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Phytochemistry 66 (2005) 165–173
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Cyanogenic glucosides in grapevine: polymorphism, identification and developmental patterns

T.K. Franks [a,b,*], Y. Hayasaka [a,c], S. Choimes [a,b], R. van Heeswijck [a,b,✠]

[a] *Cooperative Research Centre for Viticulture, PO Box 154, Glen Osmond, SA 5064, Australia*
[b] *School of Agriculture and Wine, The University of Adelaide (Waite Campus), PMB 1 Glen Osmond, SA 5064, Australia*
[c] *The Australian Wine Research Institute, PO Box 197, Glen Osmond, SA 5064, Australia*

Received 23 September 2004; received in revised form 20 November 2004

Abstract

Twelve grapevine (*Vitis vinifera* L.) cultivars were surveyed for 'cyanide potential' (i.e. the total cyanide measured in β-glucosidase-treated crude, boiled tissue extract) in mature leaves. Two related cultivars (Carignan and Ruby Cabernet) had mean cyanide potential (equivalent to 110 mg HCN kg^{-1} fr. wt) ca. 25-fold greater than that of the other 10 cultivars, and so the trait is polymorphic in the species. In boiled leaf extracts of Carignan and Ruby Cabernet, free cyanide constituted a negligible fraction of the total cyanide potential because β-glucosidase treatment was required to liberate the major cyanide fraction – which is therefore bound in glucosylated cyanogenic compound(s) (or cyanogenic glucosides). In addition, cyanide was liberated from ground leaf tissue of Ruby Cabernet but not Sultana (a cultivar with low cyanide potential). Hence, the high cyanide potential in Ruby Cabernet leaves is coupled with endogenous β-glucosidase(s) activity and this cultivar may be considered 'cyanogenic'. A method was developed to detect and identify cyanogenic glucosides using liquid chromatography combined with tandem mass spectrometry (LC–MS/MS). Two putative cyanogenic glucosides were found in extracts from leaves of Carignan and Ruby Cabernet and were identified as the epimers prunasin and sambunigrin. Cyanide potential measured at three times over the growing season in young and mature leaves, petioles, tendrils, flowers, berries, seeds and roots of Ruby Cabernet was substantially higher in the leaves compared with all other tissues. This characterisation of cyanogenic glucoside accumulation in grapevine provides a basis for gauging the involvement of the trait in interactions of the species with its pests and pathogens.

Keywords: *Vitis vinifera* L; Vitaceae; Grapevine; LC–MS/MS; Cyanogenesis; Cyanogenic glucoside

1. Introduction

Release of cyanide by living organisms upon tissue disruption is termed cyanogenesis. This trait is widespread amongst plants and is most often attributed to the coupled occurrence of cyanogenic glycoside(s) with at least one degrading enzyme (β-glycosidase; Seigler, 1998). The capacity for cyanogenesis is modulated by the amount of cyanogenic glycoside available for degradation (i.e. cyanide potential) as well as by the activity of β-glycosidase(s) and these two components of cyanogenesis can be inherited independently (e.g. *Trifolium* spp.: Corkill, 1942; Williams and Williamson, 2001 and *Eucalyptus nobilis*: Gleadow et al., 2003). Cyanogenic glycosides and their degrading enzymes are differentially compartmentalised and thus the plant avoids toxicity (Thayer and Conn, 1981; Gruhnert et al., 1994). But when the compartment boundaries are disrupted and the cyanogenic glycosides and degrading enzyme(s) become co-located, cyanogenesis occurs.

* Corresponding author. Tel.: +61 8 83036665; fax: +61 8 83037116.
E-mail address: tricia.franks@adelaide.edu.au (T.K. Franks).
✠ Deceased September 5, 2003.

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doi:10.1016/j.phytochem.2004.11.017

As a consequence of their implications for taxonomy, population studies, animal toxicity, and pest and pathogen interactions, cyanogenesis and cyanogenic glycosides have been the subject of much review and discussion (Robinson, 1930; Conn, 1981; Nahrstedt, 1985; Poulton, 1990; Jones, 1998; Seigler, 1998; Møller and Seigler, 1999; Jones et al., 2000; Gleadow and Woodrow, 2002). With respect to interactions of plants with pests and pathogens, antagonistic, stimulant and neutral effects have been attributed to cyanogenic glycosides and cyanogenesis (Møller and Seigler, 1999).

A thorough biochemical and genetic characterisation of the synthesis of the cyanogenic glycoside, dhurrin, in *Sorghum bicolor* culminated recently with the transfer of three genes that constitute the entire pathway for dhurrin biosynthesis from that species to *Arabidopsis thaliana*, which does not accumulate cyanogenic glycosides naturally (Tattersall et al., 2001). This engineered accumulation of dhurrin in *Arabidopsis* corresponded with acquired resistance to the flea beetle, *Phyllotreta nemorum*, demonstrating directly that cyanogenic glucosides can provide protection from an insect pest (Tattersall et al., 2001).

At least 60 different cyanogenic glycosides have been identified in plants (Seigler, 1991). They are most often derived from one of five amino acids (L-tyrosine, L-phenylalanine, L-valine, L-isoleucine and L-leucine; Seigler, 1998) and are generally *O*-β-glucosides of α-hydroxynitriles (Fig. 1; Jones et al., 2000). Cyanogenic glycosides have typically been detected and identified by isolating active fractions using chromatography, followed by structure determination using nuclear magnetic resonance spectroscopy and/or mass spectrometry (MS) (e.g. Erb et al., 1979; Goodger and Woodrow, 2002; Seigler et al., 2002). Amongst other alternative approaches, selective isolation of glycosylated compounds followed by GC–MS analysis of trifluoroacetylated derivatives has been used to detect and identify cyanogenic glucosides (in passion fruit; Chassagne et al., 1996). To our knowledge, however, the LC–MS/MS technique for screening for glycosides by monitoring their characteristic and common fragmentation (Qu et al., 2001) has not previously been applied to the detection and identification of cyanogenic glycosides.

In grapevine (*Vitis vinifera* L.), appreciable cyanogenesis from ground leaves has been detected previously in one of four tested cultivars (Deibner, 1967). For the cyanogenic cultivar (Carignan rouge),[1] other tissues (including seeds and berries) were acyanogenic by comparison. Since the absence of cyanogenesis from the different grapevine cultivars and the different grapevine tissues may have been a consequence of either the absence of the cyanogenic compounds (i.e. cyanide potential is low) or the absence of appropriate enzyme(s) for degrading cyanogenic compounds, exogenous β-glucosidase was added to extracts of seed and ripe berry skin of Carignan rouge. However, cyanide release was still negligible and other tissues and cultivars were not investigated (Deibner, 1967).

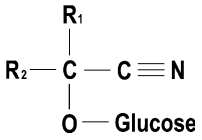

Fig. 1. General structure of cyanogenic glucosides.

Here, we expand on the work of Deibner (1967) by measuring the cyanide potential of various grapevine cultivars and grapevine tissues and by identifying the major cyanogenic glucosides involved. This information may be useful for assigning to the trait function with respect to interactions of *V. vinifera* with its pests and pathogens.

2. Results

2.1. Cyanide potential and cyanogenesis in grapevine leaves

The cyanide potential of mature leaves collected in spring from 12 grapevine (*V. vinifera*) cultivars was determined by measuring the free cyanide in boiled crude extract after treatment with β-glucosidase (Fig. 2). Two cultivars (Carignan and Ruby Cabernet) had appreciable cyanide potentials (mean = 110 mg HCN kg^{-1} fr. wt) that were ca. 25-fold greater than those measured for

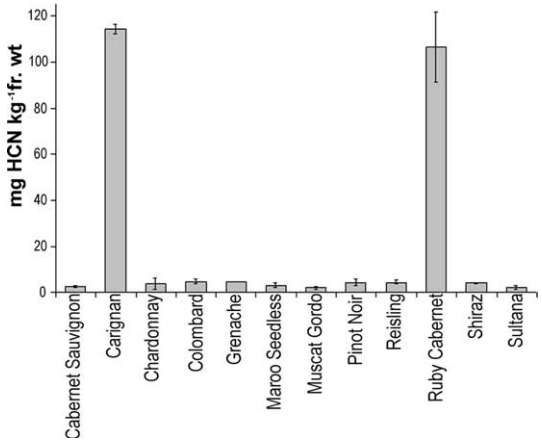

Fig. 2. Cyanide potential (cyanide equivalent to mg HCN kg^{-1} fr. wt) of mature leaf extracts from 12 grapevine cultivars at spring. Values are the mean of three replicates (or two replicates for Grenache) and replicates were assays of extracts prepared from different plants. Bars are ± SD.

[1] While no reference to Carignan rouge appears in authoritative texts (Viala and Vermorel, 1909; Galet, 1990), we assume that it is a synonym for Carignan (*syn* Carignan noire).