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Evaluation of the nodulated status of *Vigna unguiculata* probed by the JIP-test based on the chlorophyll *a* fluorescence rise

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Introduction

Under nitrogen-limiting conditions, soil bacteria of the genera *Rhizobium* form symbiotic associations with leguminous plants. Infectious rhizobia penetrate the root cortex and form nodules. Within the nodules, the bacteria enlarge and differentiate into nitrogen-fixing bacteroids. In return for carbohydrates, bacteroids exchange readily assimilated forms of nitrogen with their hosts.

Measurements were made during 6 weeks on the three first mature leaves appearing on the *Vigna* plants. The 2 seconds-curves provided by the PEA instrument **(Fig.1.)** were analysed and normalised with the program BIOLYZER (developed by R. Maldonado-Rodriguez).

Results

The fluorescence (O-K-J-I-P-curves) of leaves from *Vigna* plants grown in media with different KNO_3 or from plants inoculated with *Rhizobium* (NGR) are shown in **Fig.1.** The experimental traces of the fluorescence rise O-K-J-I-P are double normalised for single turnover events as $W_{(t)} = (F_{(t)} - F_{50\mu s})/(F_{2ms} - F_{50\mu s})$. The insert shows the difference of the fluorescence ΔW = W (of the sample x) – W (of the sample with 20 mM KNO_3) where x corresponds to the sample (from top to the bottom) with 0/0.5/1/NGR/10 mM KNO_3.

Each set of curves belonging to a particular treatment was averaged and compared with the other treatments. The results show an increasing driving force (DF) quantifying the potential of plant photosynthesis (Srivastava *et al*. 1999) as a function of the nitrate concentration.

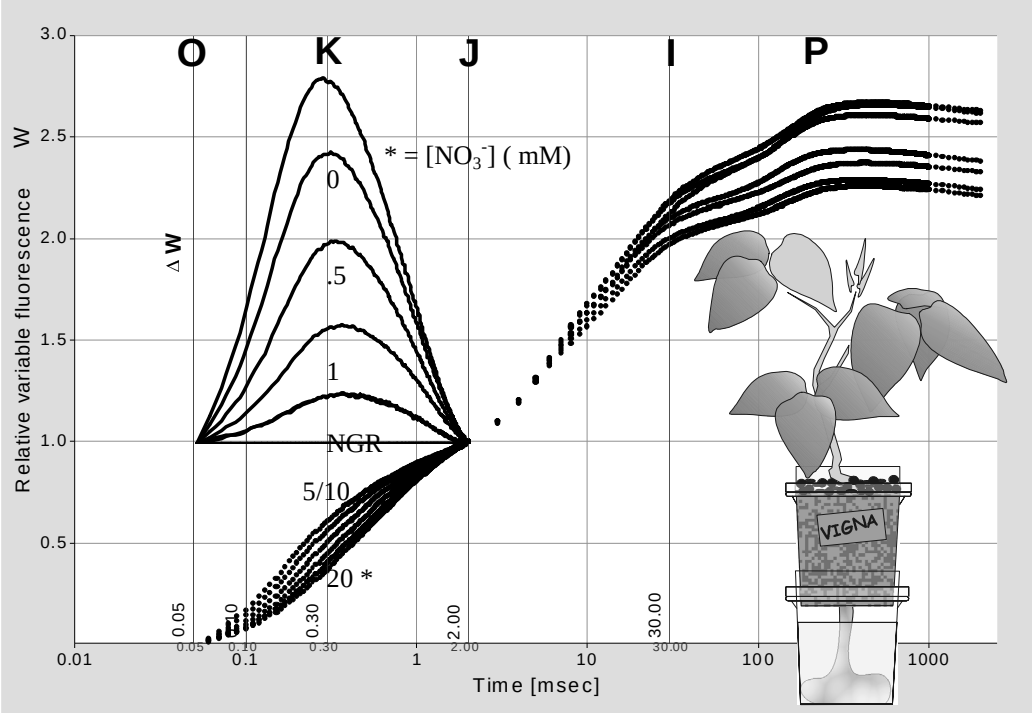
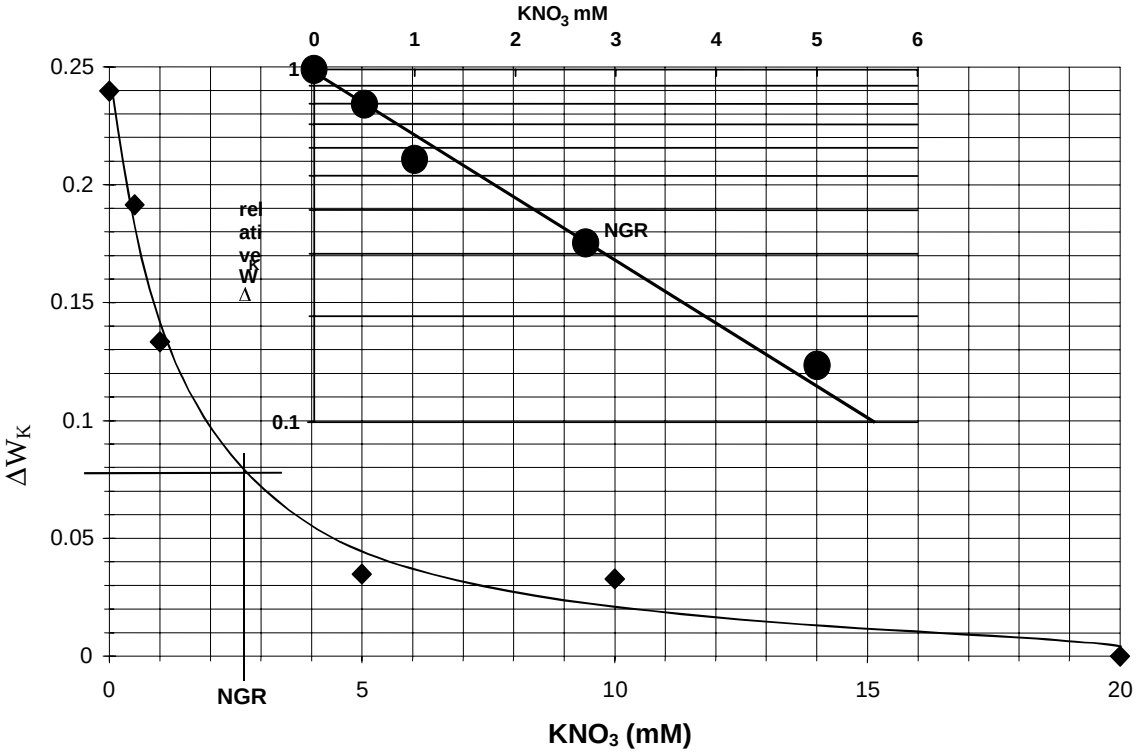


Fig.1. Fluorescence rise O-K-J-I-P (see text)

Fig.2. Nitrogen deficiency (see text)



Using the data in **Fig. 2** it was possible to determine that the presence of *Rhizobium* sp. NGR234 equals the effect of a concentration of about 3 mM nitrate in the nutrient solution. The nitrate deficiency can be detected using the correlation of ΔW measured at 300µs = ΔW_K versus the KNO_3 concentration in the medium **(Fig.2.)**. The symbiosis of the plants with *Rhizobium* (NGR) establishes an estimated nitrate supply comparable to 3 mM KNO_3, which corresponds to about 70% of the saturating KNO_3 concentration under which conditions the plants show a normal healthy fluorescence rise of the type O-K-J-I-P with the K-peak more pronounced in stress condition (Srivastava *et al.* 1996).

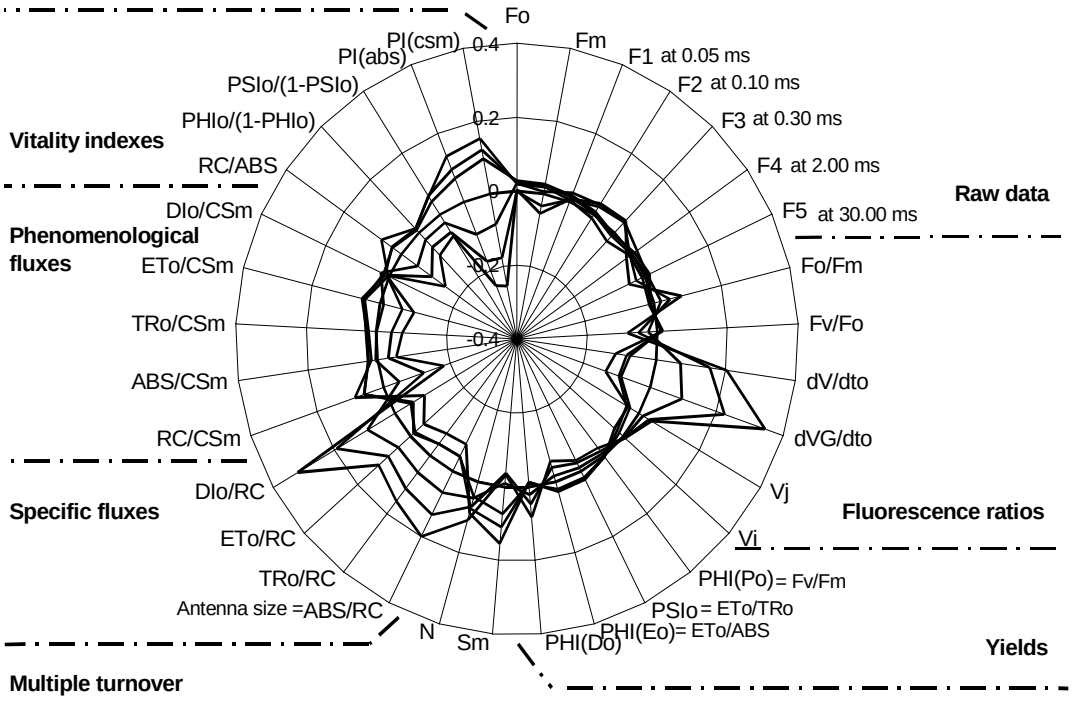


Fig.3. Radar plot of fluorescence data (see text)

According to the JIP-test many parameters can be derived **(Fig.3.)** and presented as a Radar Plot. Each axis corresponds to a measured or derived parameter. The values for each sample are indicated as relative deviations of the reference sample cultivated with *Rhizobium* (NGR = 1). The sequence of the samples is from positive to negative deviations e.g. for the expression PI_{ABS} the sequence is in mM KNO_3 : 20, 10, 5, NGR, 1, 0.5, no KNO_3. Note that e.g. for DIo/RC the sequence is just the opposite. For the equations of each expression see Strasser *et al.* (2000) and Srivastava *et al.* (1999). The energy fluxes are labeled as absorption ABS, trapping TRo, electron transport beyond Q_A^- as ETo.

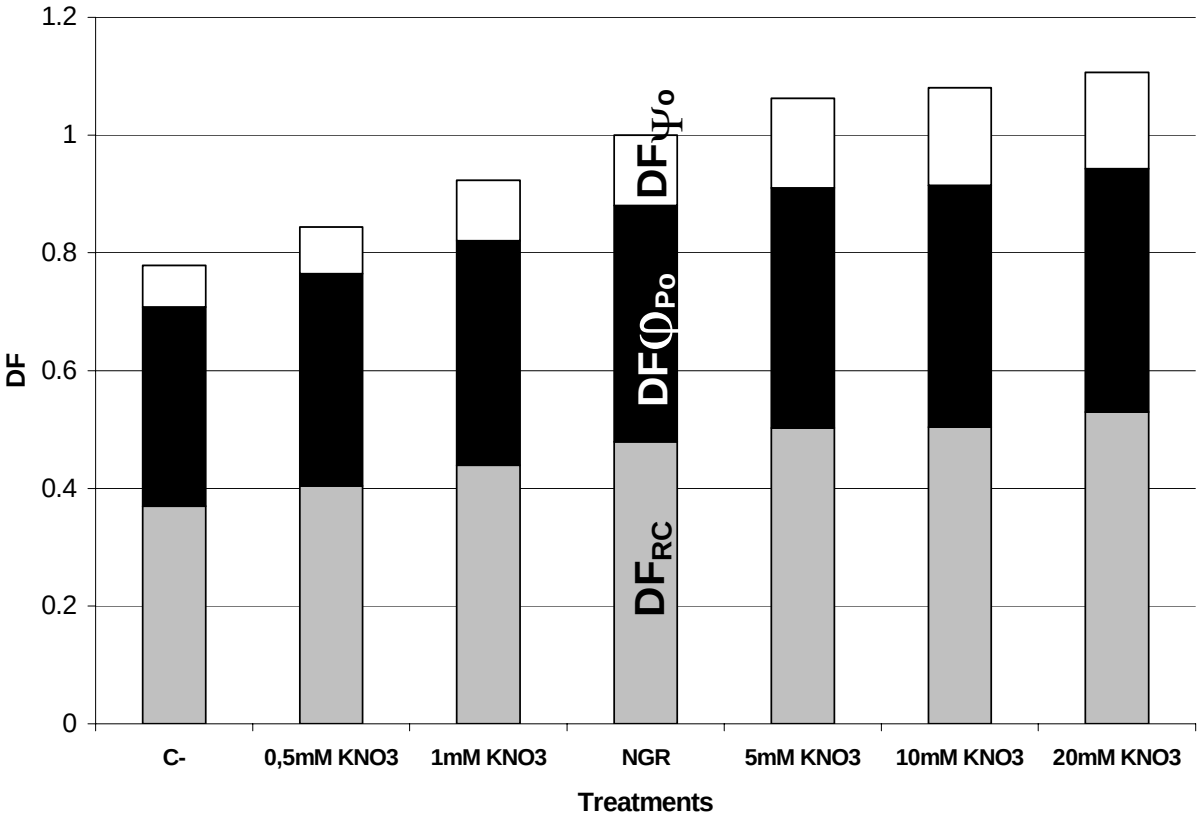


Fig.4. Decomposition of the Driving Force into its components (see text)

The deconvolution of the photosynthetic driving force (DF_{ABS} = log PI_{ABS}) into components due to the density of reaction centers (DF_{RC}), due to the quantum yield of primary photochemistry ($DF\varphi_{Po}$) and due to the probability that Q_A^- feeds an electron further into the electron transport chain ($DF_{\Psi o}$) is shown in **Fig.4.** PI_{ABS} stands for driving force calculated with the fluorescence values $F_{50\mu s}$, F_m, $F_{300\mu s}$ and F_{2ms}.

Conclusion

The photosynthetic behaviour of a plant is manifested in its fast fluorescence rise O-K-J-I-P. The JIP-test allows to derive several independent parameters which form a constellation typical for the actual state of the plant. The data show that the JIP-test is sensitive to nitrogen deficiencies and to the degree of nodulation by *Rhizobium*. Therefore *in vivo* screening for nitrogen deficiency is feasible.

Acknowledgments

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