

A simple analytical method for dhurrin content evaluation in cyanogenic plants for their application as a biofumigant

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INTRODUCTION

Cyanogenic plants appear an additional option to Brassicaceae plants in biofumigation technique for controlling some soil borne pathogens and pests. In these last years, several studies on their application as green manure have been reported in the literature with positive results, also in nematode control. Their wider application would contribute to improving biodiversity and amending soil with different active compounds, making it possible to delay the development of resistance of some pathogens to glucosinolate degradation products. With the aim of characterizing a large number of genotypes and tissue of Sorghum and Sudangrass plants for their dhurrin content, a simple analytical procedure was defined.

The enzymatic system

Sorghum and Sudangrass contain the cyanoglycoside dhurrin which is involved in a two step enzymatic hydrolysis that gives rise to the formation of hydrogen cyanide.

In plant cell tissues, as for glucosinolates and myrosinase, dhurrin and hydrolytic enzymes are separated and they come into contact only when plant tissues are lesioned or disrupted, producing the bioactive compound (Conn, 1991).

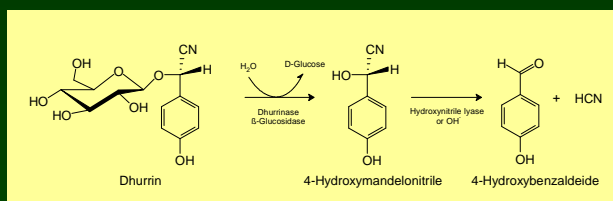


Figure 1. Reaction scheme of cyanidric acid production by dhurrin hydrolysis via dhurrinase and hydroxynitrile lyase

The extraction method

Freeze-dried, finely grounded plant tissue samples were extracted in the presence of activated carbon in MeOH (1:50 w/v) by sonication for 25 min at room temperature.

The extraction procedure was checked for recovery efficiency with spiked dhurrin standard, obtaining values ranging between 87-98%.

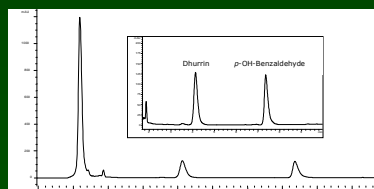


Figure 2. HPLC profile of methanolic extract of sorghum plant tissue.



HPLC analysis

Dhurrin was evaluated using a modified HPLC procedure (Johansen *et al.*, 2007), by an HPLC Agilent 1100 equipped with an Eclipse XDB-C18 (150 x 4.6 mm) column and a diode array as detector. The chromatography was performed with a gradient of water (A) and acetonitrile (B): isocratic 10% B for 1 min, linear gradient to 30% B for 7 min and linear gradient to 10% B in 2 min, monitoring the absorbance at 232 nm.

The peak corresponding to dhurrin was identified by comparing the retention time to that of standard dhurrin obtained from Extrasynthese (France).

Two types of analysis were applied and compared:

- **external standard method:** quantitative determinations were made by reference to a standard curve constructed with authentic dhurrin ($R^2 = 0.99$),
- **internal standard method:** *p*-hydroxybenzaldehyde was added to the methanolic extract and a response factor of 0.36, determined with pure compounds, was considered for dhurrin content evaluation.

The HPLC analysis was checked for reproducibility both in terms of extractions and injections. RSD for replicate extraction was 2.4% and RSD between injections varied around 0.1-0.6%.

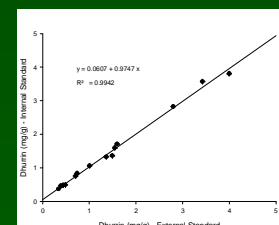
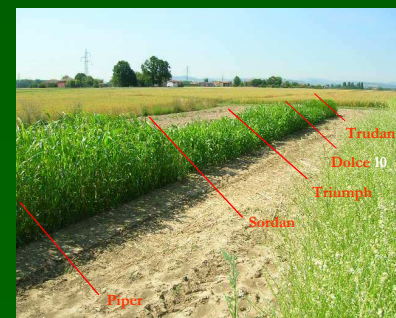
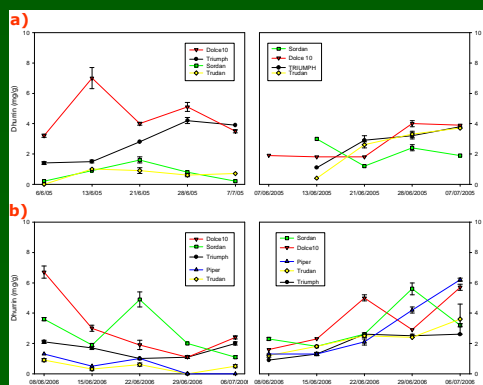


Figure 3. Correlation between external and internal standard analytical HPLC method of dhurrin evaluation in sorghum plant tissue.

Applications

Using this technique it was possible to quantify the dhurrin content on five different varieties cultivated in agronomic trials carried out in Italy in 2005 and 2006 and also to evaluate its distribution in roots and stems during cultivation. The results varied in the years also if we can evidence how Dolce 10 variety showed an interesting dhurrin content both in stems and roots, while Trudan showed the lowest content in both years.

Figure 4. Dhurrin content in plants (left) and in roots (right) during cultivation: 2005 (a), 2006 (b).



CONCLUSIONS

In this study a linear relationship was obtained between the two proposed methods of HPLC analysis of dhurrin content in plant tissues. There is a significant correlation, $R^2 = 0.99$, between the two methods and the data proved to be reproducible. The use of *p*-hydroxybenzaldehyde, easily available in common labs, thus permits simple, rapid and safe analysis avoiding use of hazardous chemicals and opens the possibility of a practical chemical evaluation of the biocidal potential of Sudangrass varieties.

REFERENCES

1. Conn, E. E. The metabolism of a natural product: lessons learned from cyanogenic glycosides. *Planta Medica*, 57 (1991): S1-S9.
2. Johansen H. *et al.* Rate of hydrolysis and degradation of the cyanogenic glycoside - dhurrin - in soil. *Chemosphere*, 67 (2007): 259-266.