic embryogenesis was achieved by culturing phylloclade explants of *Schlumbergera truncata* cv. 'Russian Dancer' (Al-Ramamneh *et al*. 2006a). Callus that developed on phylloclade explants and sub-cultured over a period of 16 months on MS-medium containing cytokinins was superior for the induction of somatic embryos compared to the callus grown for a shorter time. Subculture of callus grown in SH- (Schenk and Hildebrandt 1972) or MS-based liquid media supplemented with 7.0 µM kinetin and then transferred onto solid MS-based medium with 0.45 µM 2,4-D or without hormones resulted in differentiation into somatic embryos. SH-based medium proved better than MS-based medium when used as the first medium for the induction of somatic embryogenesis. Callus grown in SH- based liquid media supplemented with 7.0 µM kinetin and transferred onto solid MS-based medium devoid of hormones developed new friable embryogenic callus from which somatic embryos differentiated three and half months later.

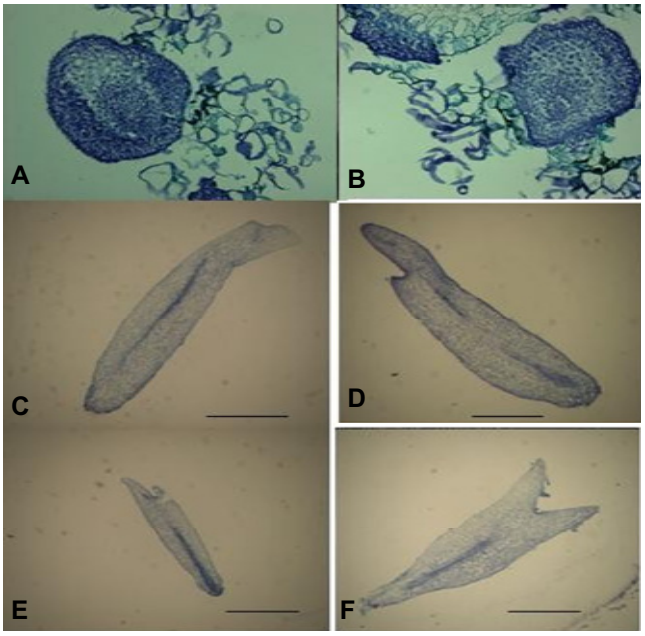

Fig. 2 Histological analysis of *Schlumbergera truncata* somatic embryos. (**A**) Globular embryo with visible protoderm (arrow) and a nearby section of callus cells preparing for somatic embryogenesis with apparent meristematic zones (arrowhead) characterized by dense cytoplasm and intensely stained nuclei (x10). (**B**) Oblong-shaped somatic embryo (x10). (**C**) Elongated embryo, with a closed basal end (arrowhead: bottom) and contained vascular system (arrow) (Bar: 350 µm). (**D**) Early torpedo-shaped somatic embryo, with a notch (arrow) in the middle of the shoot apex, giving rise to the first signs of the 2 cotyledons (arrowhead) (Bar: 240 µm). (**E**) and (**F**) Early and late-cotyledonary stage showing the gradual development of the 2 cotyledons; Bars: 300 µm (**E**); 240 µm (**F**). (From **Al-Ramamneh EA, Sriskandarajah S, Serek M** (2006a) *Plant Cell, Tissue and Organ Culture* **84**, 333-342, with kind permission Springer Science & Business Media.)

Seventy percent germination was recorded for dicotyledonous embryos, and plants derived from somatic embryos showed 81% survival rate when transferred to greenhouse conditions. The histological investigation showed that the somatic embryos progressed through the globular, oblong, elongation, torpedo and the two-cotyledon stages (Al-Ramamneh *et al*. 2006a; **Fig. 2 A-F**). It was also possible to characterise the distinct root and shoot poles and the discrete vascular system of the somatic embryos (Al-Ramamneh *et al*. 2006a; **Fig. 2C, 2D**).

During the course of somatic embryo development, different pattern(s) of cotyledon morphology and shoot apex structure of the embryos was observed. In addition to the normal dicotyledonous embryos, anomalous embryos with multiple cotyledons developed (**Fig. 3A-C**). About 75% of embryos in the cotyledonary stage were of the anomalous

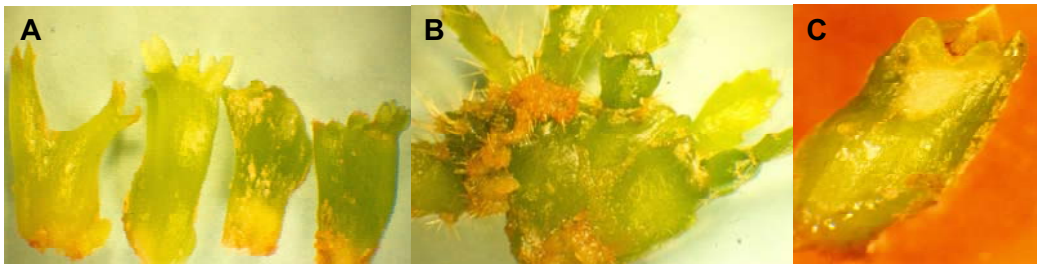

Fig. 3 Anomalous embryos of *Schlumbergera truncata*. (**A**) Embryos show multiple finger-like cotyledons (x6) (**B**) which formed later the new phylloclades (x6). (**C**) Vase-like embryos with cotyledons arranged in a circle at the embryo apex, with the new growth primordia located in the centre (x18). (From **Al-Ramamneh EA, Sriskandarajah S, Serek M** (2006a) *Plant Cell, Tissue and Organ Culture* **84**, 333-342, with kind permission Springer Science & Business Media.)

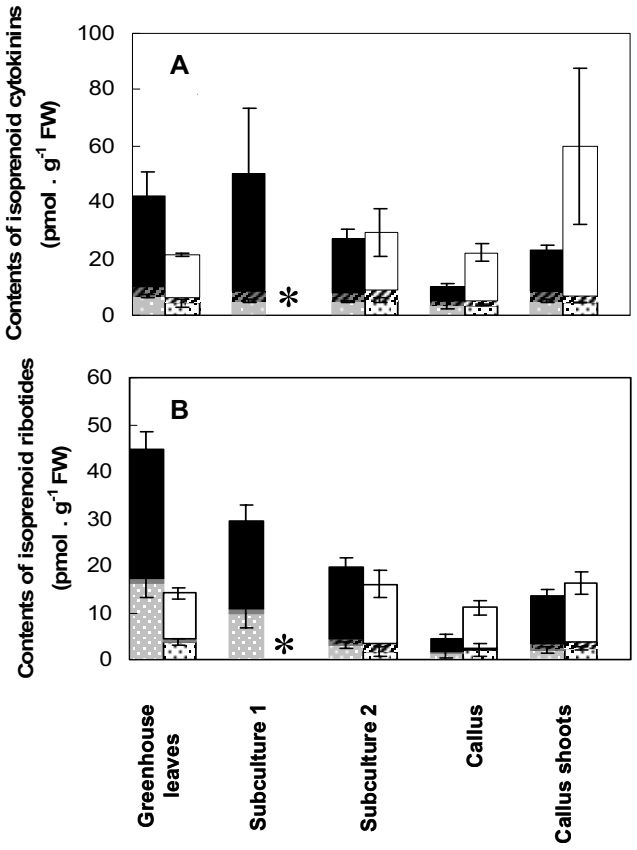

Fig. 4 (**A**) Total pool of isoprenoid type cytokinins in greenhouse leaves, during 2 subsequent stages of *in vitro* subculturing, callus culture and callus shoots. The data of the endogenous cytokinins are pooled according to the type. Lower bar: zeatin-type (*cis*-zeatin + *trans*-zeatin + *cis*-zeatin 9-riboside + *trans*-zeatin 9-riboside + zeatin 9-glucoside), middle bar dihydrozeatin-type (dihydrozeatin + dihydrozeatin 9-riboside + dihydrozeatin 9-glucoside) and upper bar iP-type (iP + iPR+ iP 9-glucoside). **B.** Total pool of isoprenoid ribotides. The data of the endogenous cytokinins are presented according to the type. Lower bar: zeatin-type (*cis*-zeatin 9-riboside-5′-monophosphate + *trans*-zeatin 9-riboside-5′-monophosphate), middle bar: dihydrozeatin 9-riboside-5′-monophosphate and upper bar: iPR-5′-monophosphate. Left (black bars), *Rhipsalidopsis* (cv. 'CB4'); right (white bars), *Schlumbergera* (cv. 'Thor-Olga'). All data are expressed as pmol.g^{-1} FW. n= 3. Error bar = STERR. (From **Sriskandarajah *et al.*** (2006) *Journal of Plant Growth Regulation* **25**, 79-88, with kind permission Springer Science & Business Media.)

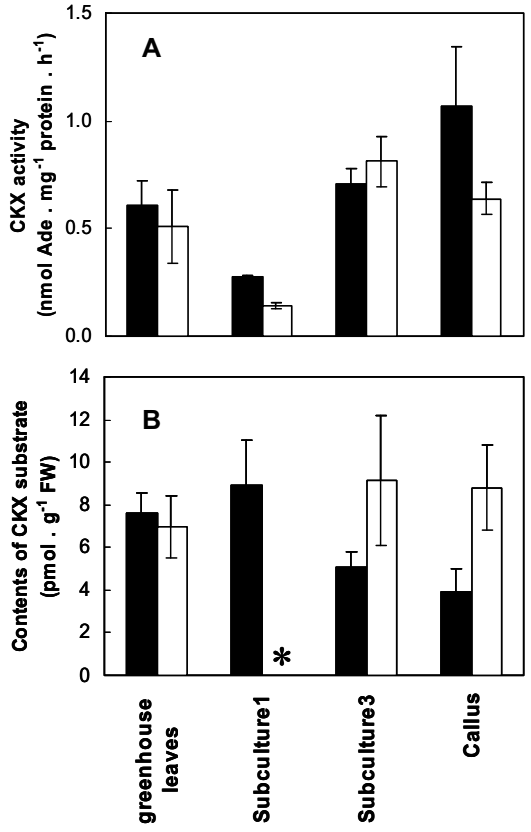

Fig. 5 Cytokinin oxidase/dehydrogenase (CKX) activity (A) and total amount of cytokinins susceptible to CKX catalysed degradation (iP + iPR + zeatin + zeatin 9-riboside) (B) in greenhouse leaves, during 2 subsequent stages of *in vitro* subculturing and callus culture. Left (black bars), *Rhipsalidopsis* (cv. 'CB4'); right (white bars), *Schlumbergera* (cv. 'Thor-Olga'). All CKX data are expressed as nmol Ade.mg protein^{-1}.h^{-1}, cytokinin data are expressed as pmol.g^{-1} FW. n= 3. Error bar = STERR. * Not analysed. (From **Sriskandarajah *et al.*** (2006) *Journal of Plant Growth Regulation* **25**, 79-88, with kind permission Springer Science & Business Media.)

type (Al-Ramamneh *et al*. 2006a). In *Rhipsalidopsis*, using a similar procedure, Al-Ramamneh (2006) reported the formation of white, transparent and round structures on the callus of cv. 'CB5' grown on MS-based medium supplemented with 2,4-D. These structures were thought to be intermediate structures between somatic embryos, roots and possibly shoots. However, these structures failed to develop into recognizable embryos.

Other *in vitro* techniques were also used for cacti micropropagation. Micrografting was attempted *in vitro* with prickly pear cactus (*Opuntia* spp.). Estrada-Luna *et al*. (2002) found that horizontal graft was an easy and reliable method to graft micropropagated prickly pear. Within 28 days vascular connections occurred in the callus bridge between rootstocks and scions in all genetic combinations. Growing the grafts under *in vitro* conditions avoided problems with fungal and bacterial contamination or scion stress by conditions such as dehydration. In addition, establishing *in vitro* grafting methods for cacti may benefit from enhanced growth and development (Burger 1985).

DETERMINATION OF MORPHOGENESIS

It is clear that a critical hormonal balance, represented by the ratio of cytokinins to auxins, controls the morphogenic response in *Schlumbergera* and *Rhipsalidopsis*. Apparently, embryogenesis and adventitious shoot organogenesis were promoted by an increase in cytokinins through a long period of incubation of cultures on media with high cytokinin to auxin ratio until a critical balance was reached. This, in turn, appeared to have controlled the fate of the somatic cells (Sriskandarajah and Serek 2004; Al-Ramamneh 2006). It was clear that the presence or absence of auxins in the last solid media used was the decisive factor for the determination of the morphogenic response for somatic embryogenesis or shoot formation. Somatic embryo-induction in *Schlumbergera* was favoured when MS medium without hormones was used as the final medium, whereas inclusion of 2,4-D or IAA in the medium favoured adventitious shoot regeneration. However, the length of exposure to cytokinins and the duration of callus growth in the liquid media, which is required to reach the critical hormonal balance, varied between the different cultivars of *Schlumbergera* and *Rhipsalidopsis*.

Cactus, in general, is known to produce high levels of auxins (Hubstenberger *et al*. 1992), and this was evident in the ready rooting ability of a wide range of cultures from both *Schlumbergera* and *Rhipsalidopsis* (Sriskandarajah and Serek 2004; Al-Ramamneh *et al*. 2006a). Early research work suggested that sources of auxin and cytokinin needed for maximal production of axillary shoots interact strongly with cactus species (Clayton *et al*. 1990). Flower buds and phylloclade initiation was found to be controlled by varying the concentrations of growth regulators like BA and GA_3 (Boyle and Marcotrigiano 1997).

Studies conducted by Sriskandarajah *et al*. (2006) provided some insights on the role of phytohormones on the

formation of organs *in vitro* in these two cactus species. These studies suggested a correlation exists between the concentrations of the endogenous phytohormones and the corresponding enzymes that are responsible for mediating the regeneration process (**Fig. 4, 5**). Leaf tissues obtained from the greenhouse-grown mother plants, the first and third *in vitro* sub-cultures, and callus tissues were analysed to compare the non-regenerative initial explants and cultures with enhanced regenerative capacity. It was found out that isopentenyl-type derivatives were the main cytokinins present in the two cactus genera and the total content of isopentenyl-type cytokinins in greenhouse-grown leaves of *Rhipsalidopsis* was more than twice the amount found in greenhouse-grown leaves of *Schlumbergera*. This increase was more pronounced in *Rhipsalidopsis* than in *Schlumbergera* and it was negatively correlated with the total contents of isoprenoid cytokinins that serve as substrates for oxidase/dehydrogenase (CKX). The activity of CKX increased two to threefold during sub-culturing. The total peroxidase (POX) activity in greenhouse-grown leaves of both genera was low (0.01-0.028 µM mg/l protein per min), and the activity increased significantly to twofold during sub-culturing, more specifically in the tissue of *Rhipsalidopsis*. In studies with Petunia, high organogenic capacity was correlated to an increased level of endogenous isoprenoid cytokinins along with enhanced CKX activity (Auer *et al*. 1999). Similar studies in plants such as *Rosa hybrida* also indicate that the difference in the organogenic ability could be related to endogenous cytokinin levels and CKX activity (Kapchina-Toteva and Yakimova 1977). It was concluded that the enhanced auxin metabolism (biosynthesis, conjugation/deconjugation and POX activity), in combination with an enhanced CKX activity, shifts the auxin and cytokinin pool, favouring adventitious shoot formation in *Rhipsalidopsis*, whereas the lack of accumulation of POX activity, in combination with auxin autotrophy, makes *Schlumbergera* more recalcitrant (Sriskandarajah *et al*. 2006).

AGROBACTERIUM TUMEFACIENS-MEDIATED GENETIC TRANSFORMATION

The highly efficient *in vitro* regeneration systems established for *Schlumbergera* and *Rhipsalidopsis* (Al-Ramamneh 2006) has opened up the windows for genetic improvement in these plants. A protocol for *Agrobacterium tumefaciens*-mediated genetic transformation of *Rhipsalidopsis* cv. 'CB5' was developed (Sriskandarajah and Serek 2004; Al-Ramamneh *et al*. 2006b). Transformed shoots were obtained by using the bacterial strain LBA4404 (van der Fits *et al*. 2000). Plasmid constructs used carry the *npt*II gene, as a selectable marker, and the reporter *uid*A gene (GUS). The results obtained by GUS expression studies, ELISA assay and Southern hybridization analysis confirmed the presence of the *uid*A and *npt*II genes in the selected transgenic callus lines. Elisa and southern blot analyses were conducted to confirm the presence of the *npt*II gene and its product in the genome of the transgenic callus lines (Al-Ramamneh *et al*. 2006b; **Fig. 6A, 6B**). Hybridization of the digested DNA of the transgenic callus lines (using different restriction enzymes) to the DNA fragment of the *npt*II gene yielded a single to multiple hybridization bands. The formation of multiple bands indicated a multiple transformation events. The callus used would have been a clump of different mixed cells. Analysing determined morphogenic structures such as shoots from complete plantlets can give more clear information on the integration pattern of the transgene. The formation of transgenic *Rhipsalidopsis* shoots was confirmed by GUS assay as detected by the dark blue colour expressing in the entire shoots tested. With this approach, a transformation efficiency (expressed as the percentage of the number of GUS-positive shoots to the total number of callus explants inoculated) of 22.7% was demonstrated. These studies also revealed some of the factors that could influence transformation efficiency.

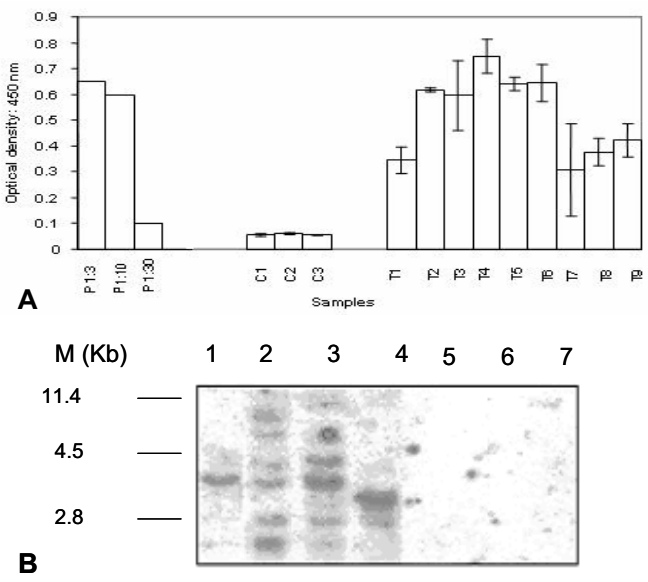

Fig 6 Analysis of *Rhipsalidopsis* transgenic calluses. (**A**) OD values obtained by ELISA reader at 450 nm for the immuno-detection of NPTII transgene expression of *Rhipsalidopsis* callus lines. *T1-T9* Independent transformed callus lines; *C1-C3* Non-transformed callus lines; *P* Positive controls provided with the kit at three dilutions (1:3, 1:10, 1:30). (**B**) Southern analysis of genomic DNA of transgenic *Rhipsalidopsis* calluses with kan1 probe M: *Pst*I digested λ DNA ladder. Lane 1: DNA from first putative transgenic callus digested only with *Hind*III. Lanes 2, 3 and 4: DNA from the second putative transgenic callus digested with *Hind*III, (*Hind*III and *Eco*R1), and (*Hind*III and *Xba*1), respectively. Lanes 5, 6 and 7: DNA from non-transformed control callus digested with *Hind*III, (*Hind*III and *Eco*R1), and (*Hind*III and *Xba*1), respectively. (From **Al-Ramamneh *et al*.** (2006b) *Plant Cell Reports* **25**, 1219-1225, with kind permission Springer Science & Business Media.)

These include the starting explants, selection system, bacterial strain, induction of *vir*-genes by acetosyringone, inclusion of a washing step with cefotaxime (two times; 5 min each) for explants after the co-culture and nutritional stress.

Strategies are needed to overcome the flower and bud abscission of cacti due to exposure to high temperature (Hammer 1992), exogenous ethylene (Serek and Reid 1993) and during long distance shipment of plants (Cameron and Reid 1981). A mutant *etr1-1* allele (Chang *et al*. 1993) that confers insensitivity to ethylene was used to engineer prolonged vase-life in carnation (Bovy *et al*. 1999), Petunia (Gubrium *et al*. 2000) and *Campanula* (Sriskandarajah *et al*. 2004). Limited number of experiments using a strain harbouring the plasmid pBEO210 (Bovy *et al*. 1999), which contains the *etr1-1* gene under the control of the flower specific promoter *fbp1* (Angenent *et al*. 1992; Angenent *et al*. 1993) and *npt*II gene under control of the cauliflower mosaic virus (CaMV) 35S promoter were conducted with both cactus species without success (Al-Ramamneh 2006).

CONCLUDING REMARKS

The recalcitrant nature of cactus species was discussed in the respective studies although great strides have been made in the micropropagation of several cactus species (Ramirez Serrano and Teixeira da Silva, in press). There have been limited studies on the biotechnology of these two cactus species, however, the methods established for multiplication via axillary and adventitious shoot regeneration and somatic embryogenesis for *Schlumbergera* and *Rhipsalidopsis* could be useful for *in vitro* culture of slow growing plants. Application of genetic engineering methods for cactus is still in the preliminary stage, however, the very recent results from *Rhipsalidopsis* species indicate promising future for modifying plants with useful genes.

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