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Screening for hydroxynitrile lyase activity in non-commercialised plants

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ABSTRACT

Hydroxynitrile lyases are used for the synthesis of enantiomerically pure cyanohydrins which are of great importance in the pharmaceutical and fine chemical industries. In this study, the hydroxynitrile lyase activity of 100 plants from 40 families was investigated, first by screening for cyanogenic activity, followed by a hydroxynitrile lyase activity assay. Of the 100 plants, four were found to be cyanogenic and exhibited specific hydroxynitrile lyase activity: *Adenia* sp. (0.44 U/mg), *Adenia firingalavensis* (2.88 U/mg), *Adenia fruticosa* (1.99 U/mg) and, *Adenia pechuelii* (2.35 U/mg), all from the family Passifloraceae. This is the first report of hydroxynitrile lyase activity in these plants.

1. Introduction

In plants, a cyanohydrin is broken down by a hydroxynitrile lyase into hydrogen cyanide and an aldehyde or ketone. This reaction, known as cyanogenesis, occurs in over 3000 plant species and serves as an ingenious self defence mechanism — the hydrogen cyanide released by these cyanogenic plants protects them from further microbial, fungal and animal attacks (Conn, 1981; Erdman, 2003; Jones, 1998; Sharma et al., 2005; Ueatrongchit et al., 2010; Zagrobelny et al., 2008). However, in principle, each enzymatic reaction is completely reversible, and it is this reversible reaction (Fig. 1) which is of interest.

The chiral cyanohydrins produced using this reversible reaction — the condensation of hydrogen cyanide with an aldehyde or ketone is of great importance to the pharmaceutical and fine chemical industries where they are used as building blocks in the production of various essentials such as pesticides, medicines, agrochemicals, etc. (Dadashipour et al., 2011). Hydroxynitrile lyase can also be used to detoxify cyanogenic food plants (Fokunang et al., 2001; Hasslacher et al., 1996).

The first discovery of hydroxynitrile lyase occurred in 1837, by two German chemists — Justus von Liebig and Friedrich Wohler. This was found in an *Amygdalus communis* (bitter almond) extract (Hosel, 1981). According to literature, the two main families containing cyanogenic plants and therefore contributing hydroxynitrile lyases are the Euphorbiaceae and Rosaceae. Despite the numerous discoveries of other hydroxynitrile lyases since, the application of these naturally occurring enzymes in enantioselective biocatalytic synthesis was limited due to the difficulty of obtaining a sufficient amount and most importantly, lack of novel substrate specificity (Asano et al., 2005; Dadashipour et al., 2011; Hernandez et al., 2004; Hughes et al., 1994; Sharma et al., 2005; Wajant et al., 1995; Xu et al., 1988).

Although recombinant DNA technology has now provided a way to mass produce these enzymes for industrial applications, there is still a lack of novel substrate specificity.

One solution to this is to identify multiple plants exhibiting hydroxynitrile lyase activity as there is the potential that each such plant may contain a hydroxynitrile lyase with novel substrate specificity, partially addressing the second issue. The aim of this study is to identify such new plants.

2. Materials and methods

2.1. Plant identification and collection

Apical buds from 100 plant species, comprising 40 families (Table 1) were collected in triplicate from the Pretoria National Botanical Gardens (National Herbarium, South African National Biodiversity Institute).

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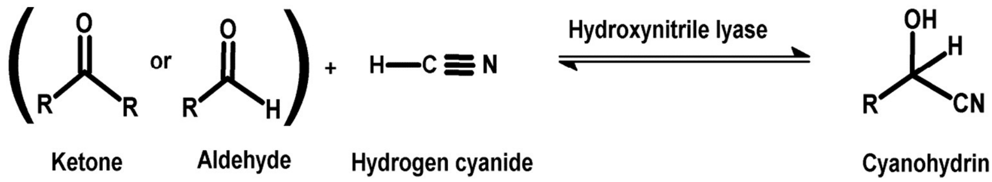

Fig. 1. General reaction catalysed by hydroxynitrile lyase.

These plants were specifically selected from families in which cyanogenesis had been reported previously.

The microtitre plates containing the samples were kept on ice at all times during sample collection to prevent wilting and were stored at −80 °C within 4 h.

2.2. Cyanogenic activity

Cyanogenic activity was tested using the Feigl–Anger test (Feigl and Anger, 1966; Takos et al., 2010) which relies on the oxidation of a tetrabase in the presence of hydrogen cyanide (a by-product of cyanogenesis) to create a distinct blue spot on a specially prepared detection paper after tissue disruption by a single freeze–thaw cycle. A Whatman 3MM filter paper, cut to the dimensions of 8 cm × 11 cm to fit the microtitre plate was used. The solution was then prepared by separately dissolving 75 mg of copper ethylacetoacetate (Sigma-Aldrich) and 75 mg of the tetrabase 4,4-methylenebis (N,N-dimethylalanine) (Sigma-Aldrich) in 7.5 ml of chloroform (Merck) each and then combining both solutions. The filter paper was then saturated with this combined solution and allowed to dry. After drying, the resultant

Table 1
Plants used in this study.

Family	Species
Acanthaceae	*Barleria obtusisepala* C.B. Clarke
Aizoaceae	*Ruschia* sp. C.f. *indurata*, L.c. *Trichodiadema* sp.
Anacardiaceae	*Searsia lancea* (L.F) F.A. Barkley
Apocynaceae	*Orbea melanantha* (Schltr.) Bruyns, *Pachypodium namaquensis* (Wyley ex Harv.) Welw., *Pachypodium lamerei* Drake, *Strophanthus amboensis* (Schinz) Engl. & Pax, *Acokanthera oblongifolia* (Hochst.) Benth. & Hook.f. ex B.D. Jacks., *Catharanthus roseus* (L.) G. Don, *Rauvolfia caffra* Sond.
Asclepiadaceae	*Huernia zebrina* (Phillips) L.C. Leach
Asparagaceae	*Asparagus densiflorus* (Kunth) Jessop
Asteraceae	*Kleinia stapeliiformis* (E. Phillips) Stapf, *Senecio barbertonicus* Klatt
Boraginaceae	*Ehretia rigida* (Thunb.) Druce
Cactaceae	*Rhipsalis baccifera* (J.S. Muell.) Stearn
Cannabaceae	*Celtis africana* Burm. f.
Celastraceae	*Elaeodendron croceum* (Thunb.) DC., *Gymnosporia tenuispina* (Sond.) Szyszyl., *Putterlickia verrucosa* (E. Mey. Ex Sond.) Szyszyl.
Crassulaceae	*Kalanchoe beharensis* Drake, *Adromischus* sp., *Adromischus filicaulis* (Eckl. & Zeyh.) C.A. Sm. subsp. *filicaulis*, L.c *Adromischus diabolicus* Toelken
Cucurbitaceae	*Xerosicyos danguyi* Humbert
Cycadaceae	*Cycas thouarsii* R. Br.
Dichapetalaceae	*Dichapetalum cymosum* (Hook.) Engl.
Didiereaceae	*Alluaudiopsis fiherenensis* Humbert & Choux, *Alluaudia procera* (Drake) Drake, *Alluaudia dumosa* (Drake) Drake, *Alluaudia humbertii* Choux, *Decarya madagascariensis* Choux
Dioscoreaceae	*Dioscorea dregeana* (Kunth) T. Durand & Schinz
Ebenaceae	*Euclea* sp. (Pretoria National Botanical Gardens 18236/73)
Euphorbiaceae	*Euphorbia tortirama* R.A. Dyer, *Euphorbia fruticosa* Forssk, *Euphorbia platyclada* Rauh, *Euphorbia stellispina* Haw., *Euphorbia leistneri* R.H. Archer, *Euphorbia pseudocactus* A. Berger, *Euphorbia cylindrica* Marloth ex A.C. White, R.A. Dyer & B. Sloane, *Euphorbia clivicola* R.A. Dyer, *Euphorbia buruana* Pax, *Euphorbia jansenvillensis* Nel, *Euphorbia fusca* Marloth, *Euphorbia gummifera* Boiss., *Euphorbia* C.f. *aeruginosa*, *Euphorbia bupleurifolia* Jacq., *Euphorbia lignosa* Marloth, *Euphorbia pulvinata* Marloth, *Euphorbia aeruginosa* Schweick, *Euphorbia monteiroi* Hook., *Euphorbia antso* Denis., *Euphorbia invenusta* (N.E.Br.) Bruyns, *Euphorbia schubei* Pax, *Euphorbia lugardae* (N.E.Br.) Bruyns, *Euphorbia ritchiei* (P.R.O Bally) Bruyns, *Euphorbia guentheri* (Pax) Bruyns, *Euphorbia virosa* Willd., *Euphorbia dregeana* E. Mey. Ex Boiss., *Euphorbia cupularis* Boiss., *Spirostachys africana* Sond., *Sclerocroton ellipticus* Hochst., *Croton sylvaticus* Hochst., *Croton gratissimus* subsp. *Gratissimus*, L.c. *Jatropha curcus*
Fabaceae	*Burkea africana* Hook., *Philenoptera violaceae* (Klotzch) Schrire, *Peltophorum africanum* Sond., *Bauhinia galpinii* N.E.Br., *Colophospermum mopane* (Benth.) Leonard,
Geraniaceae	*Pelargonium ceratophyllum* L'Her., *Pelargonium klinghardtense* R. Knuth, *Pelargonium crassicaule* L'Her.
Gesneriaceae	*Streptocarpus* sp. Pink cultivar
Hernandiaceae	*Gyrocarpus americanus* Jacq.
Icacinaceae	*Pyrenacantha cordata* Villiers.
Juncaceae	*Juncus effusa* L.
Lamiaceae	*Tetradenia fruticosa* Benth.
Malvaceae	*Grewia flavescens* Juss.
Meliaceae	*Turraea obtusifolia* Hochst.
Menispermaceae	*Tinospora fragosa* Verdoorn & Troupin
Moringaceae	*Moringa drouhardii* Jum.
Pandanaceae	*Pandanus epiphyticus* Martelli
Passifloraceae	*Adenia* sp. (Pretoria National Botanical Gardens 14638/69), *Adenia* sp., *Adenia firingalavensis* (Drake ex Jum.) Harms, *Adenia fruticosa* Burtt Davy, *Adenia pechuelii* (Engl.) Harms, *Adenia gummifera* (Harv.) Harms
Pedaliaceae	*Ceratotheca triloba* (Bernh.) Hook.f.
Phyllanthaceae	*Bridelia catharthica* subsp. *Carthartica*
Polygalaceae	*Polygala myrtifolia* L.
Pteridaceae	*Adianthum* sp.
Rosaceae	*Leucosidea sericea* Eckl. & Zeyh.
Rubiaceae	*Vangueria infausta* Burch., *Xeromphis obovata* (Hochst.) Keay
Solanaceae	*Solanum tomentosum* L., *Solanum seaforthianum* Andrews
Zamiaceae	*Encephalartos friderici-guilielmi* Lehm.