

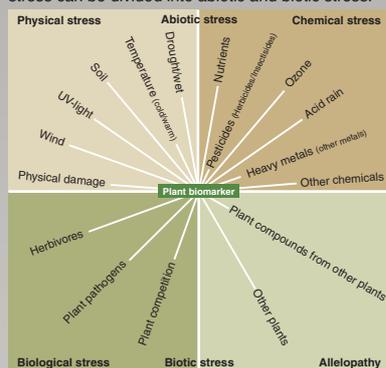
Plant Biomarker Pattern

Screening Programme for Detection of Phytochemical Differences in Plants Exposed to Stress

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Introduction

Environmental stress is a well-known phenomenon to organisms, especially plants. The different kinds of stress can be divided into abiotic and biotic stress.



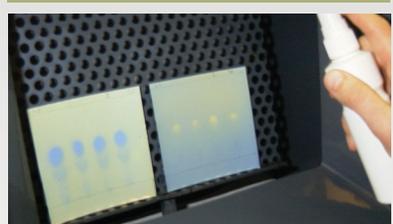
When a plant is exposed to stress, phytochemical changes appear. It is possible to detect phytochemical changes before morphological changes, if they are present. The phytochemical changes depend on the stress type, the concentration and the extent of exposure.

A new method for detection of biomarkers has been developed. The method is based on the detection of a reproducible biomarker pattern, consisting of at least two biomarkers, in exposed plant material compared with non-exposed plant material.

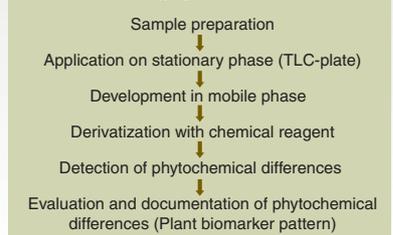
Biomarkers are indicators, signalling changes in the phytochemical composition of the plant. The method can be used as an early warning system, where the phytochemical response to a given stress exposure appears before morphological changes, as well as a simple tool to determine whether plants have been exposed to a specific stress (1). The determination of a biomarker pattern can be used to investigate plants exposed to stress compared with non-exposed plants and forms the basis of the application of the developed screening programme.

Concept

A screening programme is developed to investigate phytochemical differences in plants exposed to stress compared with non-exposed plants.



Screening process



Method

The method used to screen for phytochemical differences is Thin Layer Chromatography (TLC).

In the mobile phase passes over a stationary phase in such a way that a mixture of substances is separated into its individual components on the basis of their polarity and chemical structure. Substances separated on a stationary phase, that are not directly visible and do not react to UV light, can be detected by reaction with derivatization reagents (2).

Modern Thin Layer Chromatography as a method is:
 – simple
 – highly flexible
 – inexpensive
 – versatile.

TLC is suitable in the investigation of phytochemical differences in plants.



Camag TLC Scanning and VideoScan equipment.

Sample preparation

Plant extracts used for screening are obtained using fresh, frozen or freeze-dried plant material.

The plant material is either pressed or extracted with suitable solvents and filtered or centrifuged before application on stationary phase (TLC-plate).

Screening programme

The screening programme covers the most general groups of compounds found in plants. The following groups of phytochemical compounds are included in the programme: Unspecific compounds, organic acids, lipids, phenolic compounds, carbohydrates, terpenoids and N-, S- and P-containing compounds.

These groups of phytochemical compounds are screened by use of different stationary phases, mobile phases and derivatization reagents. Each group of compounds is covered by several derivatization reagents in order to be able to verify the results obtained with a single derivatization reagent.

TLC-systems

- Stationary phases (TLC-plates)**
 HPTLC Aluminium sheets, Silica gel 60, Merck 1.05547.
 HPTLC Aluminium sheets, Silica gel 60 F₂₅₄, Merck 1.05548
 TLC Aluminium sheets, Cellulose, Merck 1.05552
- Mobile phases**
 1-butanol : acetic acid : water (4:1:5) (upper phase)
 1-butanol : 50% formic acid (2:1)
 2-propanol : acetic acid (2:1)
 1-propanol : 25% ammonia (11:9)

Screening

– groups of compounds and derivatization reagents

- Alcohols and phenolic compounds**
 • Iron(III)chloride
- Carbohydrates**
 • Naphthoresorcinol – sulphuric acid
 • β-naphthol – sulphuric acid
 • Thymol – sulphuric acid
- N-containing compounds**
 • Fluorescein – ammonia
 • Ninhydrin
 • Bismuth(III)nitrate – potassium iodide
- Organic acids and lipids**
 • Bromocresol green – bromophenol blue – potassium permanganate
 • 2,7-dichlorofluorescein
 • Fluorescein
 • Rhodamine 6G
 • Rhodamine B
 • Bromocresol green
- P-containing compounds**
 • Ammonium molybdate
 • Cobalt(II)chloride
- S-containing compounds**
 • Methylene blue
 • Palladium(II)chloride
- Terpenoids**
 • Phosphoric acid
 • Sulfuric acid
 • Zinc chloride
- Several of the above-mentioned groups**
 • Vanillin – sulphuric acid
 • Diphenylboric acid 2-aminoethylester
 • Iodine – potassium iodide
 • Molybdatophosphoric acid
 • Anisaldehyde – sulfuric acid
 • Silver nitrate - ammonia

Evaluation

The TLC-plates are evaluated using advanced CAMAG TLC-equipment supported by The Danish Agricultural and Veterinary Research Council.

Utility of the screening programme

The screening programme, in its present form or in a more simplified form, can be utilized in several different areas as a preliminary broad screening:

- Analysis of organically- and conventionally cultivated plant foods
- Demarcation of herbicide polluted areas
- Demarcation of herbicide exposure in spraying free zones close to water steams and lakes
- As a new tool for farmers to obtain an optimal effect using a reduced amount of herbicide
- Monitoring of sulphur- and chemical stress resulting in the death of sea-grasses



(1) Ravn (2001): An assay method and kit for testing biological material for exposure to stress using biomarkers. International Patent (PCT) application, priority data: PA 2000 00874, 30 may 2000 (WO 01/92879 A1).
 (2) Harborne, J.B., Biochem. Syst. Ecol. (1999) 27, 335-367



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In 2000 an international PCT patent application: "An assay method and kit for testing biological material for exposure to stress using biomarkers" (WO 01/92879 A1), PCT /DK01/00377) was filed at the European Patent Office with the purpose of international patent protection.