General and Applied Plant Physiology – 2009, Volume 35 (3–4), pp. 172–178
©2009 ISSN 1312-8183 Published by the Institute of Plant Physiology – Bulgarian Academy of Sciences
Available online at http://www.bio21.bas.bg/ipp/

Special Issue (Part I) – Proceedings of the XI National Conference on Plant Physiology
18–19 November 2009, Sofia, Bulgaria

VARIABILITY AMONG FIVE BULGARIAN WHEAT CULTIVARS FOR SEEDLINGS RESPONSE TO IRON DEFICIENCY

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Received: *15 September 2009* Accepted: *22 February 2010*

Summary. Iron-deficiency chlorosis brings about significant yield losses in numerous crops. The introduction of resistant genotypes is one possible solution to the problem. In this study the development of Fe deficiency symptoms (growth depression and yellowing of the youngest leaves) at early stages of growth in five Bulgarian cultivars of bread wheat grown hydroponically was compared. Chlorophyll fluorescence measurements were also carried out in order to assess the extent to which the stress had damaged the photosynthetic apparatus. Although at day 10 some effects of Fe deficiency on root length and chlorophyll content were observed, a differentiation among the cultivars could not be found. At day 17 the shoot growth was also affected and the chlorophyll as well as the carotenoid contents were lower. Sadovo 1, which is standard for the registration of new varieties in Bulgaria, was the most Fe-efficient among the studied cultivars, characterized by the smallest decrease in root length, shoot biomass, chlorophyll and carotenoid contents, and relative growth for the 7-day period. In Gladiator 113, Iskur 45 and Sadovska belija, together with a greater drop in the abovementioned parameters, lower actual PS II efficiency and higher amount of light, dissipated thermally by the PS II antenna were found, thus suggesting a greater susceptibility to Fe deficiency. Under optimum Fe supply Lozen 6 had the greatest biomass and relative growth, and high chlorophyll content. Under Fe deficiency it was characterized by the largest reduction in relative growth and in root length, but by a comparatively low decrease in chlorophyll content and by unchanged fluorescence parameters. The detected variability in the early responses of the studied cultivars to Fe deficiency might be important for the early establishment of seedlings and thus, it might be related to iron-efficiency in field conditions.

Key words: chlorophyll fluorescence, chlorosis, growth, iron deficiency, wheat cultivars

Abbreviations: Chl – chlorophyll; Fe – iron; PS II – photosystem II

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INTRODUCTION

Iron-deficiency chlorosis is widespread and brings about significant yield losses in numerous crops, including wheat, when grown on calcareous soils. The introduction of genotypes resistant to Fe-deficiency chlorosis development is one possible solution to the problem. (Jolley et al., 1996). Lately, several studies have reported that when wheat suffers from Fe deficiency, differences among genotypes exist with regard to the release of phytosiderophores, which are non-protein amino acids highly effective in mobilizing sparingly soluble Fe (Tolay et al. 2001**,** Shen et al. 2002**,** Zhang et al. 2003). But in addition to Fe uptake, other factors such as internal Fe utilization and seed reserves also contribute to Fe deficiency susceptibility, so it is important to consider the integral plant reaction. Chlorophyll synthesis and chloroplast development on one side, and meristematic growth on the other, are both sensitive to Fe supply and might react or not react simultaneously to Fe deficiency (Gogorcena et al. 2001, Gruber and Kosegarten, 2002). In this study the development of Fe deficiency symptoms (growth depression and yellowing of the youngest leaves) at early stages of growth in five Bulgarian cultivars of bread wheat (*Triticum aestivum* L.) was compared. Chlorophyll fluorescence measurements were also carried out as they assess the extent to which the photosynthetic apparatus had been damaged and thus can give insight into plant's ability to tolerate environmental stresses (Maxwell and Johnson, 2000). The objects of the study include high yielding cultivars, some used in agriculture (Sadovo 1 and Sadovska belija) and others of breeding interest due to their highly productive spike (Gladiator 113), disease resistance (Lozen 6), high grain quality (Iskur 45) or high tolerance to excess copper (Sadovska belija).

MATERIALS AND METHODS

After seed germination on moistened filter paper, at day 3 seedlings were transferred into containers with half-strength Hoagland-Arnon nutrient solution I with micronutrient supply according to a modified Hoagland's "A–Z" solution (Hoagland and Arnon, 1938). Control (+Fe) and Fe deficient (-Fe) plants were supplied with 100 μM and 1 μM of Fe, respectively, in the form of Fe(III) ethylenediamine tetraacetate. The nutrient solutions were changed at days 8, 11 and 14, and the analyses were made at day 10 and 17. The plants were grown under controlled conditions in a growth chamber. The content of Chl a, Chl b and carotenoids in the youngest fully expanded leaves was measured in 80% acetone extracts and calculated according to McKinney (1941). Chlorophyll fluorescence was measured in leaf discs from the same leaves by a pulse modulation chlorophyll fluorometer (PAM 101, H. Walz, Effeltrich, Germany) after 5 min of dark adaptation, using actinic light at 100 μmol m^{-2} s^{-1} and saturating light at 3500 μmol m^{-2} s^{-1} photon flux density. The minimal Chl fluorescence in the dark (F_0), maximum fluorescence in dark and in light (F_m and F_m', respectively), fluorescence at steady-state photosynthesis (F_t) and fluorescence after switching off the actinic illumination (F_0') were recorded. Variable fluorescence $F_v = F_m – F_0$. Maximum quantum yield of PS II = F_v/F_m; actual quantum yield of PS II $\Phi_{PS\,II} = (F_m' – F_t)/F_m'$;

photochemical quenching qP= $(F_m'–F_t)/(F_m'–F_0')$; non-photochemical quenching NPQ = $(F_m–F_m')/F_m'$ and qN = $(F_m–F_m')/(F_m–F_0')$ were calculated according to Maxwell and Johnson (2000). The intrinsic PS II efficiency $\Phi_{exc} = (F_m'–F_0')/F_m'$ and the relative amount of light absorbed by PS II and dissipated thermally $D = F_0'/F_m'$ were calculated according to Abadía et al. (1999). The relative growth was calculated as $(W_{17}–W_{10})/W_{10}$ where W is the total plant fresh weight at day 10 or 17 (de la Guardia and Alcántara, 2002). Means of at least two separate experiments with 3-10 replicates are represented.

RESULTS AND DISCUSSION

As early as day 10 some changes caused by Fe deficiency were observed. With a drop of 27-36% the root length was the most sensitive parameter (Table 1). Still, there was no difference among the five cultivars. The shoot growth was not altered (data not shown). Some decrease in Chls, but not in carotenoids in the first leaf was found. The difference in the reaction to Fe deficiency among the cultivars comes from the difference in Chl content under optimum Fe supply. For instance, Lozen 6 with the greatest decrease had the highest Chl content when supplied with 100 µM Fe, and had relatively high content under 1 µM Fe, while Gladiator 113 had the lowest Chl content under +Fe conditions, and almost the same content under -Fe. It seems that 1µM Fe in nutrient solution together with the seed Fe was enough to meet the needs for Chl synthesis in plants with lower Chl content under optimum conditions, but not enough for plants with higher requirements.

One week later the above-mentioned parameters continued to be the most sensitive to Fe supply. Sadovo 1 was with the smallest decrease in root length (by 24%), while Lozen 6 was with the largest (by 46%). The Fe-deficient plants from Lozen 6 increased their root length on average by only 1 cm between days 10 and 17, while for the other cultivars the increase was by 4-5 cm, and for the Fe-sufficient plants - by 7-10 cm. Despite of the considerable decreased root length, the root dry biomass did not change significantly due to Fe deficiency (data not shown). The roots visually appeared thicker, brownish, with more root hairs. This response might be related to advantageous modifications, resulting in phytosiderophores release, and thus, in more effective Fe absorption (Zhang et al., 2003). Based on the changes in root length Sadovo 1 might be qualified as the least susceptible cultivar to Fe-deficiency, whereas Lozen 6 - as the most susceptible. But changes in root parameters should be discussed together with changes in shoots, and especially together with chlorosis development, since a strong root growth reaction might be associated with more intense or efficient mechanisms for absorbtion and/or translocation of Fe when it is in low concentration. Indeed, Fe deficiency chlorosis resistance in soybean was not found to be correlated with root length (Vasconcelos et al., 2008). By day 17 Fe deficiency had already affected the shoot growth. While the decrease in shoot length was within a small range, by 4-11%, the fresh biomass reduction was by only 8% for Sadovo 1, and by 20-26% for the other four cultivars. Plants from Lozen 6 had the highest shoot biomass under both optimum and suboptimum Fe supply, while the opposite was true for Sadovska belija.

Table 1. Effect of iron deficiency on growth, pigment content and chlorophyll fluorescence parameters of wheat plants.

Parameter	Sadovo 1			Sadovska belija			Lozen 6			Iskur 45			Gladiator 113		
	+Fe	-Fe	%*	+Fe	-Fe	%*	+Fe	-Fe	%*	+Fe	-Fe	%*	+Fe	-Fe	%*
Day 10															
Root length [cm]	15	11	**73**$^+$	14	9	**64**$^+$	17	12	**71**$^+$	15	11	**73**$^+$	15	10	**67**$^+$
Chlorophyll [mg/g fr.wt.]	1.890	1.699	**90**$^+$	1.722	1.565	**91**	1.903	1.627	**85**$^+$	1.704	1.519	**89**$^+$	1.546	1.535	**99**
Day 17															
Root length [cm]	22	16	**76**$^+$	21	13	**62**$^+$	24	13	**54**$^+$	25	15	**60**$^+$	24	15	**63**$^+$
Shoot length [cm]	29	27	**93**$^+$	26	25	**96**$^+$	27	24	**89**$^+$	26	24	**92**$^+$	27	25	**91**$^+$
Shoot fresh biomass [g/plant]	0.441	0.404	**92**	0.338	0.270	**80**$^+$	0.576	0.429	**74**$^+$	0.489	0.369	**75**$^+$	0.428	0.330	**77**$^+$
Chlorophyll [mg/g fr.wt.]	2.257	1.489	**66**$^+$	2.076	1.134	**55**$^+$	2.279	1.451	**64**$^+$	2.041	1.047	**51**$^+$	2.146	0.981	**46**$^+$
Chl. a/Chl. b	2.18	2.34	**107**$^+$	2.13	2.21	**104**	2.07	2.15	**104**	2.19	2.25	**103**	2.22	2.31	**104**
Carotenoids [mg/g fr. wt.]	0.147	0.134	**91**	0.146	0.097	**67**$^+$	0.130	0.108	**83**$^+$	0.141	0.096	**68**$^+$	0.165	0.106	**64**$^+$
F_v/F_0	2.849	2.405	**84**	2.722	2.186	**80**	2.819	2.914	**103**	4.174	3.229	**77**	2.933	2.270	**77**
F_v/F_m	0.733	0.702	**96**	0.723	0.680	**94**	0.740	0.738	**100**	0.773	0.745	**96**	0.737	0.692	**94**
Φ_{PSII}	0.532	0.477	**90**	0.545	0.440	**81**$^+$	0.549	0.512	**93**	0.537	0.442	**82**$^+$	0.560	0.486	**87**$^+$
Φ_{exc}	0.667	0.595	**89**	0.673	0.559	**83**$^+$	0.665	0.637	**96**	0.702	0.599	**85**	0.667	0.599	**90**$^+$
qP	0.799	0.791	**99**	0.809	0.782	**97**	0.827	0.805	**97**	0.771	0.738	**96**	0.839	0.810	**97**
qN	0.338	0.365	**108**	0.272	0.407	**149**$^+$	0.323	0.403	**125**	0.421	0.528	**125**	0.235	0.355	**151**$^+$
NPQ	0.354	0.353	**100**	0.258	0.394	**153**$^+$	0.342	0.452	**132**	0.591	0.711	**120**	0.205	0.331	**162**$^+$
D	0.333	0.405	**122**	0.327	0.441	**135**$^+$	0.335	0.363	**108**	0.298	0.401	**135**	0.333	0.401	**120**$^+$

* Percent of the values in Fe-sufficient (+Fe) plants to the respective value in Fe-deficient (-Fe) plants.

$^+$ signs indicate that these two values were significantly different at P = 0.05 according to Student's t-test.

At day 17 the drop in Chl content was more pronounced than at day 10. This time the second leaf, which had very likely developed in conditions of exhausted seed Fe reserves (Shen et al. 2002), was analyzed. On the other hand, the Fe mobilization from 1 μM solution was favoured by phytosiderophores release, which might vary among cultivars (Tolay et al. 2001**;** Shen et al. 2002**)**. Although Sadovo 1 and Lozen 6 continued to demonstrate the highest Chl content when supplied with 100 μM Fe, they were characterized by the smallest decrease under Fe deficiency (by 34 and 36%, respectively). The greatest decrease was observed in Gladiator 113 and Iskur 45 (by 54 and 49%, respectively). A small, significant only for Sadovo 1, increase in chl. a/chl. b ratio by 3-7%, indicating a smaller photosynthetic unit size, was found. Carotenoid concentrations were not as strongly reduced as Chl concentrations, but in general the cultivars' order was the same.

The chlorophyll content is often regarded as the best measure of iron availability inside the plants. Some additional information about the extent to which the energy absorbed by photosynthetic pigments is utilized, and more precisely, about the functioning of PS II, might be obtained from chlorophyll fluorescence measurements. As characterized by the ratios F_v/F_m and F_v/F_o the chlorotic plants from all cultivars demonstrated an insignificant decrease in the dark adapted PS II efficiency, i.e. in maximum quantum yield if all PS II centers were open. On the other hand, at steady-state photosynthesis, the actual PS II efficiency ($\Phi_{PS\ II}$), decreased significantly in Sadovska belija, Iskur 45 and Gladiator 113, and this decrease was mainly due to changes in intrinsic efficiency (Φ_{exc}), while the proportion of open PS II reaction centers, assessed by "photochemical quenching" i.e. by qP, did not change. In Sadovska belija and Gladiator 113 Fe deficiency increased the amount of light dissipated thermally by the PS II antenna, as assessed by D, qN and NPQ. Iskur 45 was the only cultivar with significant changes in the fluorescence parameters at day 10 - the values for Φ_{exc}, qN and D in chlorotic plants were respectively 89, 121 and 124% of the values in control plants (data not shown). A similar decrease in actual PS II efficiency and an activation of photoprotective mechanisms due to Fe deficiency had also been found by other authors. Usually the lower $\Phi_{PS\ II}$ had resulted from reduction in both of Φ_{exc} and qP. A sustained decrease in the dark adapted PS II efficiency might be expected only when leaves have lost most of their Chl (Abadía et al. 1999; Gogorcena et al. 2001; Donnini et al. 2003).

We applied the method, proposed by de la Guardia and Alcántara (2002) in order to compare the cultivars for their responses to Fe deficiency. When the relation between the relative growth and the Chl content at day 17 was studied (Fig.1), the position of Sadovo 1 in the upper right corner of the graph confirmed the results from the previous analyses that it was the most Fe-efficient among the studied cultivars. This cultivar, recognized as a standard for productivity in Bulgaria since its release in 1972, because of its ecological plasticity and sustainable yield under various conditions, appeared to be less affected by Fe deficiency at early growth stage. The opposite was true for Gladiator 113, occupying the lower

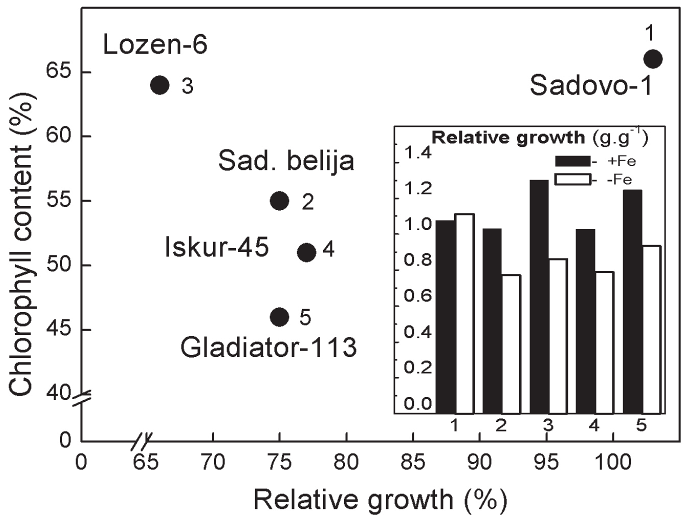

Fig.1. Effect of Fe deficiency on the relationship between chlorophyll content at day 17 and relative growth between days 10-17. Both indices are expressed as percentage of the values under 1 μM Fe to the values under 100 μM Fe. The absolute values for the relative growth are represented in the lower right corner of the figure. The absolute values for Chl content are as in Table 1.

left corner of the graph. Iskur 45 and Sadovska belija also belong to the group of susceptible cultivars, this classification also being confirmed by the results from fluorescence measurements. Lozen 6 was characterized by a comparatively low decrease in Chl content, but by the greatest decrease in relative growth. Under optimum Fe supply it had the greatest biomass and high Chl content at days 10 and 17, and the greatest relative growth for this period. Although one might speculate that in -Fe conditions its photosynthetic functions were more preserved as also supported by chlorophyll fluorescence measurements, it fell behind in meeting its higher growth potential. Moreover, this was the cultivar with the largest reduction in root length. In a previous study Lozen 6 had demonstrated resistance to some plant diseases and 5-10% higher productivity than Sadovo 1 (unpublished results). Still, a more prolonged study of its reactions to Fe -deficiency is necessary. The detected variability in the early responses of the studied cultivars to iron deficiency might be important for the early establishment of seedlings and thus, it might be related to iron efficiency in field conditions.

Acknowledgements: The author is grateful to Mrs. Ana Trifonova for excellent technical assistance.

REFERENCES

Abadía J, F Morales, A Abadía, 1999. Photosystem II efficiency in low chlorophyll, iron-deficient leaves. Plant Soil, 215: 183-192.

De la Guardia MD, E Alcántara, 2002. A comparison of ferric-chelate reductase and chlorophyll and growth ratios as indices of selection of quince, pear and olive genotypes under iron deficiency stress. Plant Soil, 241: 49-56.

Donnini S, A Castagna, L Guidi, G Zoechi, A Ranieri, 2003. Leaf responses to reduced iron availability in two tomato genotypes:T3238FER (iron efficient) and T3238fer (iron inefficient). J Plant Nutr, 26: 2137-2148.

Gogorcena Y, N Molias, A Larbi, J Abadía, A Abadía, 2001. Characterization of the responses of cork oak (*Quercus suber*) to iron deficiency. Tree Physiol, 21: 1335-1340.

Gruber B, H Kosegarten, 2002. Depressed growth of non-chlorotic vine grown in calcareous soil is an iron deficiency symptom prior to leaf chlorosis. J Plant Nutr Soil Sci,165: 111-117.

Hoagland DR, DI Arnon, 1938. The water-culture method for growing plants without soil. Calif Agric Exp Station Circular, 347: 1-39.

Jolley V, K Cook, N Hansen, 1996. Plant physiological responses for genotypic evaluation of iron efficiency in Strategy I and Strategy II plants - A review. J Plant Nutr, 19: 1241-1255.

Maxwell K, GN Johnson, 2000. Chlorophyll fluorescence - a practical guide. J Exp Botany, 51: 659-668.

McKinney, 1941. Absorption of light by chlorophyll solutions. J Biol Chem, 140: 315-332.

Shen J, F Zhang, Q Chen, Z Rengel, C Tang, C Song, 2002. Genotypic difference in seed iron content and early responses to iron deficiency in wheat. J Plant Nutr, 25: 1631-1643.

Tolay I, B Erenoglu, V Römheld, H Braun, I Cakmak, 2001. Phytosiderophore release in *Aegilops tauschii* and *Triticum* species under zinc and iron deficiencies. J Exp Botany, 52: 1093-1099.

Vasconcelos M, G Li, C Li, M Grusak, 2008. Physiology of iron deficiency chlorosis resistance in soybean. In: XIV International Symposium on Iron Nutrition and Interactions in Plants, October 11-16 2008, Beijing, China, p. 67 (Abstract).

Zhang A, F Yu, F Zhang, 2003. Alien cytoplasm effects on phytosiderophore release in two spring wheats (*Triticum aestivum* L.). Gen Res Crop Evol, 50: 767-772.