Short Communications/Kort Mededelings

Cyanogenic glycosides in leaves and callus cultures of Schlechterina mitostemmatoides

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Leaf material and callus cultures of Schlechterina mitostemmatoides (Passifloraceae), an endangered species from northern KwaZulu-Natal, was screened for cyanogenic glycosides. Both leaf and callus material tested positive. Extracts from both sources were further investigated by thin-layer chromatography. This indicated that the callus culture produced the same cyanogenic compound as the intact plant.

Keywords: Callus culture, cyanogenic glycosides, Passifloraceae, Schlechterina mitostemmatoides, thin-layer chromatography

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Schlechterina mitostemmatoides Harms (Passifloraceae) is a creeper growing in the coastal forests of northern Zululand and Mozambique. The Zulu name for the species is Mhulalanyosi and the plant is used for cleansing steam baths. It is reported to be a favourite food plant for elephants. As S. mitostemmatoides is now scarce in the wild, efforts to micropropagate the plant by in vitro culture were undertaken. It is well known that many members of the Passifloraceae contain cyanogenic glycosides (Russell & Reay 1971; Siegler 1977, Adsersen et al. 1993) but S. mitostemmatoides has never been investigated for cyanogenesis. Leaf and callus material were therefore screened in this study for cyanogenic compounds.

Callus culture of S. mitostemmatoides was initiated from juvenile leaves which had been surface sterilized for 10 min in 3.5% NaOCl. The explants were placed on Murashige and Skoog medium supplemented with 3% sucrose, 100 mg l-1 myo-inositol, 1 mg l-1 NAA and 1 mg l-1 BA, at a pH of 5.8, for the induction of callus. This medium was also used for maintenance of the callus and the cultures were kept in the light at 26°C.

Two tests that detect cyanide release from cyanogenic glycosides were used to screen leaf and callus material for the presence of these compounds. Fresh leaf (50 mg) and callus material (500 mg) were transferred to 25-ml Erlenmeyer flasks. In order to break down cyanogenic glycosides, and thereby release cyanide, 100 μl of 0.1 mg l-1 β-glucosidase or 1 ml chloroform was added to the flasks. Chloroform breaks the cell walls, releasing cyanogenic glycosides, which come into contact with any glycosidases stored in other cells. Amygdalin (0.01 mg and 0.1 mg) in aqueous solution was used as a standard. Controls contained either 1 ml chloroform or 100 μl of the β-glucosidase solution. Strips of sodium picrate paper (Whatman no. 1 filter paper dipped in a solution of 5 g sodium carbonate, 0.5 g picric acid and 100 ml water, and dried) were hung in the closed flasks. After 30 min, the strips in the flasks containing the amygdalin standard as well as leaf and callus material turned reddish-brown, indicating the presence of cyanide, whereas the controls remained unchanged.

Figure 1  Thin-layer chromatograms of extracts from leaf and callus cultures of S. mitostemmatoides. Cyanide released from the cyanogenic glycosides was detected by sandwiching TLC plates with picrate paper. Cyanide bands on the picrate paper corresponding to the TLC chromatograms are indicated in the cyanide lane. Amygdalin was used as standard (ST). Colours in the chromatogram: B: brown, BL: blue, BLW: bluish-white, BW: brownish-white, GP: greyish-purple, O: orange, P: purple, PK: pink, R: red, W: white, Y: yellow.
A second test was performed similarly, using different test strips. These strips were prepared by dipping Whatman no. 1 filter paper firstly into a solution of 1 mg ml⁻¹ guaiacum gum in ethanol, drying, and then into 0.1 mg ml⁻¹ CuSO₄. Strips in flasks containing the amylodulin standard, leaf and callus material turned prus-sian blue in colour, indicating the presence of cyanide. The guaiac gum control strips did not change colour.

To investigate the chromatographic properties of the cyanogenic glycoside(s) present in the leaf and callus material, leaves and callus were extracted for cyanogenic glycosides. Cellus (2 g) or fresh leaf material (150 mg) were cut into small pieces and boiled in 80% ethanol for 5 min. The extracts were cooled, filtered, and the filtrates dried in vacuo. The residues were redissolved in eluent and applied to Merck Silica 60 F₂₅₄ TLC plates. Only one-third of the leaf extract was applied, whereas all of the callus extract was used. Amygdalin (5 µg) was used as standard. The TLC plates were developed (one ascent) using ethyl acetate:chloroform:methanol:water (40:30:12:10:8) (Brumner et al. 1983). To detect the cyanogenic compounds, the TLC plates were sandwiched by a modified method from Tantisawie et al. (1969). The plate was sprayed with 0.1 mg ml⁻¹ β-glucosidase. A piece of synthetic net fabric and a sheet of sodium picrate paper were placed in an oven at 80°C for 30 min. The bands were visible on the sodium picrate paper in both the leaf and callus extracts, indicating the presence of cyanide. The guaiac gum in ethanol.

**Plant-derived smoke and seed germination: is all smoke good smoke? That is the burning question**

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Smoke derived from burning a natural mixture of plant species stimulates the germination of seed from a wide range of plants. It is not known, however, whether smoke derived from individual species is equally effective in promoting seed germination. The germination of Themeda triandra seed following exposure to smoke, generated by individually burning the leaf material of 27 different species common to the montane grasslands of the Drakensberg, is reported. Germination of T. triandra seed was not promoted in response to the smoke of all 27 species tested.

Rook aakomstig van die brand van 'n mengsel van natuurlike plantspieses stimuleer die ontkieming van 'n wy e reeks saad. Dit is egter nie bekend of rook van individuele plantspieses eew effektyf is in die stimulerings van saadontkieming nie. Die ontkieming van Themeda triandra-saad na blootstelling aan rook wat verkry is deur die verbranding van blaaamateriaal van 27 verskillende spesies vanaf die graslande van die Drakensberg is ondersoek. Onciiming van T. triandra-saad is nie deur rook van al 27 spesies gestimuleer nie.

**Keywords:** Plant-derived smoke, seed germination, smoke source, Themeda triandra.

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Smoke derived from the burning of natural mixtures of plant species stimulates the germination of seed from a wide range of plant species common to fire-dependent floral communities (de Lange & Boucher 1990; Baxter et al. 1993; Brown 1995a). Seeds of T. triandra exposed to smoke derived from burning a mixture of species do not lose the enhanced effect of such exposure during storage. This provides an effective means of pre-treatment for seeds which may prove difficult to germinate (Baxter & van Staden 1994). In addition, smoke has been shown to stimulate germination of soil-stored seed (de Lange & Boucher 1990). Neither the mechanism by which smoke acts to stimulate seed germination, nor the active component(s) in plant-derived smoke are

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