Plants adapted to small mammal-pollination show characters like visually inconspicuous, bowl-shaped flowers near ground level, stiff stamens, easily accessible nectar and characteristic scent. The South African Pineapple lily *Eucomis regia* is hypothesised to be small mammal-pollinated on the basis of sharing these characters and as it differs from insect-pollinated *Eucomis* species mainly in scent. Under natural conditions and in the laboratory mice and an elephant shrew were observed to become dusted with pollen as they licked nectar in the flowers. Pollen and dye was transferred to stigmas. Live-trapped mice had large amounts of *E. regia* pollen in the fur around the snouts and in the faeces. Selective exclusion of vertebrates, but not insects, led to significant reduction in seed set. Controlled pollination experiments showed that *E. regia* is self-incompatible and thus entirely dependent on pollinators for seed production. Spectral reflectance of floral tepals is very similar to the green bracts and leaves, rendering flowers inconspicuous to insects. The scent of flowers and nectar is reminiscent of boiled potatoes due to the presence of the sulphur compound methional, confirmed by analysing headspace scent samples with gas chromatography–mass spectrometry. Also the nonane derivative ethrievicomin was found in two of the three studied populations, the first record in flowers. Choice experiments showed that mammals are strongly attracted to the scent of flowers and methional. *E. regia* resembles other *Eucomis* species pollinated by spider-hunting wasps and carrion-flies in floral morphology and colour as well as nectar properties, but differs heavily in floral scent. Available evidence thus suggests that pollination systems in *Eucomis* are mediated mainly by scent rather than visual cues.

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**Poster abstracts**

**Anthelmintic phloroglucinol derivatives and antifungal activity of fractions from Leucosidea sericea (Rosaceae)**

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The leaf of *Leucosidea sericea* is used traditionally as anthelmintic and in treatment of ophthalmia in South Africa. Anthelmintic activity had been reported from this plant. The aim of this study was to identify and characterized the compounds responsible for the anthelmintic activity using bio-assay guided fractionation. Bio-guided phytochemical investigation of the ethyl acetate portion from the acetone crude extract of *L. sericea* was achieved using repeated silica gel column chromatography techniques. The structures of isolated compounds were identified by interpretation of their NMR and IR data and by comparison with reported values. The anthelmintic assay was conducted using guidelines approved by the World Association for Advancement for Veterinary parasitology. The antifungal activity was determined using a microplate dilution method, while cytotoxicity on Vero monkey cells was tested using the MTT assay. The isolated compounds were identified from their NMR data as β-sitosterol, agrimol G and a mixture of agrimols G and A. The Egg hatch assay EC50 for agrimol G was 0.52 mg/ml, while that of the mixtures of agrimols G and A was 0.28. The larval development test EC50 for agrimol G was 0.08 mg/ml and 0.11 mg/ml for mixture of agrimols G and A. This study is the second report of compound been isolated from this plant species. The compounds isolated have in vitro anthelmintic activity.

**Microsatellite DNA fingerprinting and cultivar identification in sugarcane using a semi-automated genetic analyser**

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Microsatellites or simple sequence repeats (SSR) have become the genetic marker of choice internationally for fingerprinting sugarcane varieties. SSR markers are able to identify co-dominantly inherited multi allelic loci, which makes it an ideal marker for fingerprinting of complex aneu-polyplids such as sugarcane. In the past, radioisotope (α–32P dCTPs) labelling was used to visualize the amplified microsatellite DNA fragments on auto-radiographs. In recent years, due to increasing costs and limited availability of isotopes, researchers have made use of alternative visualization techniques. Modern technological advances in electronics and genomics have made genetic analysers popular for fragment analysis. Genetic analysers produce electropherograms from fluorescently labelled primers that are incorporated into DNA fragments during the polymerase chain reaction (PCR) amplicon synthesis. Advanced software is used to analyse and identify the allelic variation between amplified loci in different genotypes. At the South African Sugarcane Research Institute (SASRI), a technique was developed for fingerprinting released sugarcane varieties using an ABI 3500 genetic analyser and SoftGenetics GeneMarker™ software. All methods were successfully optimised to minimise PCR artefact formation and develop a high-throughput fingerprinting method making use of the ABI 3500 genetic analyser. Using the genetic analyser for fingerprinting resulted in a cost reduction of approximately 25% and reduced turnaround time from a couple of weeks to a few hours. The more cost effective and quick fingerprinting technique has been welcomed by the sugar industry. SASRI have already started to create a fingerprinting database of its released varieties including the breeding populations.

**A comparative study of the pollen morphology of Prototulbaghia Vosa and other genera in the Alliaceae**

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The southern African Alliaceae Borkh. is represented by four genera (*Allium* L., *Nothoscordum* Kunth, *Tulbaghia* L. and *Prototulbaghia* Vosa) and 28 species. *Prototulbaghia* is a monotypic genus endemic to the Leolo Mountains that are situated in the Sekhuwhuneland Centre of Endemism. The pollen morphology of this genus has not been documented before, and the aim is thus to describe the pollen morphology of *Prototulbaghia siebertii* Vosa and
to compare it with the pollen of Nothoscordum borbonicum Kunth, Tulbaghia similleri P. Beauv. et T. violaceae Harv. Floral material of the four species was collected from the gardens of the North-West University (NWU) campus and a special collection held at the NWU-botanical garden. Pollen was examined with scanning electron- and light microscopy. The pollen morphology of the four species is perprolate and monosulcate, and the surface sculpturing is reticulate and heterobrochate. The pollen of Prototulbaghia siebertii however, displays a unique characteristic as the grains fold breadth-wise causing the tips to touch. Hence, the grain displays a triangular shape and has a disulcate appearance. Two hypotheses can be formulated to explain this phenomenon. The folding can either be due to the process of harmomegathy or a still unknown event that occurs during the development of the pollen grain. These processes will be investigated further to determine the mechanism of the folding, whether it is a unique taxonomic character for the genus, and whether it is of evolutionary significance within the Alliaceae.

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**Regulation of bioactive secondary metabolite production in in vitro-derived greenhouse-grown Aloe arborescens**

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Auxins and cytokinins are known to interact at different levels in synergistic, antagonistic or additive ways to produce or regulate physiological effects. The effects of cytokinins as well as auxin and smoke-water (SW) treatments applied during the shoot regeneration and rooting phases respectively, on secondary metabolite production of in vitro-derived Aloe arborescens were evaluated after two months of growth in the greenhouse. The cytokinins used during shoot regeneration were 6-benzyladenine (BA), meta-topolin (mT) and their derivatives while indole-butric acid (IBA) and SW were used for rooting. In MemT-regenerated shoots, the treatment with either SW or IBA during rooting significantly increased total phenolic and flavonoid contents when compared to those rooted without plant growth regulators (PGR). On the other hand, mT, MemTR and MemTTHP regenerated shoots which were rooted on PGR-free medium, yielded a significantly higher flavonoid content when compared to those rooted on SW and/or IBA-containing medium. Regenerated shoots from BAR-containing medium rooted using SW treatment gave a significantly higher iridoid level compared to those rooted using IBA or without PGR. Conversely, significantly lower iridoid levels were observed with regenerated shoots from BA, mT and mTR-containing media rooted with IBA or SW when compared to those rooted without PGR. Regenerated shoots from mTR and MemT-containing media rooted with SW showed a significantly reduced radical scavenging activity when compared to those rooted without PGR. Shoots regenerated from BA, BAR and MemTTHP-containing media and rooted with SW demonstrated a significantly higher radical scavenging activity when compared to those rooted without PGR. Taken together, the cytokinin type used during shoot proliferation stages and the rooting treatment applied, individually and interactively had a significant carry-over effect on the production of bioactive secondary metabolite in A. arborescens.

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**The role of cytokinins on the antioxidant capacity and phenolic acid content during tissue culture and acclimatization of Merwilla plumbea**


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Merwilla plumbea (Lindl.) Speta, a member of the Hyacinthaceae is a popular South African medicinal plant which is currently threatened in the wild. Cultivation using tissue culture techniques could alleviate pressure on wild populations. Nevertheless, there is no adequate literature on the phytochemical and pharmacological efficacy of tissue cultured M. plumbea. In addition effects of the series of events that occur during tissue culture and acclimatization on the production of bioactive constituents remain intricate and poorly-understood. Hence, we evaluated the effect of five cytokinins (isoprenoid and aromatic) on the antioxidant activity and phenolic acid content of M. plumbea during tissue culture and acclimatization stages. Meta-topolin riboside-treated and control plantlets had the best ORAC activity during tissue culture and acclimatization stages, respectively. Generally, the antioxidant activity increased in the aerial parts and decrease in the underground parts after acclimatization. Remarkably, the level of phenolic acids such as protocatechuic acid, 4-hydroxybenzoic acid, caffeic acid and vanillic acid were generally higher in the plant parts and stages which demonstrated better antioxidant activity. The current findings highlight the great chemical variations that could result from the type of applied cytokinin. The use of the right cytokinin is required to guarantee the presence of bioactive chemicals and subsequently the efficacy of micropropagated M. plumbea.

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**Are long-term stored medicinal plants as active as the freshly harvested materials?**

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Medicinal plant materials are usually stored for a period of time by plant gatherers, traders or traditional healers before usage. Questions that frequently arise are: Do such plant materials retain their biological efficacy? Are they safe for consumption after a long period of storage? In the present study, the iridoid and phenolic contents as well as the antioxidant and mutagenic properties of five South African medicinal plants stored for 16 years were compared to those of freshly harvested materials. The iridoid content of the freshly harvested materials of Ocotea bullata, Protorhus longifolia and Ziziphus mucronata were significantly higher when compared to the stored materials. Stored Acokanthera oppositifolia material had a significant higher iridoid content compared to the freshly harvested material. The total phenolic and flavonoid contents recorded in all freshly collected materials (except Artemisia afra) were significantly higher than the