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*Abstract: To detect the stress state of sugar beet suffering magnesium restriction, three stages of development were studied. Leaf reflectance and solvent extraction were used to analyse the pigments. Chlorophyll fluorescence monitoring has been carried out with direct and modulated techniques. Although Mg is the atom in the centre of the chlorophyll molecule, its deficiency becomes apparent in the plant at several levels (such as electron transport and non-light dependent rate constants) before the chlorophyll content. Distinct depressions in chlorophyll concentration parallels interveinal chlorosis toward the distal end of the recently mature leaves. Therefore, the fluorescence transient is a potential biosensor that monitors photosynthetic activities for basic, agricultural and ecological projects.*

### **Introduction**

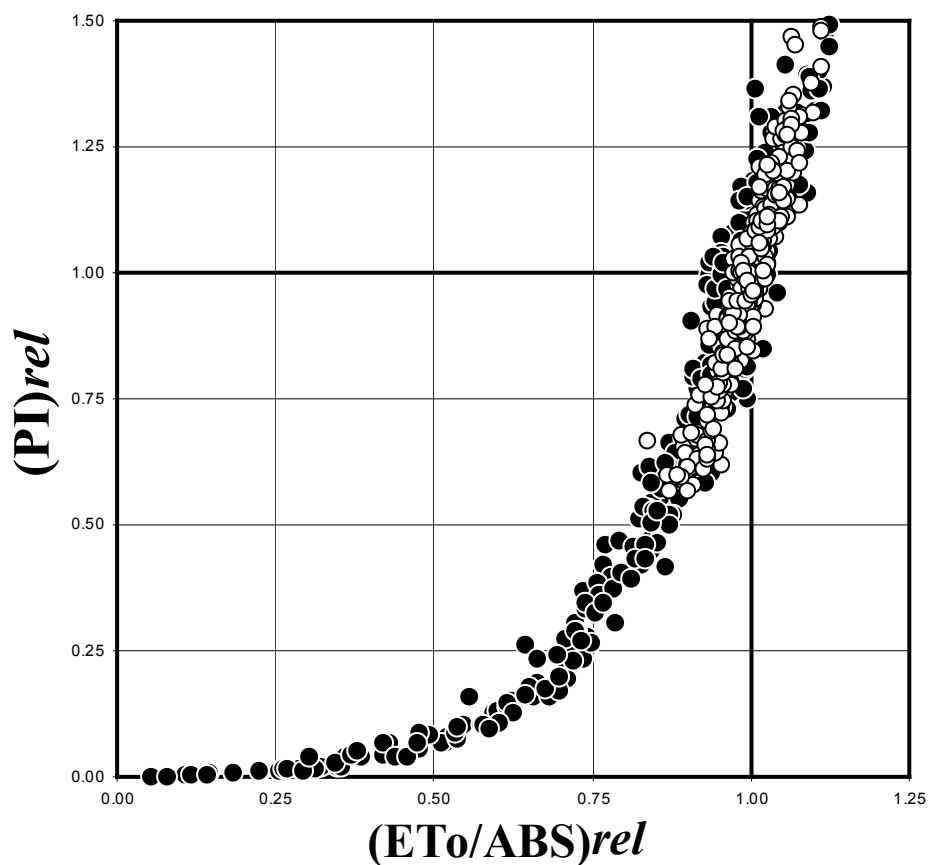
Mineral nutrition of sugar beet is an important issue in agriculture, as fertilisers constitute a large proportion of the variable cost of production. The early diagnosis of mineral deficiencies is a keen feature because it helps to prevent any loss of crop yield and decrease of crop quality. In this context, analytical plant and soil sampling is helpful but time consuming and does not convey any accurate information on plant vitality. Therefore, probing plant physiology using alternative methods to adjust the standard analytical procedures has become prevalent in agricultural management. Optical techniques such as leaf reflectance (Gamon and Surfus, 1999) and chlorophyll fluorescence (Maxwell and Johnson, 2000) can assay the qualitative and quantitative changes in photosynthetic processes.

Although magnesium deficiency is a common disorder in crop plants (Bennett, 1997), its impact on plant metabolism is not fully understood. It is not easy to establish the primary effects on the photosynthetic activity. Actually, Mg is vital to many metabolic activities, including photosynthesis (Terry and Hulrich, 1973). However the deficiency does not seem to affect first chlorophyll, hence it has repercussions on source leaf metabolism and sucrose translocation (Cakmak et al, 1994). Somewhat less emphasis has been placed on the dynamic description of the photosynthetic apparatus suffering Mg deficiency. Therefore, the aim of this work is to provide new insights in the Mg deficiency installation process.

### **Materials and methods**

Seeds of *Beta vulgaris* L. var. Claudia have germinated with an interval of 6 weeks to provide three growth stages. Plants were grown in a green-house at  $20 \pm 2$  °C with a 12/12 photoperiod (at least a minimum of 80  $\mu\text{mol}/\text{m}^2\text{s}$ ). In the hydroponics tide system, a full nutrient solution with and without Mg was used. Two Hansatech Instruments (Kings' Lynn, UK) fluorometers were used: the direct *Plant Efficiency Analyser* and the modulated *Fluorescence Monitoring System II*. The direct fluorescence induction kinetics OJIP (Strasser et al, 1995) were analysed according to Srivastava *et al*, 1999. Pigment concentration was determined in acetone extracts (Lichtenthaler, 1987) and *in situ* by the leaf reflectometer GER 1500 (GER Corporation). The normalised difference vegetation index NDVI and the photochemical reflectance index PRI were determined (Gamon and Surfus, 2000). Mineral content of organs was analysed by atomic absorption spectrometry (Perkin Elmer AAS 3110).

**Fig.1.** Correlation of the relative Performance Index, as a function of the relative yield ( $ETo/ABS$ )*rel* on a daily basis after withholding Mg from the nutrient solution. • Mg deficiency; ○ Control - most recently matured leaves of the young plant growth stage.



**Fig.2.** Effect of withholding Mg from the nutrient solution (at day 0) on the relative driving force  $\Delta DF_{rel}$  for three growth stages (*A.* juveniles; *B.* young; *C.* mature plants) on the most recently matured leaves of the sugar beet, through the days of treatment.  $\Delta DF_{rel} = (DF_{Mg\ def}) / (DF_{avg\ ctrl})$ .

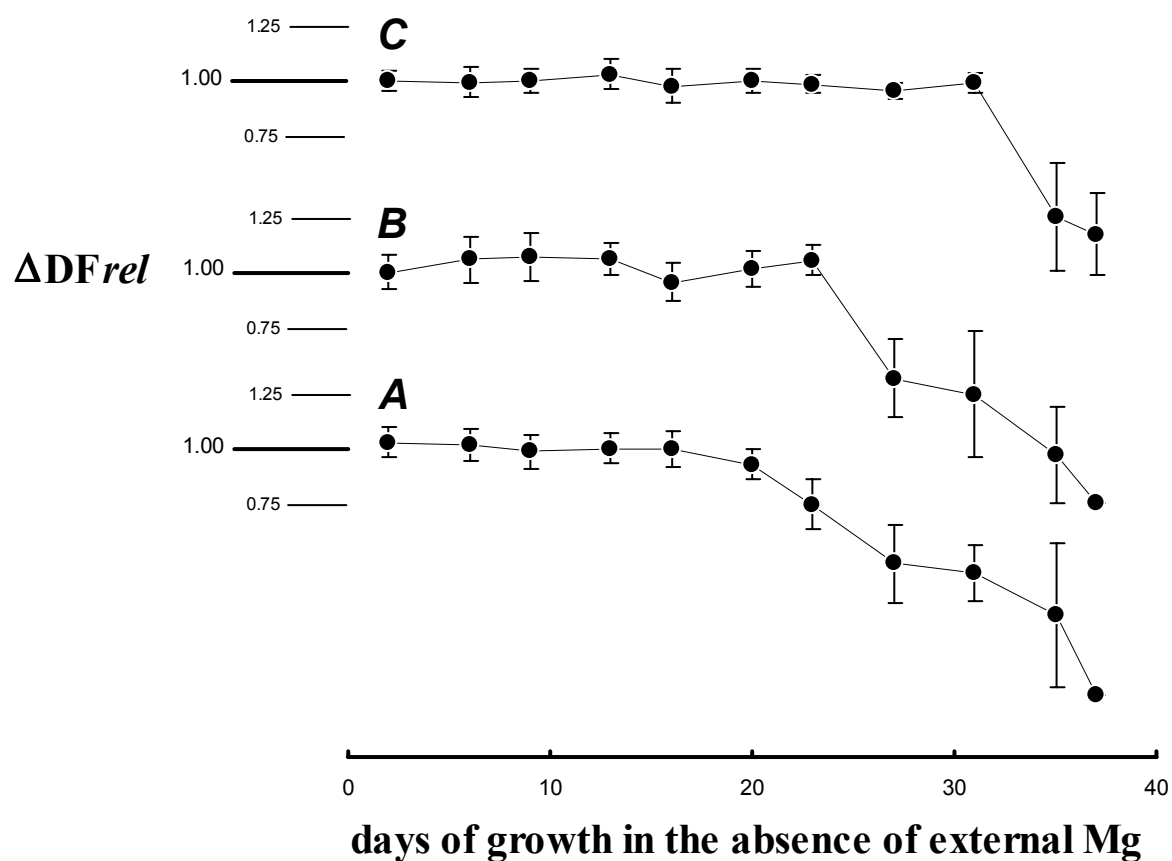


Fig.3. Multi-parameter plot representation. The average values of the JIP-test expressions on the 37th day of treatment for the expanding (hatched zone) and most recently matured leaves (sprinkled zone) of the young plant growth stage are plotted on a logarithmic scale. Parameters reviewed in Alaka et al, 1999.

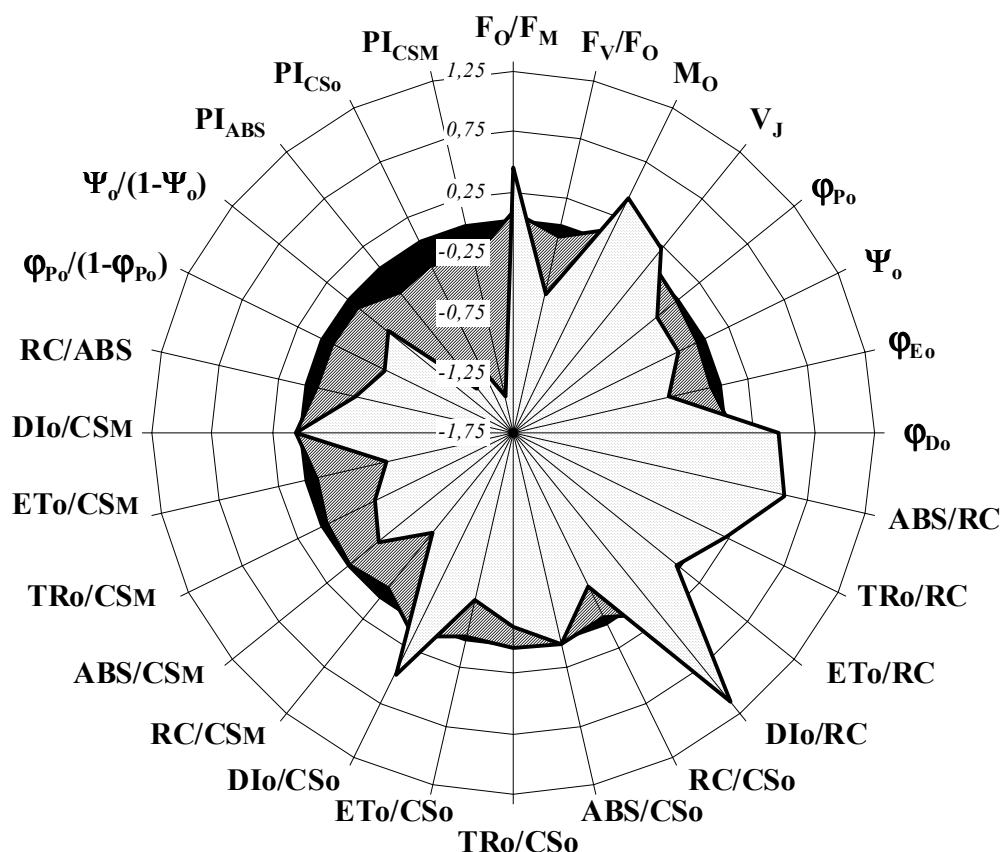
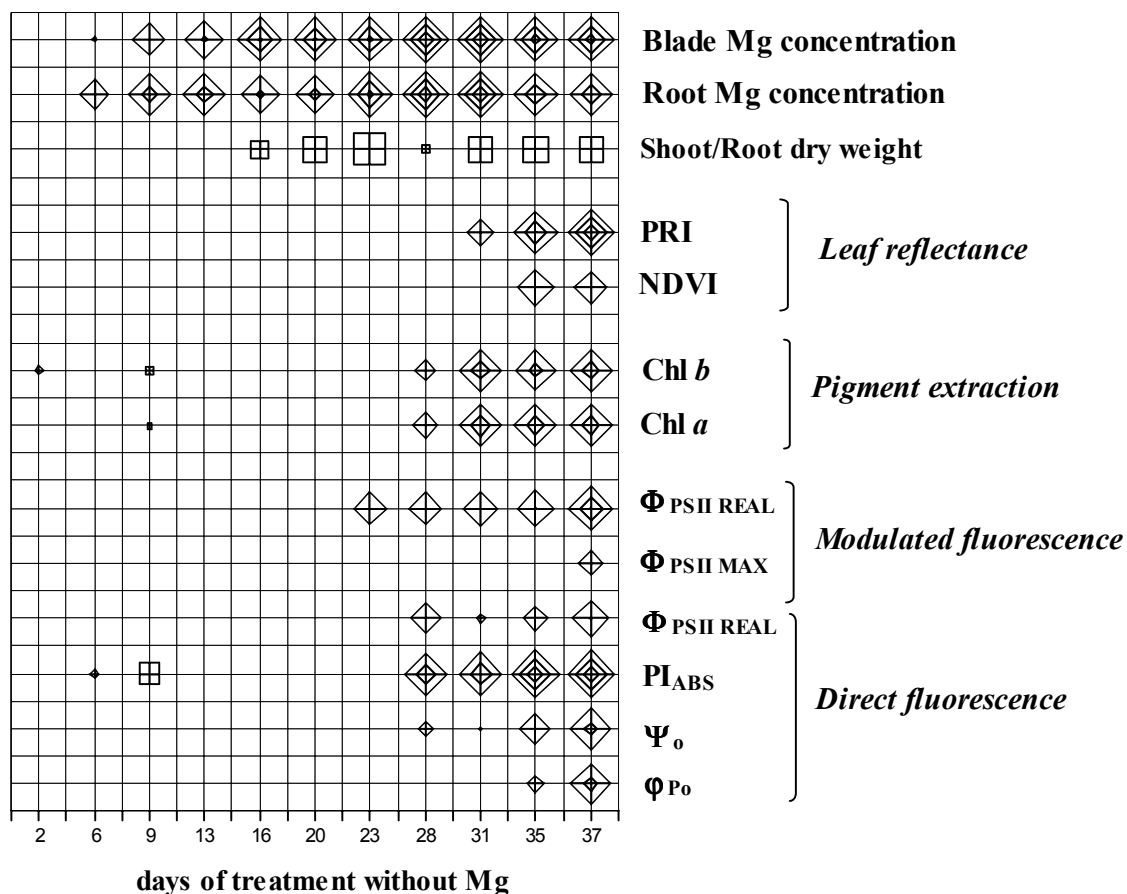


Fig.4. Carpet plot showing the variation of each parameter probed by optical or analytical methods, expressed respectively to the corresponding control value, versus the days after withholding Mg. The plotted values are visualised by the number of the contour lines, with successive lines corresponding to values differing by 0.20. Diamonds and squares respectively indicate negative and positive deviations.



## Results

In mature leaves, the performance index PI and the electron transport rate ETo/ABS have been determined from about 600 measurements (300 control and 300 Mg deficient samples). The lack of Mg in the nutrient solution decreases both expressions (fig.1.). Due to the exponential behaviour of the performance index, the driving force DF has been defined as  $\log PI$ . This is shown for juvenile, young and mature plants suffering Mg deficiency, as a function of the time without Mg. Moreover, the deficiency successively appears in juvenile, young and mature plants (fig.2.). The JIP-test, based on the fluorescence rise, allows the determination of many expressions quantifying the PSII behaviour. The relative values of the Mg deficient sample per control is shown in the so-called multi-parameter plot (fig.3.). A typical pattern reflecting the stress encountered by Mg deficient plants becomes apparent. It depends on the physiological state of the leaves. The carpet plot (fig.4.) allows the comparison of the parameters from different experimental techniques as a function of the days without Mg. Slight interveinal chlorosis appear after 30 days of treatment, however several experimental parameters shown in fig.4. reveal the effect of the deficiency several days before. Upon the decreasing of Mg content in the organs, the shoot/root ratio for the dry matter increases immediately to a certain extent, due to a weight loss in the root and an increase in the leaf. It seems that the transfer of assimilates between the sink organs to the roots is blocked (Cakmak et al, 1994 ) and this should be the origin of a gradual change of most measured parameters. The most important variations, which reach values superior to 80 %, have been noted for the photochemical reflectance index PRI and for the performance index PI.

## Conclusion

The ability to sample non-invasively plants coping with nutrient deficient environment offers prospects for detecting the stress state encountered by the crop. For the assessment of the photosynthetic activity on a leaf scale, the techniques described in this application show high potential capabilities. In order to gather useful information about the fast fluorescence transient, the JIP-test turns out to be an effective tool to diagnose Mg deficiency. It satisfies the demand for rapid screening test (measuring time : one second) and set with handy equipment to improve field management.

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