Cell proliferation and ultrastructure in the roots of young wheat seedlings induced by oxidative stress

S. Dmitrieva, F. Minibayeva, A. Ponomareva, L. Gordon
Kazan Institute of Biochemistry and Biophysics, Russian Academy of Sciences, Kazan, Russian Federation

Desiccation stress in plants is often accompanied by universal stress responses such as the accumulation of oxygen radicals resulting in oxidative stress. This in turn may lead to the depression of the early development of seedlings after the germination of orthodox seeds. To study the negative effects of oxidative stress on the growth of plant cells, we investigated the production of reactive oxygen species (ROS), the changes in mitotic index, growth and cell ultrastructure induced by the classical pro-oxidant paraquat (methylviologen). We also tested the effects of salicylic acid (SA), a signal molecule involved in plant stress tolerance and a growth regulator but that can also facilitate an increase in ROS and down regulate the cell cycle and cell proliferation. Both SA and paraquat at concentrations of 0.01–1.0 mM caused the accumulation of superoxide radicals and H_2O_2, decreased cell mitotic index and inhibited root growth in 5 day old wheat (*Triticum aestivum* L.) seedlings. These effects were accompanied by the changes in cell ultrastructure. Treatment of roots with 1 mM SA for 6 h caused degradation of the membrane lipids and an increase in the number of peroxisomes. Paraquat (0.001–0.10 mM) induced the accumulation of myelin-like bodies, reduced the electron density of the mitochondrial matrix, and caused collapse of the tonoplast. A common characteristic of both SA and paraquat induced effects on cell ultrastructure was the appearance of numerous autolytic vacuoles containing mitochondria, cytoplasm and other organelles. Thus, the accumulation of ROS can result in the depression of plant growth and cell proliferation and further, can induce autophagy like processes.

doi:10.1016/j.sajb.2007.04.043

A protocol for the production of adventitious shoot explants for future cryopreservation of *Ekebergia capensis* using a temporary immersion system (RITA®)

E. Hajari, P. Berjak, N.W. Pammenter, P. Watt
Plant Germplasm Conservation Research, School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban, South Africa

Ekebergia capensis Sparrm. is an indigenous African species that produces unstorable, recalcitrant seeds. This poses major problems for the long–term storage of such germplasm. Work with excised axes has revealed that the shoot apical meristem sustains lethal damage consequent upon excision from the cotyledons, although adventitious buds can be induced to form at the wound sites. The phenomenon of shoot apex necrosis, which seems to be common when axes of tropical species are excised (see abstract by Goveia et al.), prompted our research group to investigate the potential of alternative explants for cryopreservation of the genetic resources of recalcitrant-seeded species. The present contribution reports the preliminary findings on the development of a protocol for the production of adventitious shoots from intact root explants of *Ekebergia capensis*. Roots produced by *in vitro* germination of excised embryonic axes, were placed in a temporary immersion bioreactor (RITA®) containing liquid media supplemented with the cytokinin, BAP. After 24 h, the roots were plated on standard semi-solid germination medium. After 4 weeks, adventitious shoots were produced from the root explants. This protocol was performed using seeds obtained from two locations *viz*. Port Elizabeth (33°30′ S) and Mtunzini (28°22′ S), and a difference in the response of germplasm from these two locations was observed. (For further provenance-related differences in the responses of seeds of *E. capensis*, see the Abstract and poster of Bharuth et al.) The adventitious shoots produced in this manner were cultured on elongation medium for further growth and development. Once shoots were sufficiently large, they were rooted on medium supplemented with no auxin or only a low IBA level. This work has important implications for the storage of species that produce recalcitrant seeds as it provides a potential strategy for the production of an alternate source of explants that can be used for the cryopreservation of germplasm.

doi:10.1016/j.sajb.2007.04.044

Assessment of a strategy to curtail fungal proliferation in non-orthodox seeds during storage

V. Reddy, P. Berjak, N.W. Pammenter
Plant Germplasm Conservation Research, School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban, South Africa

Recalcitrant seeds usually host a range of active bacterial and fungal contaminants at harvest. The storage conditions necessary to maintain viability of intact tropical recalcitrant seeds are high humidity and relatively high temperatures which are also conducive to fungal and bacterial proliferation. A number of deteriorative changes accompany the presence of fungi during storage of recalcitrant seeds, and the hydrated storage lifespan, particularly of seeds of tropical species can be considerably extended if fungal activity is curtailed. Simple surface sterilization is effective only in removing contaminants on the seed surfaces, thus to implement successful long-term storage and axis cryostorage, elimination of intraseminal fungi is imperative. In this study the use of systemic fungicides applied as a preliminary soak before hydrated storage was evaluated as one method of removing internal fungal contaminants from the recalcitrant seeds of the tropical species *Trichilia dregeana*. Three different fungicides namely: Orius 200 EW® (active ingredient tebuconazole [200 g l^{-1}]); Heritage®, a systemic translaminar fungicide (active ingredient azoxystrobin [0.5 g g^{-1}]); and Celest 100FS® (active ingredient phenylpyrole [100 g l^{-1}]) were

assessed in this investigation. The seeds were treated with the three different fungicide solutions (distilled water in the case of the control) for 5 h after an initial 10 min treatment with 1% NaOCl (w:v) and rinsing with three successive applications of sterile distilled water. The proliferation of bacteria was minimised by the addition of chlorohexidine gluconate (Hibitane®) to the fungicide solutions. The viability of the treated seeds after different storage periods was assessed and the effectiveness of the fungicides to curtail fungal proliferation was evaluated. The data from this study show that loss of viability occurred within 32 d for seeds stored hydrated following NaOCl treatment only, while those seeds treated with the three different fungicides retained some viability even after 64 d in hydrated storage, with Orius (tebuconazole) emerging as the most efficacious. Two different concentrations of tebuconazole (5 and 1 g l^{-1}) were used to treat *T. dregeana* seeds before hydrated storage. When the seeds were removed from the fungicide treatments, a discolouration was observed in those treated with the higher concentration of Orius, yet ~60% of these seeds germinated after 64 d of hydrated storage. Fungal proliferation in storage was apparent only on the control (water-soaked) seeds, occurring within 8 d. However, when axes from seeds from the different fungicide treatments were cultured on a nutrient medium, fungal persistence was still apparent, but the species proliferating (one each after Heritage and Celest treatment and two following Orius application) were different, depending on the fungicide applied. Shoot development in the axes from the three fungicide treatments was rapid: In contrast, no shoot development occurred from axes of untreated seeds over a 6-week culture period. Progress towards obtaining axenic (pure) isolates of the fungal species associated with the cultured axes from the different treatments is currently underway. It therefore appears that systemic fungicide treatments of recalcitrant seeds have the potential to improve hydrated storage longevity of *T. dregeana* seeds, but, to eliminate all the fungal inoculum, must be tested in various combinations and concentrations.

doi:10.1016/j.sajb.2007.04.045

Cryostorage of callus produced during indirect organogenesis in *Eucalyptus grandis*×*urophylla*

P.G. Simelane [a], M.P. Watt [b], N. Edwards [c], D.J. Mycock [a]
[a] *School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa*
[b] *School of Biological and Conservation Sciences, University of KwaZulu-Natal, Durban, South Africa*
[c] *Mondi Business Paper, Trahar Technology Centre, Hilton, South Africa*

The South African Forestry industry forms an essential part of the economy of the country. *Eucalyptus* species are mainly planted in low altitude areas of KwaZulu-Natal and Mpumalanga and are used for timber, pulp and paper production, poles and firewood. Members of the genus are also used commercially for the production of essential oils and tannins which are extracted from leaves. The industry invests considerable effort in research towards increased plantation yields and, as a result, micropropagation approaches are now routinely used to support clonal programmes. *In vitro* conservation is presently an active area of research and this study established the successful use of organogenic callus in cryopreservation of the germplasm. An indirect organogenesis technique developed in our laboratories was used to produce propagules of a *Eucalyptus grandis*×*Eucalyptus urophylla*. Assessment of developmental processes in this material focused mainly on identifying callus origin, the vascular connection between callus and shoots and the shoot-root junction. The histological development over a two month period will be described. Identification of the cells that are responsible for initiation and differentiation of callus can also assist in determining the appropriate micropropagatory developmental stages for cryostorage. Using this approach it was established that 52–55% of callus (22 days old) that was prepared for cryostorage by drying to a water content of 49–56% (wet mass basis), exposed to cryoprotectants (dimethyl sulphoxide and sucrose) and then slowly frozen (±1 °C/min) was viable after thawing. Collectively the data add to our growing understanding of the biological processes underlying both micropropagation and cryopreservation of *Eucalyptus*.

doi:10.1016/j.sajb.2007.04.046

Chlorophyll fluorescence and CO_2 assimilation of desiccation-tolerant cyanobacterial crustaceous layer of tropical inselberg rock surfaces after rehydration following one and four-year air-dried stage

Z. Tuba [a,b], N. Lei [c], E. Péli [a], T. Pócs [d], S. Porembski [e], Z. Laufer [a]
[a] *"Plant Ecology" Departmental Research Group of the Hungarian Academy of Sciences, Szent István University, Páter K. u. 1., 2100 Gödöllö, Hungary*
[b] *Department of Botany and Plant Physiology, Faculty of Agricultural and Environmental Sciences, Szent István University, Páter K. u. 1., 2100 Gödöllö, Hungary.*
[c] *Department of Botany, Foshan University, Ch-528000 Foshan, PR China*
[d] *Department of Botany, Eszterházy K. Teachers Training College, Leányka u. 2., 3301 Eger, Hungary*
[e] *Department of Botany, Institute of Biodiversity, University of Rostock, Wismarsche str. 8, 18051 Rostock, Germany*

Changes in chlorophyll fluorescence and net CO_2 assimilation were studied in previously air-dry desiccation-tolerant inselberg cyanobacterial crustaceous layers from two tropical locations, after various periods of time. The cyanobacterial crustaceous layers on granatic inselberg rock surface from Tanzania and on gneissic inselberg rock surface from French Guayana were studied. Photosynthetic $C0_2$ assimilation rates