

Determination of replacement of some inorganic elements in pulvinus of bean (*Phaseolus vulgaris* cv. Gina 2004) at chilling temperature by the WDXRF spectroscopic technique

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Abstract

In this study, bean seedlings (*Phaseolus vulgaris* cv. Gina 2004) were exposed to chilling temperatures until leaves are wrinkled (9 day), that is, showed nyctinastic movement. Pulvinus were subsequently were cut from the leaves. Concentrations of inorganic elements (P, S, Cl, K, Ca, Cu) in the pulvinus were measured by wavelength-dispersive X-ray fluorescence (WDXRF) spectrometry. Results indicated that concentration change (%) was not significant for Ca (0.82) but it was significant for K, P, Cl, S, and especially Cu concentrations (5.4%, 12.8%, 40.2%, 43.7%, 365%, respectively) in pulvinus of plants exposed to chilling temperature compared with control group.

We hypothesize here the presence of association between nyctinasti movement brought about by pulvinus at chilling temperature in bean and changes of K, P, Cl, S and especially Cu concentrations measured by WDXRF analysis method.

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1. Introduction

Studies of leaf movements have elucidated some basic scientific truths. Analyses of these movements in the early 18th century provided the first clue that organisms have internal clocks [1], and subsequent studies are providing useful information on light–rhythm interaction in biological time keeping. Investigation of seismonasty in *Mimosa pudica* and the carnivorous plants has revealed that electrical signals propagate through plants [2,3] as well as animals and are an important means of communication among the different cells of the plant body [4].

In nyctinastic plants, there are two cells group (extensor motor cells and flexor motor cells) that causes movement of pulvinus [5]. Extensor motor cells increase turgor during leaf opening and decrease turgor during

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closure, while flexor motor cells act in a opposite manner [4]. Leaf movements are turgor regulated and dependent upon redistribution of ions, mainly K^+ , among cells in different regions of the pulvinus, the motor organ at the base of the leaflet [6]. The uptake of K^+ is coupled to the efflux of H^+ [7–10]. However, other researchers have supposed that this movements is related to pH [11], Ca–K [12] and Cl [13] in the leaf tissues.

The concentrations of K, Na, Ca, and Mg in extracts of tissue from *Albizia* [14], *Samaea* [15], *Phaseolus vulgaris* [16], *P. coccineus* [13] and *Trifolium* [12] have been measured with the atomic absorption spectrophotometer. It has been determined that K levels are unusually high, particularly in the pulvinus [4].

There are very close relations between inorganic elements and metabolism of living organisms. For example, S is needed for protein synthesis. P is important for nucleic acid synthesis, and the production of important cofactors such as ATP and NADP. K plays an important role in regulation of the osmotic potential. It also activates many enzymes involved in respiration and photosynthesis [17]. Ca is required for the normal functioning of plant membranes and has been implicated as a second messenger for various plant responses to both environmental and hormonal signals [17,18]. Cl is one of the major osmotically active solutes in the vacuoles and thus affects, for example, the turgor potential of leaves or of specialized tissues such as the pulvini in *Mimosa* [19]. Cu is associated with enzymes involved in redox reactions.

The recent advent of commercially available wavelength-dispersive spectrometers for X-ray fluorescence (XRF) measurements has provided an economical and powerful tool for environmental, clinical, chemical, geological and industrial analysis. XRF is a non-destructive, fast, multi-element technique for analyzing the surface layer and determining major, as well as minor elements, in thin and thick samples of all sizes and forms. Accurate quantitative measurements, however, often depend on matrix correction procedures, which require a large number of standards.

A wide range of new research applications have been demonstrated by using the EDXRF technique [20–24].

Consequently, the objective of the present study was to examine the hypothesis that significant inorganic element (S, Cl, P and especially Cu) changes in pulvinus were correlated with nyctinastic-leaf movement that occurred under chilling stress in bean plants by using WDXRF, a new technique for biological materials.

2. Experimental

2.1. Apparatus

A wavelength-dispersive spectrometer (WDXRF, Rigaku ZSX-100e with Rhodium target X-ray) was used. This instrument was controlled by a Software ZSX computer.

The ZSX 100e wavelength-dispersive spectrometer characteristics included;

1. Analysis of elements from B to U.
2. 4 kW 70 kV end-window X-ray tube.
3. Micro area mapping down to 0.5 mm.
4. Up to five primary beam filters, 10 analyzing crystals, and eight limiting area diaphragms.
5. Optional secondary collimators.
6. Automatic sample changer.
7. Compact design.
8. Multi-window, multi-function fundamental parameters software.

The WDXRF is a technique that allows fast, accurate elemental analysis for a range of materials and is described at the company's web site [25].

2.1.1. SQX advanced semi-quantitative software

Rigaku [25] has improved their semi-quantitative software package further with the introduction of SQX. It is capable of automatically correcting for all matrix effects, including line overlaps. SQX can also correct for secondary excitation effect by photoelectrons (light and ultra-light elements), varying atmospheres, impurities and different sample sizes. Increased accuracy is achieved using Matching Library and Perfect Scan Analysis Programs.

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