Performance index as a sensitive indicator of water stress in *Triticum aestivum* L.

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ABSTRACT

Polyphasic chlorophyll *a* fluorescence represents a promising tool for detection of plant tolerance to various environmental stresses. In pot vegetation experiments, plants of seven winter wheat varieties were screened for their drought tolerance. The drought stress was initiated in plants by withholding water at the beginning of anthesis. While water content was measured continuously as relative water content (RWC), fast chlorophyll *a* fluorescence kinetics was measured and analysed on dehydrating intact leaves by the JIP-test (analysis of O-J-I-P fluorescence transient). Maximum quantum efficiency of PS II photochemistry (F_V/F_M) parameter was almost unaffected by dehydration until the severe water stress occurred. In contrast to this a continuous decrease of performance index (PI_{abs}) parameter (Strasser et al. 1995) was observed from the very beginning of dehydration following the decrease of RWC. Statistically significant differences were also found in the PI_{abs} parameter among all tested varieties. The results show that PI_{abs} may serve as an index of plant/variety vitality and/or sensitivity to water stress reflecting their different drought tolerance.

Keywords: chlorophyll *a* fluorescence; drought; drought tolerance; photosynthesis; photosystem II; *Triticum aestivum* L.

Drought is one of the main abiotic constraints affecting negatively yield of many field crops. For this reason screening for improved plant drought tolerance, a rather difficult and time-consuming process, seems to be a promise for breeding programs of many agricultural crops.

Lack of appropriate physiological traits comprised in the breeding programs is the reason why plant breeders have not included more analytical approaches into the selection process. Any desirable physiological traits should be rapidly and easily measured and more efficiently evaluated than parameters of final yield (Araus et al. 2002, Svobodová and Míša 2004).

Many studies found the chlorophyll *a* fluorescence as a very sensitive probe of physiological status of leaves and plant performance in a wide range of situations (Baker and Rosenqvist 2004). Key factors of successfully applied chlorophyll *a* fluorescence measurements within the crop improvement programs are carefully selected and analysed fluorescence parameters. As it is found in many studies (Genty et al. 1987, Cornic and Briantais 1991, Havaux 1992, etc.), especially the initial phase of drought stress in plants as followed by stomatal limitation of photosynthesis is not perceived by the fluorescence parameters that can only monitor the efficiency of primary photochemical processes of photosynthesis. However, Araus et al. (1998) proposed some of these parameters as selection criteria for improvement of grain yield of durum wheat in Mediterranean conditions, where frequent drought often accompanied by high temperatures is typical.

A special group of fluorescence parameters which are derived from high resolution measurements analysis of the chlorophyll *a* fluorescence kinetics can offer detailed information on the structure and function of plant photosynthetic apparatus, mainly photosystem II (PS II). Analysis of O-J-I-P fluorescence transient by the JIP-test (Strasser et al. 1995) can be applied to derive a number of parameters quantifying the flow of energy through the PS II both at the reaction centre (RC) and at

excited cross-section (CS) levels. Force et al. (2003) also demonstrated an advantage of using the JIP-test-derived fluorescence parameters to evaluate PS II function, rather than a single fluorescence parameter, such as maximum quantum yield of PS II photochemistry (F_V/F_M). The advantage of such complex analysis also lies in the fact that it can indicate stress in plants even before the visible symptoms appear on the leaves (Christen et al. 2007).

Plant vitality could be characterized by performance index PI_{abs} (Strasser et al. 2000). This integrative parameter includes three independent parameters: (1) density of fully active reaction centers (RCs); (2) efficiency of electron movement by trapped exciton into the electron transport chain beyond the Q_A; and (3) the probability that an absorbed photon will be trapped by RCs. PI_{abs} reflects the functionality of both photosystems I and II and gives us quantitative information on the current state of plant performance under stress conditions (Strasser et al. 2004).

The objective of this study was to evaluate the applicability of the parameter(s) derived from the fast chlorophyll a fluorescence kinetics to evaluate drought stress response of wheat and distinguish drought tolerance among the tested wheat varieties.

MATERIAL AND METHODS

Plant material and cultivation. Plants of winter wheat (*Triticum aestivum* L.), varieties from different proveniences, such as Viginta (VIG), Ilona (ILO), Arida (ARI), Eva (EVA) from Slovakia, Pobeda (POB) from Serbia and Monte Negro, Stephens (STE) from USA and Amerigo (AME) from France were cultivated in pot vegetation experiments in 2004 and 2005. The 20 l plastic pots were filled with a sandy-loam soil substrate. Seeding was performed at an appropriate agrotechnical term in autumn and plants were grown in natural conditions in a vegetation cage of the Department of Plant Physiology, Slovak Agricultural University in Nitra (48°18'25"N, 18°05'11"E) for the whole season. Plants were fertilized optimally and watered regularly so that no symptoms of nutrient deficiency or water deprivation were observed. Diurnal and seasonal fluctuations of main microclimatic factors (temperature, radiation) were monitored by the LiCor-1400 datalogger (LiCor, Nebraska, USA) with standard temperature and radiation sensors mounted both above and inside the plant stand levels.

At the growth stage of anthesis a rain shelter with a transparent plastic foil was installed above the pots ensuring sufficient air circulation underneath. Half of plants from each variety were kept as well-watered (control plants) whereas the rest was submitted to dehydration by withholding water (stressed plants). The dynamics and duration of water stress was different in each experimental year reflecting the dissimilar weather conditions, especially during June 2004, which was characterized by several rainy days leading to delaying the RWC decline for a short time.

Measurements. Chlorophyll a fluorescence measurements were performed both on control and stressed plants at the beginning of water stress period and several times during the dehydration treatment always at the same time of day (morning hours). Intact flag leaves of wheat plants were adapted to darkness for 30 min using light-withholding clips. Chlorophyll a fluorescence was measured by a portable non-modulated fluorimeter Handy PEA (Plant Efficiency Analyser, Hansatech Instruments, Kings Lynn, UK). After the adaptation of leaves to darkness a single strong 1 s-light pulse (3500 μmol/m^2/s) was applied on them with the help of three light-emitting diodes (650 nm).

The fast fluorescence kinetics (F_0 to F_M) was recorded during 10 μs to 1 s. For each variety and treatment at least 10 repetitions were applied. The measured data were analysed by the JIP test (Strasser et al. 1995, 2000). Plant vitality was characterized by performance index PI_{abs} parameter (Strasser et al. 2000) calculated as follows:

$$PI_{abs} = \frac{1-(F_0/F_M)}{M_0/V_J} \times \frac{F_M - F_0}{F_0} \times \frac{1-V_J}{V_J}$$

where: F_0 means fluorescence intensity at 50 μs, F_J is fluorescence intensity at the J step (at 2 ms), F_M represents maximal fluorescence intensity, V_J is relative variable fluorescence at 2 ms calculated as $V_J = (F_J - F_0)/(F_M - F_0)$, M_0 represents initial slope of fluorescence kinetics, which can be derived from the equation: $M_0 = 4^* (F_{300\,μs} - F_0)/(F_M - F_0)$

Measured data were also used for calculation of maximum quantum efficiency of PS II photochemistry (F_V/F_M) according to the equation:

$$F_V/F_M = (F_M - F_0)/F_M$$

Immediately after the chlorophyll a fluorescence measurements also the relative water content (RWC) was determined in the same leaf samples according to Turner (1981) and calculated as: RWC = (fm – dm)/(sm – dm), where fm means fresh
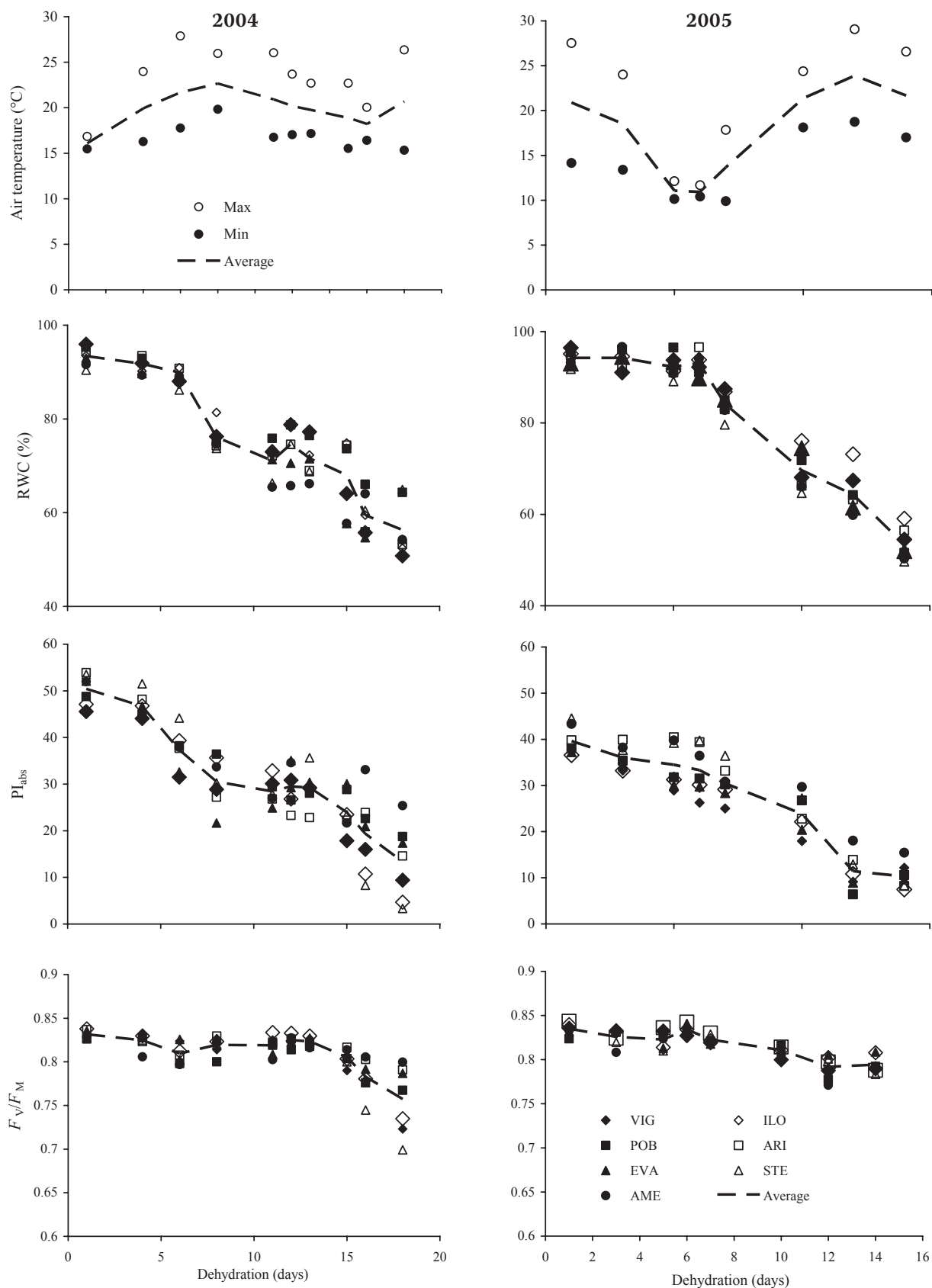
Figure 1. Parameters measured during dehydration process in 2004 (left column) and 2005 (right column). Maximum, minimum and average air temperature monitored during the dehydration period (upper left and right graphs). RWC, performance index (PI_{abs}) and maximum quantum yield of PS II photochemistry (F_V/F_M) of winter wheat varieties measured during water stress. Each point represents an average value of each variety; dashed lines are the average trends for all varieties

mass of leaf segments, sm is a mass at full water saturation measured after their 4 h rehydration in distilled water, and dm is a constant dry mass of leaf segments after 12 h drying at 80°C.

Statistical analysis. The achieved results were processed with the Microsoft Excel and Statgraphic programs. The differences among varieties and treatments were analysed by the Student t-test at 0.05 significance level.

RESULTS AND DISCUSSION

Few days after withholding water, the relative water content (RWC) decrease was observed in leaves indicating the onset of water stress (Figure 1).

Although plant primary processes of the PS II photochemistry are quite resistant to mild water deficits (Cornic and Briantais 1991, Havaux 1992), in our experiments from both experimental years, a continuous decrease of performance index (PI_{abs}) parameter was observed as a result of plant dehydration characterized by the RWC decrease. On the contrary, the maximal quantum efficiency of PS II photochemistry (F_V/F_M) was almost unaffected until the last third of dehydration period, which is in accordance with the findings of Genty et al. 1987, Christen et al. 2007 and Oukarroum et al. 2007, considering generally the F_V/F_M parameter as very insensitive to early changes of plant photosynthesis due to water stress. Correlation between the F_V/F_M and RWC only shows a significant F_V/F_M decrease at the RWC of 70% and less (Figure 2). This also corresponds to the authors who consider the 30% of leaf water deficit as critical for functioning of plant photosynthetic machinery (Chaves 1991, Cornic et al. 1992). However, photosynthetic parameters could be a good tool to screen crop genotypes for conditions of climate change (Brestič et al. 2007).

Many studies found that non-stomatal limitation of photosynthesis may occur at the RWC of 70% with a direct effect on inhibition of primary photochemistry (Chaves et al. 2002, Cornic and Fresneau 2002). A considerable decrease of these parameters at the end of the dehydration period
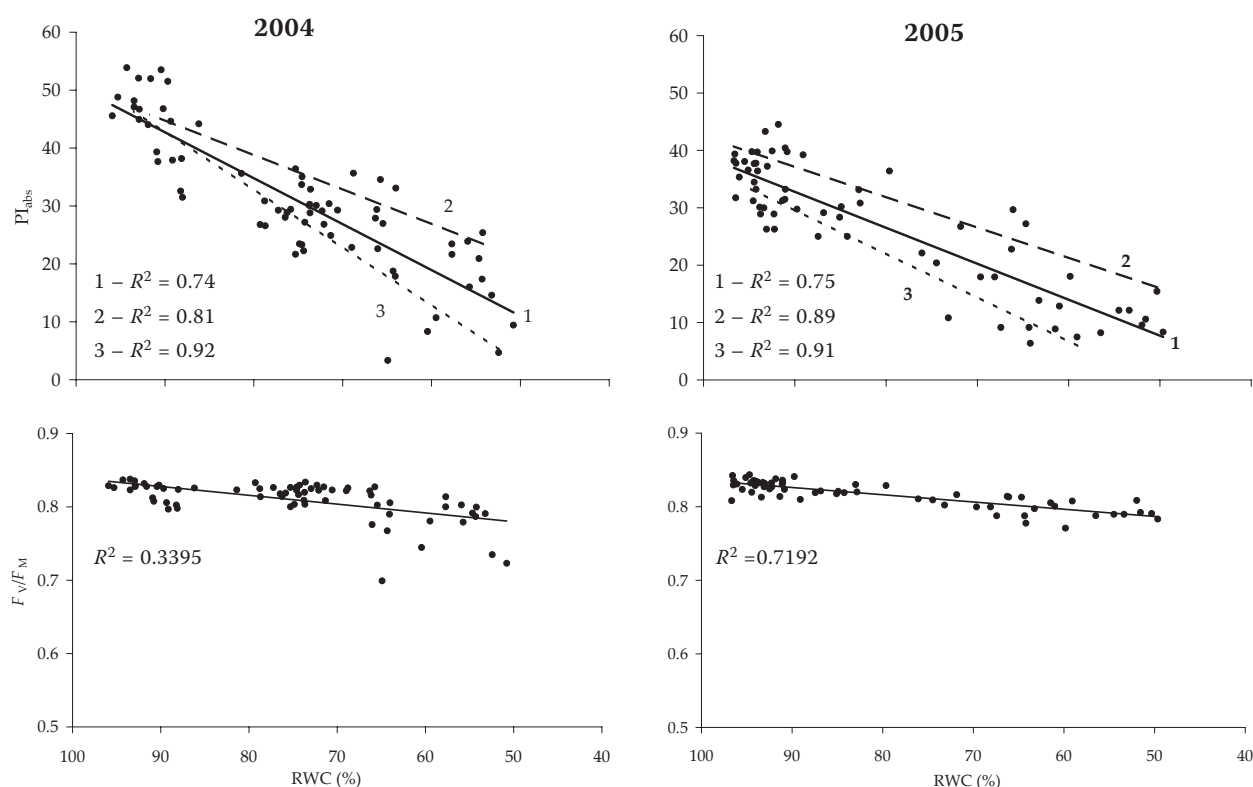

Figure 2. Correlation between performance index (PI_{abs}) or maximum quantum efficiency of PS II photochemistry (F_V/F_M) and relative water content (RWC) recorded in observed genotypes during dehydration process in two years. The solid lines (no. 1) show linear trend calculated for all observed genotypes. In performance index correlations, the long-dashed lines (no. 2) show linear trends for genotype Amerigo; the short-dashed lines (no. 3) show linear trends for genotype Ilona. These two genotypes were significantly different compared to other five observed genotypes, which show trends similar to average or strongly non-linear (cv. Stephens)
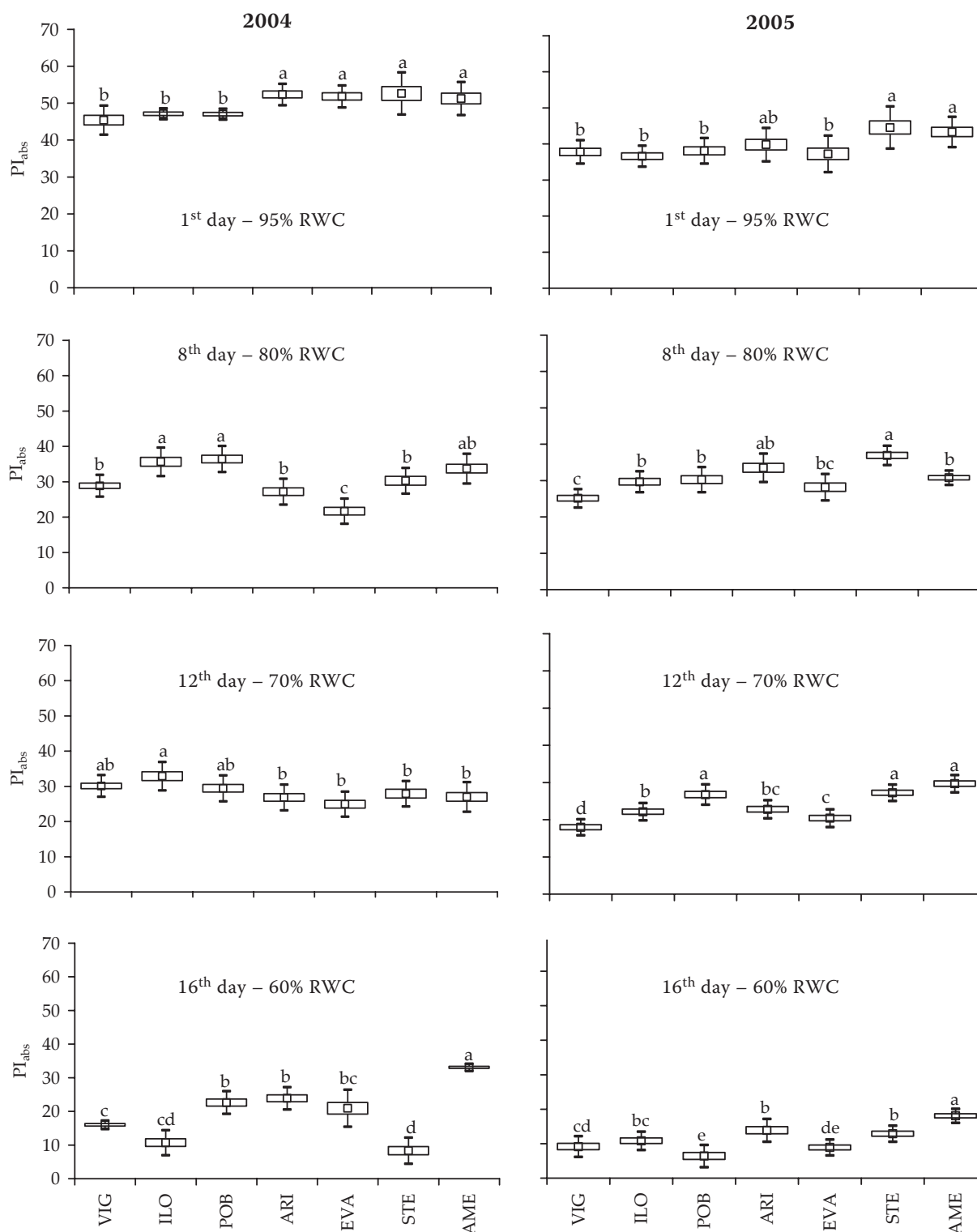
Figure 3. Performance index (PI_{abs}) of control (well-watered) and stressed (approx. 80, 70 or 60% RWC) leaves of tested wheat varieties. Each point represents mean value; each box represents standard error and each bar shows standard deviation. Letters above the boxes help to classify the values (and the varieties) into the following groups: a, b, c, d – varieties with statistically significant differences between measured values, and ab, bc, cd, de – statistically non-significant differences between measured values (Student *t*-test, *P* = 0.05)

is probably more related to leaf senescence with a typical decline of the PS II quantum efficiency (Lu and Zhang 1998).

Nevertheless, our results show that PI_{abs} decline occurred in advance of achieving this critical RWC level. The correlation between the PI_{abs} and RWC

was almost linear (Figure 2). More detailed statistical analysis of measured values showed that the lowest varietal differences were obtained in control plants, only Stephens and Amerigo achieved significantly higher PI_{abs} values compared to other varieties in both experimental years (Figure 3). Moderate (approx. 80% RWC) and severe (approx. 70% RWC and less) drought stress led to dividing the tested varieties into groups, where Eva and Viginta were more sensitive to drought, whereas Stephens, Ilona, Amerigo and Pobeda were more tolerant to drought. The most significant differences were found in this parameter among the tested varieties at approx. 60% RWC, which can be reported as a critical water deficit. In both experimental years, the highest PI_{abs} values were reached by Amerigo. This variety is probably able to delay (at least to some extent) the leaf senescence at severe water deficits, which is considered to be important and favourable trait called "stay-green character" (Spano et al. 2003).

The statistical analysis showed, in general, relatively low differences in PI_{abs} among the tested varieties at 70–80% RWC. It is supposed to be caused by pre-selection of wheat varieties for our testing. There are no extreme pre-selected varieties, all of them are highly productive and originate from temperate zones of Slovakia, Serbia, France and North-West USA. However, in the case of more severe drought stress (RWC declined to the values of 70 or 60%) more significant statistical differences among examined varieties were observed and trends were more obvious (Figures 1 and 3).

Performance index PI_{abs} is found to be a very sensitive parameter in different crops and in most of environmental stress situations (Strasser et al. 2000, Jiang et al. 2006, Christen et al. 2007, Oukarroum et al. 2007), which is in accordance with our results achieved on winter wheat plants under drought stress. Van Heerden et al. (2007) observed also a very good positive correlation between CO_2 assimilation capacity and PI_{abs} values under water stress.

Chlorophyll *a* fluorescence as a non-invasive and efficient method is very advantageous with a potential for use in plant screening for stress tolerance. The main problem, especially in selection for improved drought tolerance, is the lack of reliable and sufficiently sensitive parameters of selection. Our results show that performance index is able to reflect the effect of water deficit on plant vitality and, providing that a suitable screening protocol is designed, also to differentiate tested crop varieties as more or less drought tolerant.

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