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Recognizing the Sources of Stress in Wheat and Bean by Using Chlorophyll Fluorescence Induction Parameters as Inputs for Neural Network Models

By

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Summary

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Bean and wheat plants were exposed to either ozone, drought or flooding stress in a pot experiment for three weeks. By measuring the fast kinetics of chlorophyll fluorescence induction at anthesis, a large dataset for characterizing the stress effects and for developing models was created. The specific differences in the effects of the individual stress types on chlorophyll fluorescence could be used as stress-specific fingerprints. Artificial neural network models were trained to recognize these fingerprints. The correct classification rate of the trained models was in the range of 71-97 % for individual measurements, depending on the classification task, stress type and plant species.

This study shows that a combination of measurements of the fast kinetics of fluorescence induction and the use of these data as inputs for neural network models offers the possibility to extract more information about the specifity of causes for stress effects (drougth, flooding, ozone or unstressed) than the isolated consideration of individual photosynthetic parameters.

Introduction

Environmental stress conditions affect physiological behaviour of plants in multiple ways. The effects on chlorophyll fluorescence (CF) are only one of different aspects, and frequently CF is even not the primary target of the stress influence.

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Instead, the losses of rubisco-activity and rubisco-protein are early indicators of oxidative stress, e.g. caused by ozone (REICHENAUER & al. 1997). Nevertheless, sooner or later indirect effects are also exerted on the primary photochemical processes of electron transport. These reactions are analytically accessible via CF-measurements.

Measurements of the fast kinetics of CF offer an opportunity to investigate photosynthetic responses under field conditions on many leaves in a short time. Consequently, this method has been frequently used as a screening tool for analyzing stress resistance and reactions in field crops and cultivars (e.g. ozone - CIOMPI & al. 1997, SOJA & al. 1998, water stress - LU & ZHANG 1999, YORDANOV & al. 1999, heat stress - REKIKA & al. 1997, flooding - GUIDI & SOLDATINI 1997, cold tolerance - FRACHEBOUD & al. 1999, salt stress - PERCIVAL & GALLOWAY 1999).

The potential of interpreting the kinetics of the chlorophyll fluorescence induction curve has been expanded through the works of STRASSER & STRASSER 1995 and STRASSER & al. 2000. These analyses, called JIP-test according to certain stages of the induction curve during the first half second after illuminating a predarkened leaf, aim at the context of light absorption and electron transport in photosystem II to generate assimilatory power. The authors have derived several new phenomenological and biophysical expressions for describing the dynamics of electron flux in a photosynthetic sample. The potential of this test to screen for stress effects on photosynthetic performance has been shown e.g. by MATOUŠKOVÁ & al. 1999, CLARK & al. 2000 and NUSSBAUM & al. 2001.

This work is based on the assumption that not all CF parameters will be affected in the same way if environmental stresses are as diverse as ozone, drought and flooding. Hence the parallel analysis of the reaction pattern of different CF expressions should reveal different mechanisms how CF is influenced by these stresses. However, the results should not only be assessed with standard statistical techniques but should also be used as inputs for the development of models that could serve as a differentiation tool to distinguish different possible sources of stress from one another. For such classification tasks artificial neural network models (ANN) offer a high potential. ANN are not process-oriented but statistical models which can reveal non-linear relationships between multiple input parameters to explain one or several outputs. The development of ANN requires large datasets for training as they can be provided by CF-measurement campaigns with efficient induction curve monitoring instruments.

The objective of this study was twofold:

Analysis of the main differences in growth and chlorophyll fluorescence reactions to different stresses in two crop species (wheat and bean).

Development of classification models to distinguish different sources of stress and testing their performance.

Material and Methods

Bean (*Phaseolus vulgaris* cv. Maxi) and wheat (*Triticum durum* cv. Extradur) were grown in 8-l pots (n=5) with standard growth substrate (Frux Einheitserde ED 73; wheat: 2 l substrate per plant, bean: 4 l substrate per plant). Three weeks before anthesis, three types of stress treatments started in small open-top chambers:

- ozone stress (90 $nl.l^{-1}$ ozone for 8 $h.d^{-1}$),
- drought stress (withholding irrigation till reaching a soil water capacity (S.W.C.) of 35 % and maintaining this level)
- flooding stress (lower 50 % of root zone was flooded; 100 % S.W.C. in upper 50 %). Control plants were kept at ozone concentrations of <40 $nl.l^{-1}$ and at S.W.C. of 60 to 70 %.

At flowering stage, at three consecutive days fast kinetics of chlorophyll fluorescence were measured at both leaf sides of identical leaves (Hansatech PEA, 15 min pre-darkening). Only leaves which had reached their full length at the start of the stress treatment were used. In wheat, usually this was the flag leaf; in bean a corresponding leaf. Measurements were taken only at parts of the leaves without visual injury. From the fluorescence intensities at 5 points of the induction curve (0.05, 0.1, 0.3, 2 and 30 ms), the classical CF parameters as well as additional CF-expressions according to STRASSER & al. 2000 were calculated. This database of about 800 measurements was further submitted to standard variance-analytical methods, principal component analysis (PCA) and multiple regression analysis.

The CF data (absolute values) were used as inputs for the development of artificial neural network models (Statsoft: STATISTICA, Neural Networks™). For the development of the models the data set was split in training, verification and test data (3:1:1). The inputs were partly chosen according to the PCA-results to ensure independence of the used parameters, partly the models were allowed to self-optimize the number of inputs. The models were designed to fulfill classification tasks with the correct recognition of the treatment as desired output. Sensitivity analysis, a technique to assess the relative contribution of the input variables to the performance of a neural network, was used after establishing the networks: each input variable was set unavailable and the performance of the network was tested for this case.

Total chlorophyll concentration ($C_a + C_b$ with a Minolta SPAD 502) was measured at the same attached leaves as used for the CF-measurements. Finally plants were harvested and aboveground dry matter was determined.

Results

Productivity and chlorophyll concentration

Bean plants experienced flooding stress as most damaging. Both dry matter and chlorophyll concentration were reduced by 30-40 % in this treatment (Table 1). Drought had an adverse effect on dry matter only, but not on chlorophyll. Ozone affected beans similar to wheat: a reduction was only statistically significant in chlorophyll concentration but not in dry matter production.

Considering dry matter productivity, wheat was most affected by drought (-40%) and by flooding (-30%), and least impaired by ozone (Table 1). Enhanced chlorophyll degradation was only evident under ozone stress but not in the other treatments.

Chlorophyll fluorescence[2]

For bean the flooding treatment that had caused the highest reductions in productivity and chlorophyll concentration, also produced the most distinct changes in the chlorophyll fluorescence parameters (Fig. 1). A part of the reaction

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[2] For abbreviations of chlorophyll fluorescence expressions see legend to Table 2.

Table 1. Above-ground dry matter of the experimental plants after three weeks of stress treatment and chlorophyll concentrations of the measured leaves. Absolute values are means ± s.d.; means in columns followed by different letters are significantly different (Duncan-test, $\alpha = 5$ %).

bean treatment	dry matter (g / plant)	relative means	chlorophyll a+b (SPAD values)	relative means
control	14.0 ± 1.7 a	100	32.5 ± 0.3 a	100
ozone	11.0±4.1 ab	79	26.6 ± 1.7 b	82
drought	9.7 ± 0.7 bc	69	30.2 ± 1.5 a	93
flooding	8.1 ± 1.7 c	58	23.8 ± 2.2 c	73
P of ANOVA	0.013		< 0.001	
wheat treatment	**dry matter (g / plant)**	**relative means**	**chlorophyll a+b (SPAD values)**	**relative means**
control	12.1 ± 0.4 a	100	50.5 ± 1.8 a	100
ozone	12.2 ± 0.9 a	101	41.5 ± 2.2 b	82
drought	6.8 ± 0.6 c	56	50.8 ± 0.6 a	101
flooding	8.7 ± 1.1 b	72	48.1 ± 2.1 a	95
P of ANOVA	< 0.001		< 0.001	

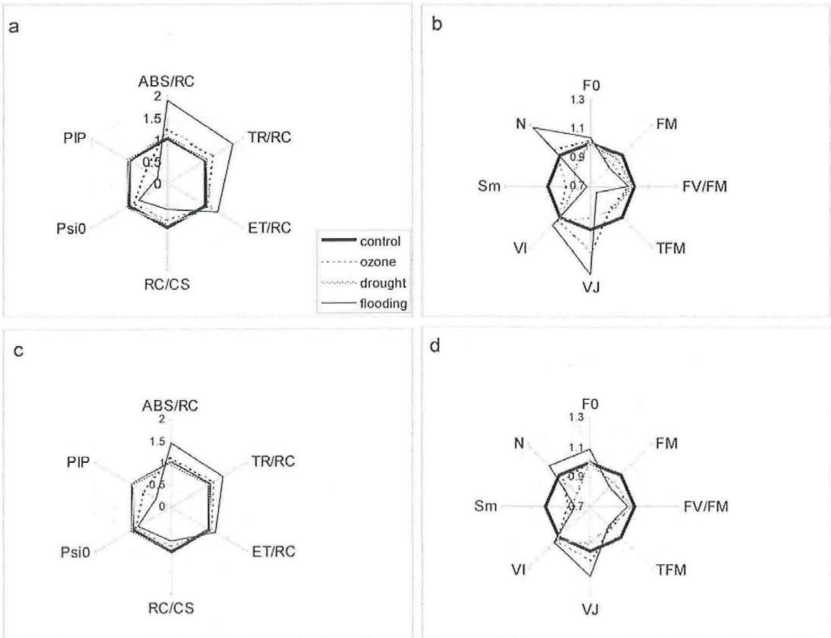

Fig. 1. Chlorophyll fluorescence (CF) behaviour of bean plants under ozone, water and flooding stress relative to non-stressed control plants. The individual graphs show the technical CF expressions (b, d) and the specific and phenomenological fluxes (a,c) of the upper leaf side (a, b) and the lower leaf side (c, d). Abbreviations see Table 2.

Table 2. Chlorophyll fluorescence of bean plants under ozone, water and flooding stress. Absolute values followed by different letters are significantly different (Duncan-test, α = 5 %). Abbreviations: F_0 = initial fluorescence intensity; F_M = maximal fluorescence intensity; $F_V = F_M - F_0$, maximal variable fluorescence; t_{FM} = time to reach F_M; Area = area between fluorescence curve and F_M; V_J, V_I = relative variable fluorescence at the time steps J and I; $M_0 = TR_0/RC - ET_0/RC$, indicates the net rate of closure of the RCs; RC = active reaction centre; S_m = area normalized to Fv (a measure for the energy needed to close all reaction centres); S_m/t_{FM} = average fraction of open RCs during the time needed to complete their closure = average redox state of Q_A (primary bound plastoquinone) in the time span from 0 to t_{FM}; N = turnover number (how many times QA has been reduced in the time span from 0 to t_{FM}); ABS/RC, TR_0/RC, ET_0/RC = specific fluxes for absorbing, trapping, electron transport; φ_{P0} = maximum quantum yield of primary photosynthesis; ψ_0 = efficiency that a trapped exciton can move an electron into the electron transport chain; φ_{E0} = probability that an absorbed photon will move an electron into the electron transport chain; CS = cross section; ../CS = phenomenological fluxes; PI_P = performance index relatively to photochemical events; PI_N = performance index relatively to non-photochemical events;. For further explanations see STRASSER & al. 2000.

bean	upper leafside				lower leafside				p (ANOVA)		
treatment	control	ozone	drought	flood.	control	ozone	drought	flood.	treatment	leafside	interaction
F_0	339 b	345 ab	342 b	353 a	270 d	272 d	272 d	295 c	<0.001	<0.001	0.092
F_M	1990 a	1820 c	1900 b	1750 d	1650 e	1530 f	1520 f	1450 g	<0.001	<0.001	0.090
F_V	1650 a	1480 c	1560 b	1400 d	1380 d	1250 e	1250 e	1160 f	<0.001	<0.001	0.100
F_V/F_M	0.828 b	0.809 d	0.820 c	0.797 e	0.836 a	0.821 c	0.821 c	0.796 e	<0.001	0.005	0.058
t_{FM}	299 ab	270 b	276 b	227 c	319 a	287 ab	282 ab	280 ab	<0.001	0.009	0.316
Area	388 a	302 b	300 bc	231 d	280 c	216 d	211 d	185 e	<0.001	<0.001	<0.001
V_J	0.439 e	0.507 c	0.403 f	0.575 a	0.462 d	0.490 c	0.438 e	0.542 b	<0.001	0.769	<0.001
V_I	0.778 e	0.805 d	0.797 d	0.837 b	0.822 c	0.845 b	0.836 b	0.861 a	<0.001	<0.001	0.171
M_0	0.530 f	0.744 e	0.526 f	1.202 b	0.809 d	0.962 c	0.767 de	1.288 a	<0.001	<0.001	<0.001
S_m	24.3 a	21.2 b	19.8 c	17.7 d	21.2 b	18.1 d	17.5 d	17.0 d	<0.001	<0.001	0.037
S_m/t_{FM}	0.0830 a	0.0818 a	0.0787 a	0.0798 a	0.0691 b	0.0639 bc	0.0651 bc	0.0624 bc	0.063	<0.001	0.587
N	28.9 c	30.5 c	24.9 d	36.6 b	37.0 b	35.3 b	30.5 c	40.3 a	<0.001	<0.001	0.016
ABS/RC	1.487 g	1.871 e	1.618 f	2.799 b	2.186 d	2.512 c	2.213 d	3.181 a	<0.001	<0.001	<0.001
TR_0/RC	1.197 g	1.451 e	1.289 f	2.088 b	1.747 d	1.956 c	1.744 d	2.374 a	<0.001	<0.001	<0.001
ET_0/RC	0.667 g	0.706 f	0.763 e	0.886 d	0.938 c	0.994 b	0.977 b	1.087 a	<0.001	<0.001	<0.001
φ_{P0}	0.807 a	0.779 c	0.799 a	0.747 d	0.800 a	0.780 c	0.789 b	0.747 d	<0.001	0.031	0.067
ψ_0	0.561 b	0.493 d	0.597 a	0.425 f	0.538 c	0.510 d	0.562 b	0.458 e	<0.001	0.769	<0.001
φ_{E0}	0.453 b	0.385 d	0.478 a	0.318 f	0.431 c	0.398 d	0.443 bc	0.343 e	<0.001	0.306	<0.001
RC/CS	260 a	219 c	240 b	158 d	152 de	134 f	147 e	116 g	<0.001	<0.001	<0.001
ABS/CS	383 c	400 b	381 c	440 a	330 ef	334 e	321 f	367 d	<0.001	<0.001	0.033
TR_0/CS	308 b	311 b	305 b	329 a	264 d	260 d	253 e	274 c	<0.001	<0.001	0.245
ET_0/CS	172 b	153 c	181 a	140 d	142 d	133 e	142 d	125 f	<0.001	<0.001	<0.001
PI_P	4.53 a	2.54 b	4.57 a	1.08 d	2.79 b	2.00 c	2.77 b	1.07 d	<0.001	<0.001	<0.001
PI_N	0.258 d	0.497 b	0.252 d	0.994 a	0.373 c	0.543 b	0.384 c	0.978 a	<0.001	<0.001	0.018

centres had been inactivated but average absorption and trapping per active reaction centre increased (Table 3). ABS/RC and TR_0/RC increased by 90 and 70 %, respectively, in the upper leafside. As a consequence M_0, the net rate of closure of the RCs, was enhanced more than twofold. The number of closed reaction centers usually is increased by trapping and decreased by electron transport. Consequently the performance index for photochemical events was lowered by about 80 % in the adaxial and 60 % in the abaxial leafside by flooding. The flooding treatment had no effect on S_m/t_{FM}, the average redox state of Q_A (primary bound plastoquinone) in the time span from 0 to t_{FM}, on both leafsides. In contrast to wheat, in bean the upper leafside was generally more susceptible for all treatments than the lower leafside. Ozone significantly affected 19 and 18, drought 15 and 12 of the evaluated fluorescence parameters, respectively. In bean as well as in wheat the performance index PI_P exhibited a special sensitivity to ozone impacts: it was lowered by 40 and 30 % (upper and lower leafside), respectively. The parameters F_0, t_{FM}, S_m/t_{FM}, N and TR_0/CS were not significantly affected by ozone. In contrast, drought exerted the greatest effect on S_m, the energy needed to close all reaction centers (the more electrons are transferred from Q_A^- into the electron transport chain ET, the bigger S_m becomes, when every Q_A is only reduced once it is on its minimum) and N, the turnover number of Q_A, lowering them by 15 to 20 %.

In wheat it was ozone that had affected the chlorophyll fluorescence parameters most of all stress treatments (Fig. 2, Table 2), although dry matter production had not been lowered (Table 1). 22 of the 24 parameters evaluated at the upper leafside showed a significant difference to control when the plants were ozone-fumigated, whereas in drought or flooding stress only 17 parameters exhibited significant differences. At the lower leafside the trend was similar (23, 20 and 23, respectively), but the plants were even more susceptible and the flooding effects more pronounced. Generally F_V and F_M showed no influence of the treatments. The parameters V_I , S_m/t_{FM} and N did not react under the drought treatment. All stress treatments influenced the "Performance Indices" to the greatest extent. PI_P was lowered by ozone by approximately 50 %, by the drought treatment by 30 %, and by the flooding treatment by 25 and 40 % for the upper and lower leafside, respectively.

Neural network models

In Table 4 some features of a model family developed for the task of distinguishing between four treatments (control + 3 stress treatments) are shown. Although the use of additional inputs slightly improved the performance of the models (= correct classification rate in test measurement data which were not used during model development), the models still had some weaknesses to recognise certain treatments correctly: the bean model was only moderately successful in the correct classification of ozone stress, and the wheat model performed badly for flooding stress. The number of input variables recommended by principle component analysis (PCA) was 11 for wheat and 19 for bean (Table 7). The selected variables were not always those with the highest significant differences between the treatments.

Table 3. Chlorophyll fluorescence of wheat plants under ozone, water and flooding stress. Abbreviations see Table 2.

wheat	upper leafside				lower leafside				*P* (ANOVA)		
treatment	control	ozone	drought	flooding.	control	ozone	drought	flooding.	treatment	leafside	interaction
F_0	346 c.	377 a	361 b	356 bc	335 d	379 a	355 bc	361 b	<0.001	0.344	0.103
F_M	1940 a	1910 ab	1940 a	1900 ab	1850 c	1910 ab	1910 ab	1880 b	0.197	0.003	0.076
F_V	1590 a	1530 bc	1580 ab	1550 abc	1520 c	1530 c	1550 abc	1520 c	0.056	0.002	0.110
F_V/F_M	0.821 a	0.803 d	0.814 b	0.813 b	0.819 a	0.801 d	0.813 b	0.808 c	<0.001	0.060	0.394
t_{FM}	392 a	338 bcd	323 cd	354 b	392 a	332 bcd	320 cd	339 bcd	<0.001	0.315	0.843
Area	521 a	364 cd	426 b	427 b	515 a	340 d	414 b	371 c	<0.001	<0.001	0.041
V_J	0.359 de	0.450 a	0.383 c	0.390 c	0.350 e	0.451 a	0.375 cd	0.415 b	<0.001	0.449	0.005
V_I	0.728 de	0.817 a	0.741 d	0.762 c	0.723 e	0.824 a	0.736 de	0.783 b	<0.001	0.218	0.067
M_0	0.439 d	0.666 a	0.572 bc	0.537 c	0.408 d	0.673 a	0.539 c	0.599 b	<0.001	0.819	0.013
S_m	33.7 a	24.6 c	27.9 b	28.5 b	34.7 a	23.2 c	27.5 b	25.2 c	<0.001	0.035	0.017
S_m/t_{FM}	0.0872 ab	0.0742 c	0.0876 ab	0.0824 bc	0.0903 a	0.0716 c	0.0865 ab	0.0765 c	<0.001	0.320	0.262
N	40.8 a	35.2 c	41.2 a	38.3 b	40.4 ab	33.7 c	38.9 ab	35.1 c	<0.001	0.001	0.304
ABS/RC	1.516 d	1.888 ab	1.881 ab	1.722 c	1.453 d	1.913 a	1.809 bc	1.826 ab	<0.001	0.985	0.024
TR_0/RC	1.217 c	1.460 a	1.485 a	1.361 b	1.166 c	1.479 a	1.431 a	1.425 ab	<0.001	0.797	0.025
ET_0/RC	0.779 cd	0.794 c	0.913 a	0.823 b	0.758 d	0.806 bc	0.892 a	0.826 b	<0.001	0.318	0.194
φ_{P0}	0.803 a	0.776 d	0.790 b	0.791 b	0.803 a	0.774 d	0.792 b	0.783 c	<0.001	0.115	0.057
ψ_0	0.641 ab	0.550 d	0.617 b	0.610 b	0.650 a	0.549 d	0.625 b	0.585 c	<0.001	0.448	0.005
φ_{E0}	0.514 a	0.428 d	0.488 b	0.483 b	0.522 a	0.425 d	0.495 b	0.459 c	<0.001	0.288	0.002
RC/CS	252 a	230 b	217 d	233 b	251 a	228 bc	221 cd	226 bcd	<0.001	0.436	0.214
ABS/CS	381 c	429 a	406 b	398 b	365 d	431 a	397 b	407 b	<0.001	0.311	0.029
TR_0/CS	306 c	332 a	321 b	314 bc	293 d	333 a	314 b	318 b	<0.001	0.105	0.030
ET_0/CS	196 ab	182 d	198 a	191 bc	190 c	182 d	196 a	185 d	<0.001	0.009	0.187
PI_P	5.48 a	2.90 d	3.90 bc	4.21 b	5.86 a	2.72 d	4.17 b	3.53 c	<0.001	0.495	0.005
PI_N	0.187 d	0.406 a	0.275 c	0.268 c	0.174 d	0.404 a	0.254 c	0.328 b	<0.001	0.470	0.031

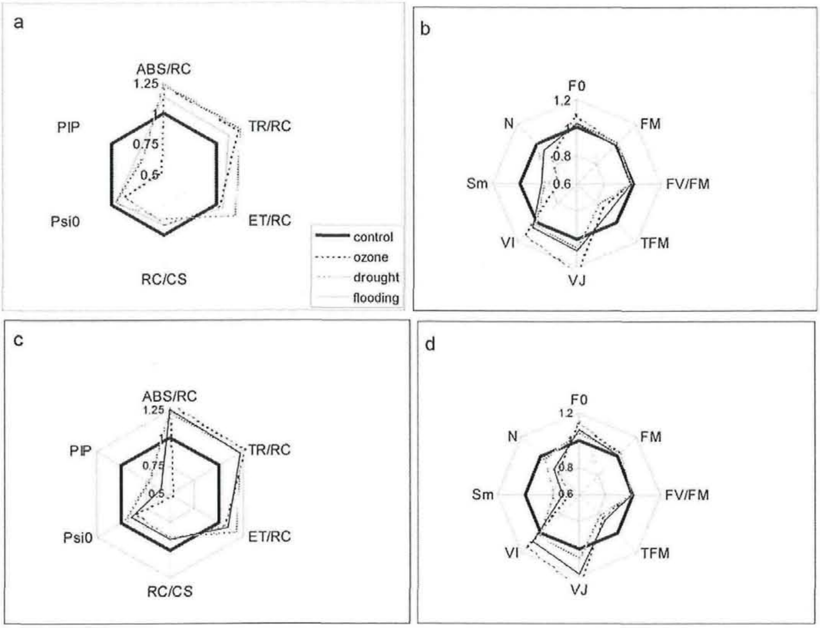

Fig. 2. Chlorophyll fluorescence reactions of wheat plants under ozone, water and flooding stress relative to non-stressed control plants. Abbreviations see Table 2.

Table 4. Characteristics of artificial neural network models (ANN) to distinguish 4 treatments (control, ozone, drought, flooding) of wheat and bean.

plant species	number of inputs	ANN hidden layers	hidden nodes	correct classification rate (in %) control	ozone	drought	flooding	total
bean	19	2	12 - 12	88.8	68.8	92.6	96.7	86.7
wheat	11	2	6 - 8	90.6	94.7	84.0	44.2	78.4

Table 5. Characteristics of neural network models (ANN) to distinguish 2 treatments (control, stress) of wheat and bean.

plant species	ANN number of inputs	hidden layers	hidden nodes	correct classification rate (in %) control	stress	total
bean	7	1	3	86.7	87.1	87.0
wheat	4	1	5	89.6	89.4	89.5
wheat	25	2	13 - 12	90.6	90.5	90.5

In an alternative approach the classification task was split in two: at first models were developed that should distinguish between control plants and any stress treatment (Table 5 and 7). The first two models used the inputs recommended by PCA (or even less) and achieved acceptable classification rates of about 90 % correct. A further increase in the number of inputs did not result in greatly improved performance (third model in Table 5).

Table 6. Characteristics of neural network models (ANN) to distinguish 3 treatments (ozone, drought, flooding) of wheat and bean.

plant species	number of inputs	ANN hidden layers	hidden nodes	correct classification rate (in %) ozone	drought	flooding	total
bean	7	1	10	87.1	92.6	96.7	92.1
wheat1	7	1	5	87.4	71.3	72.6	77.1
wheat2	2	1	2	87.4	92.6	32.6	70.8

Table 7. Input variables used in the artificial neural network (ANN) models (presented in Tables 4-6) in the order of their relative contribution to the performance of the network). Abbreviations see Table 2.

	ANN Table 4		ANN Table 5		ANN Table 6		
	bean	wheat	bean	wheat	bean	wheat1	wheat2
leafside	1		1		3	3	
F_0							
F_M						6	
F_V	3	6					
F_V/F_M	13	7	5		6	4	
t_{FM}			7		7	5	
Area	9	2		1			
V_J	15		6		1	2	
V_I	4	1					1
M_0	14	8					
S_m	19		2		5		
S_m/t_{FM}		3					
N	5					7	
ABS/RC	12						
TR_0/RC	16	4	4		4		
ET_0/RC	7	5	3	4	2	1	2
φ_{P0}	17						
ψ_0	6	11					
φ_{E0}	10	9					
RC/CS	2	10		2			
ABS/CS	11						
TR_0/CS							
ET_0/CS	8						
PI_P				3			
PI_N	18						

For the plants classified as stressed, separate models were developed which only had the task to discern between ozone, drought and flooding. These models, based on the PCA-recommended selection of inputs, achieved a correct classification rate of 71-97 % (Table 6 and 7). An extremely simplistic model with only 2 inputs (V_I, the fraction of closed reaction centres at the inflection point I of the fluorescence induction curve, and ET_0/RC, the specific energy flux or the electron transport per reaction centre at the start of illumination) was surprisingly good in recognising ozone and drought effects in wheat but had no higher hit rate for flooding stress than by chance (third line in Table 6).

Discussion

Although ozone had enhanced chlorophyll degradation of wheat more than the other stress treatments, dry matter productivity was not yet impaired. Apparently photosynthetic capacity had been maintained at a high level during most of the ozone treatment with visual injury and photosynthetic impairment starting late. This is in accordance with observations of SOJA & SOJA 1995 and PLEIJEL & al. 1997 who had observed higher sensitivity to ozone in grain yield than in straw yield and who explained this difference with the higher assimilate demand of the developing ear in comparison to the build-up of vegetative dry matter.

In bean the electron flux rate expressions for photosystem II were much less affected by drought stress than by ozone stress. This was paralleled by similar changes in the chlorophyll concentration, indicating that the higher yield reductions by drought probably were rather due to stomatal limitations of CO_2-uptake whereas ozone apparently had damaged the photosynthetic capacity more profoundly. Also BOTA & al. 2004 concluded from their observations that photosynthesis depressions because of drought in *Phaseolus* are rather caused by decreased stomatal conductance during progressing drought except under very severe stress. Another characteristic feature of bean was the higher sensitivity of fluorescence parameters on the upper leaf side compared to wheat, indicating the sensitivity of bean to light stress. A distinct sensitivity of bean to a combination of light with other stresses was also observed by GUIDI & al. 2000.

Although it is sometimes assumed that changes in the parameters of the JIP-test are mainly due to differences in the chlorophyll content per leaf area, our observations do not support this assumption. Although drought treatments of bean and wheat as well as flooding stress in wheat did not decrease chlorophyll content of the leaves significantly, several fluorescence expressions e.g. describing the specific and phenomenological fluxes changed significantly. These observations show that the decrease of the leaf chlorophyll content is not a primary reaction to the stress treatments but that the primary photosynthetic processes of electron transport in photosystem II are more sensitive indicators for stress effects.

Our results show that a combination of chlorophyll fluorescence expressions is a much more potent screening tool than the use of individual measured or calculated parameters. The combination of the information provided by a Kautsky-curve with artificial neural network models offers

- a new possibility to extract more specifity from fluorescence signals otherwise unspecific for different stresses (NUSSBAUM & al. 2001).
- an extension of the application of neural network models to ecophysiology whereas hitherto they rather have been used for forecasting tasks in environmental and economical sciences (KOLEHMAINEN & al. 2001).

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