Quantative estimation of hydrocyanic acid (HCN) in some cyanogenic plants with the help of microbial glycosidase enzyme

Ramesh Chillawar

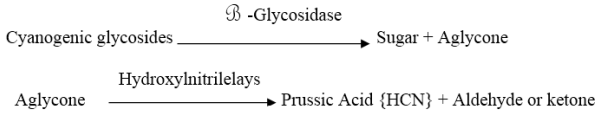
Dept of Botany; Yeshwant Mahavidalaya Nanded 431602, (M.S.).

Abstract
Cyanogenic plants are those plants which are able to synthesize cyanogenic glycosides. These glycosides when enzymatically hydrolyzed then they release Prussic acid. This is known to be a defence mechanism of plants against microbes and other invasions. In most of the cases when the microbes produces B-glycosidase enzymes for invasion into the plant tissue, the cyanogenic glycoside present in the plant is hydrolyzed with the release of HCN which may kill the pathogen. In the present study quantative estimation of prussic acid is being reported in 10 Angiospermic plants is done.

Keywords: Cyanogenic Plants, Microorganism, Angiospermic plants

1. Introduction

Cyanide C≡N exists in various forms in nature as salts of sodium, potassium and calcium beside these prussic acid is produced by number of plants of the World flora. At least 2700 species of higher plants and some micro-organisms have been shown to contain one or more nearly thirty-two compounds capable of producing prussic acid (HCN). These are known as cyanogenic plants (Seigler 1976, Moller and Seigler 1999, Jones 1998). The process of cyanogenesis is as follows:



Conversion of Cyanogenic Glycoside into Prussic Acid

Many Agriculturally and Horticulturally important plants are known to contain quite a good amount of prussic acid in them (Eyjolfsson 1970). In plant like sorghum which exist inbuilt cellular ability to synthesize B-glycosidase enzyme which can hydrolyze the cyanogenic glycoside to release Prussic acid, but in some plants prussic acid is produced even though the plants does not have the capacity to produce B-glycosidase enzyme. This happens when the plant is attacked by a pathogenic fungi or bacteria. (Malik *et al*. 1978). In the present experiment it was thought to use fungal culture filtrate as a source of hydrolytic enzyme for detection and semi-quantative estimation of Prussic acid from some angiospermic plants. In angiospermic plants, probably the release of Prussic acid from the glycoside may be induced by the B-glycosidase enzyme produced by a microbes when a pathogenic microb attack on the plant so probably the production of prussic acid by plants is a defence mechanism. In the present study by using a semi-quantative analytical method the amount of Prussic acid {HCN} produced by 10 angiospermic plants with the help of B-glycosidase enzyme present in the culture filtrate of some microbes is determined.

2. Material and Methods

The Angiospermic plants were collected from the field during the flowering and fruiting periods and correctly identified with the flora (Naik 1998, Yadav & Sardesai 2002, Almeida 2003) and the culture filtrates of two fungi *Trichoderma viride* and *Aspergillus niger* were used as a source of B-glycosidase enzyme. These microbes were cultured on Czapek's medium with CMC (Carboxy Methyl cellulose) in liquid medium. The culture filtrate was collected on sixth day of the growth of fungi. The plant part of angiospermic plant i.e.-leaf, rooot, etc. in which the cyanogenic glycoside is to be detected and the amount of prussic acid is to be estimated was weighed accurately, about 100mg using portable balance from the kit which is provided by Bradbury (Egan and Bradbury 1998, Bradbury et al. 1999). It was crushed it in mortar and pestle in distilled water.

The plant extract was taken in three small bottles and in two of them 1ml of culture filtrate was added one has control to which 1ml. distilled water was added. Then, the sodium picrate paper provided in the kit was hanged in each bottle at ambient temperature. Three sets of such experiment were arranged, a change of colour of picrate paper from yellow to brown red indicated presence of prussic acid by plants within two hours. However, in same case the result may be after 24 hours also. The amount of prussic acid canbe semi-quantitatively predicated by the appearance of different red colour shades in the testing strips of the kit. The amount of red colour is directly proportionate to the amount of Prussic acid content of the plant.

Table 1: Quantative estimation of Prussic Acid in some cyanogenic plants by using Microbial glycosidase as a Source of hydrolytic enzyme

Sr. No	Name of Plants	Family	Plant Part Tested	Test			
				T1	T2	T3	Amount of HCN (ppm)
1.	*Amygdalus communis* L.	Rosaceae	Fruit	**+Ve**	**+ Ve**	**+Ve**	400
2.	*Bougainvillea spectabilis* Willd.	Nyctaginaceae	Leaf	+Ve	+ Ve	+Ve	30
3.	*Butea monosperma* (Lamk.) Taub.	Fabaceae	Flower	-Ve	-Ve	-Ve	00

4.	*Chrozophora prostrata* Dalz.	Euphorbiaceae	Leaf	+Ve	+ Ve	+Ve	20
5.	*Diplocyclos palmatus* (L.) Jeffrey.	Cucurbitaceae	Leaf	-Ve	-Ve	-Ve	00
6.	*Ipomoea obscura* (L.) Ker-Gawl.	Convolvulaceae	Leaf	+Ve	+ Ve	+Ve	800
7.	*Justicia diffusa* Willd.	Acanthaceae	Leaf	-Ve	-Ve	-Ve	00
8.	*Linum usitatissimum* L.	Linaceae	Seed	+Ve	+ Ve	+Ve	400
9.	*Ruellia tuberose* L	Acanthaceae	Leaf	-Ve	-Ve	-Ve	00
10.	*Solanum sisymbrifolium* Lamk.	Solanaceae	Leaf	+Ve	+ Ve	+Ve	200

T1 - Test in enzyme obtained from culture of *Aspergillus niger.*
T2 - Test in enzyme obtained from culture of *Trichoderma viride.*
T3 - Test in distilled water.

3. Result and Discussion

The results of the experiment shown in the table No. (1), the results of experiment are shows that out of 10 plants tested by this method the species *Ipomoea obscura*, *Amygdalus communis*, *Linum usitatissimum*, *Solanum sisymbrifolium* released prussic acid within few minutes showing that these plant species have cyanogenic glycosides and the specific enzyme for its hydrolysis. The amount of prussic acid released is also quite significant i.e.- in between 200 to 800 ppm. The plants *Bougainvillea spectabilis* and *Chrozophora prostata* released HCN slowly within 24 hours.

Thus, from the observation it is clear that, the use of glycosidase enzymes from the culture filtrate of micro-organisms containing this enzyme to detect cyanogenesis in plants is an innovative method which was established by this experiment. Secondly it supports the hypothesis of defence mechanism of plant that the plants release Prussic acid to protect themselves in case of invasion of a microorganism.

4. References

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