

Application of photochemical parameters and several indices based on phenotypical traits to assess intraspecific variation of oat (*Avena sativa* L.) tolerance to drought

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Abstract Functionality of the photosynthetic system under water stress is of major importance in drought tolerance. Oat (*Avena sativa* L.) doubled haploid (DH) lines obtained by pollination of F_1 oat crosses with maize were used to assess the differences in plant genotypic response to soil drought. The investigations were based on the measurements of gas exchange and chlorophyll *a* fluorescence kinetics. Drought was applied to 17-day-old seedlings by withholding water for 14 days and subsequent plant recovery. Non-stressed optimally watered plants served as controls. Yield components were determined when plants reached full maturity. It was shown differences among the oat lines with respect to drought stress susceptibility (SI) and stress tolerance index mean productivity and drought susceptibility index. Sensitivity to drought of individual DH lines was significantly different, as demonstrated by the correlation between drought susceptibility index and yield components, such as dry weight (GW) or grain number (GN) of the harvested plants. GW and GN were lower in drought-sensitive genotypes exposed to drought stress compared to those resistant to drought. The principal component analysis allow to separate three groups of lines differing in their sensitivity to drought stress and indicated that tolerance to drought in oat has a common genetic background.

Keywords Chlorophyll fluorescence · Drought stress · Gas exchange · JIP-test · Oat · Yield components

Abbreviations

| | |
|----------------------|--|
| ABS/CSm | Light energy absorption |
| CF | Chlorophyll <i>a</i> fluorescence kinetics |
| DH | Doubled haploid |
| DI _o /CSm | Energy dissipated from PSII |
| DSI | Drought susceptibility index |
| <i>E</i> | Transpiration |
| ET _o /CSm | Energy used for electron transport |
| F_v/F_m | Maximum photochemical efficiency |
| GN and GW | Number and weight of grains per plant |
| g_s | Stomatal conductance |
| JIP-test | Test applied to analyze fast fluorescence kinetics |
| MP | Mean productivity index |
| PI | Overall performance index of PSII photochemistry |
| Pn | Photosynthesis rate |
| RC/CSm | Number of active reaction centers |
| SI | Stress index |
| TOL | Stress tolerance index |
| TR _o /CSm | Excitation energy trapped in PSII reaction centers |
| WUE | Water use efficiency |
| Y_C and Y_D | Yield in control and drought treatments |

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Introduction

The common oat (*Avena sativa* L.) is an important cereal cultivated worldwide, which occupies the eighth place in the world cereal production. Oat grain is an important source of feed, pharmaceutical and cosmetic products,

because it is a rich source of protein, fat, fiber and minerals (Zaheri and Bahraminejad 2012). Drought stress is one of the major causes of crop loss, as it can reduce yield components, such as the number and weight of grains. Genetic variation among genotypes is a crucial factor in plant breeding (Talebi et al. 2009). Understanding the responses of plants to drought is of great importance and constitutes a fundamental prerequisite in the development of stress tolerance in crops (Zhao et al. 2008). The relative yield performance of genotypes in drought stress or optimal conditions seems to be a common starting point in the identification of desirable genotypes for unpredictable conditions in terms of soil moisture (Mohammadi et al. 2010). Drought resistance is defined as the capacity of plants in withstanding the periods of dryness and is related to phenotypic, morphological and physiological factors (Zhang et al. 2011; Yan et al. 2012). For qualitative and quantitative evaluation, it is necessary to estimate the influence of drought during the all growing period and assess both the immediate and indirect physiological and morphological reactions of plants. The plant responses help manage the unfavorable stress conditions, either by increasing resistance to damage or sustaining metabolic functions under limited water conditions (tolerance mechanisms).

The plant reacts to soil water deficit by closing the stomata to prevent loss of water (a decrease of stomatal conductance— g_s) and inhibition of photosynthesis (P_n) and transpiration (E). Therefore, the ability to maintain the functionality of the photosynthetic system under water stress is of major importance in drought tolerance (Zlatev 2009). During drought stress, plants display osmoregulation capacity, which enable them maintaining a relatively high activity of the photosynthetic apparatus Hura et al. (2007).

Plant breeders are interested in screening techniques allowing to select drought-resistant and drought-sensitive cereal genotypes (Kahrizi and Mohammadi 2009; Kahrizi et al. 2011). Oat doubled haploids (DH) are one of the technologies currently being used in the programs aimed at developing new varieties with a combination of desirable traits in a shorter time frame. In our previous study, we have obtained a number of DH lines of oat (Marcinińska et al. 2013a), while in the current study we have tested their susceptibility to drought stress in relation to the above-described valuable characteristics of those types of lines.

Elucidating intricate relationships between fluorescence kinetics and photosynthesis contribute to our understanding of biophysical processes of photosynthesis (Sayed 2003). Handy PEA fluorometer allows measurements of chlorophyll *a* fluorescence kinetics using continuous excitation (Strasser and Govindjee 1992; Strasser et al. 2004). The procedure applied in the current study is called the JIP-test

(a test analyzing fast fluorescence kinetics), which allows to measure several photosynthetic parameters (Strasser et al. 1995; Yin et al. 2010). These include: F_v/F_m (the maximum photochemical efficiency), PI (overall performance index of PSII system), ABS/CSm (light energy absorption), TR_o/CSm (excitation energy trapped in PSII reaction centers), DI_o/CSm [energy dissipated from PSII as heat, equal to $(ABS/CSm - TR_o/CSm)$], ET_o/CSm (energy used for electron transport) and RC/CSm (number of active reaction centers). These parameters of the JIP-test are determined during the transition of the photosynthetic apparatus from a dark-adapted to a light-adapted state (Czyczyło-Mysza et al. 2013). They reflect the electron transfer and energy distribution within the photosynthetic apparatus during the primary photochemistry (Strasser et al. 2004). The possibility of applying this technique as a reliable method for screening the plants for drought tolerance has been previously reported (Li et al. 2006; Yin et al. 2010). These observations validate our approach, which should be regarded as an initial screen, which can be subsequently employed to target drought tolerance in the studied DH oat lines.

The purpose of our study was to demonstrate that on the basis of physiological processes of seedling leaves and yield evaluation after achieving full maturity by the plants, it is possible to select genotypes among oat DH lines, which exhibit different tolerance to water stress.

Materials and methods

Plant materials and growth conditions

DH lines were obtained by pollination of oat with maize at the Institute of Plant Physiology in Cracow, according to the method of Marcińska et al. (2013a). F_1 oat (*Avena sativa* L.) generation (Table 1) and sweet corn Waza (*Zea mays*) derived from the Strzelce Plant Breeding Ltd. served as the source of plant material. The seeds were sown separately in the individual 3 dm³ volume pots filled with soil composed of horticultural soil and sand (1/1 v/v). Plants were grown in an open-sided greenhouse until harvest in August. Drought stress, induced by stopping the watering, was applied from the 17th day of seedling growth with five plants as replicates. The water status in the soil was measured by HydroSense Soil Water System (Campbell Scientific 620, Inc. UK) and was set as $8 \pm 1\%$ for the control and $3 \pm 1\%$ volumetric water content (VWC) for non-watered plants. Drought treatment was continued for 14 days, until the leaves showed visual symptoms of turgor loss and $3 \pm 1\%$ VWC. After taking the measurements of gas exchange and chlorophyll fluorescence parameters, the plants were rewatered, transferred to greenhouse conditions

Table 1 List of the plant material used in the experiment: 11 doubled haploid lines of oat originated from F_1 emasculated oat generations and pollinated by sweet corn Waza

| Doubled haploid line | F_1 oat generation |
|----------------------|----------------------|
| DH1 | Szakal × Bajka |
| DH2 | Deresz × Szakal |
| DH3 | Deresz × Szakal |
| DH4 | Deresz × Szakal |
| DH5 | Krezus × STH 593 |
| DH6 | Bajka × STH 454 |
| DH7 | Bajka × STH 454 |
| DH8 | Bajka × Bingo |
| DH9 | Bajka × Bingo |
| DH10 | STH 5428 × Bingo |
| DH11 | Bajka × Chwat |

and maintained until harvest. At the final maturity, the plants were cut at the soil surface. Grain weight and grain number were measured for each plant to determine the yield. The remaining plants were weighed after drying to obtain the above-ground biomass.

Measurements of physiological parameters

Measurements of gas exchange and photochemical activity were performed in 14th day of growth in open-side greenhouse conditions on the youngest, well-developed leaf of each line. P_n , E and g_s were measured both for control optimally watered and for drought after stopping the watering during 14 days of growth. Coefficient of water use efficiency (WUE) was calculated based on the measurements of P_n and E . Gas exchange parameters of the leaf were measured using a CO_2 IRGA analyzer (CI-301PS, CID Inc., USA) with a Parkinson's assimilation chamber and a narrow type regular with a CI-301 LA light attachment.

Chlorophyll a fluorescence kinetics parameters were measured using a 230 fluorometer (Handy PEA; Hansatech Instruments, King's Lynn, UK) as it was described by Czyczyło-Mysza et al. (2013). The following parameters were calculated per excited leaf cross-section (CSm): F_v/F_m , PI, ABS/CSm, TR_o/CSm , DI_o/CSm , ET_o/CSm and RC/CSm. Data were analyzed with the JIP-test according to Strasser et al. (2000) and Force et al. (2003).

After taking the measurements of the above-described parameters, plants were grown in the greenhouse conditions as before the drought treatment and maintained until full maturity. We determined the yield components for all DH lines in the control (C) and drought-treated plants (D). Indices of sensitivity to drought stress (SI stress index, DSI

drought susceptibility index) were calculated from the grain weight based on the yield per plant and D conditions (Y_C and Y_D), both according to FAO reports Fischer and Maurer (1978) and Golbashy et al. (2010). TOL—stress tolerance index according to Hossain et al. (1990) and MP—mean productivity index according to Rosielle and Hamblin (1981).

$$SI = Y_D/Y_C * 100,$$

$$DSI = [1 - (Y_D/Y_C)]/[1 - (\overline{Y_D}/\overline{Y_C})],$$

$$TOL = Y_C - Y_D,$$

$$MP = (Y_C + Y_D)/2,$$

where Y_C and Y_D are the grain yield in C and D conditions, respectively; $\overline{Y_D}$ and $\overline{Y_C}$ are the mean grain yield from all genotypes used in the experiment.

For other measured parameters, SI indices were calculated using the following formula:

$$SI_X = X_D/X_C * 100,$$

where X_D and X_C are the CF and gas exchange parameters.

Values of the following parameters were obtained: SI_{F_v/F_m} , SI_{PI} , $SI_{ABS/CSm}$, $SI_{TR_o/CSm}$, $SI_{DI_o/CSm}$, $SI_{ABS/CSm}$, $SI_{ET_o/CSm}$ and $SI_{RC/CSm}$, SI_{P_n} , SI_E , SI_{WUE} and SI_{g_s} .

Statistical analysis

To determine statistical significance of obtained data, Pearson's linear correlation coefficients together with the probability levels of sensitivity to drought stress indices, mean productivity and yield components were calculated. Moreover, all data were calculated using ANOVA analysis of variance implemented in STATISTICA 12.0 software (Statsoft, Tulsa, OK, USA) (where DH lines and the treatment were the factors). Additionally, the distribution of normality was tested using the Shapiro–Wilk test by the same statistical program. We checked also homoscedasticity of these parameters. Drought susceptibility indices were calculated after the analysis of grain yield and parameters of gas exchange and FC for plants grown under control and drought conditions. Principal component analysis (PCA), also included in STATISTICA 12.0, was applied to assign the ranks to oat genotypes studied and to classify which of them were more susceptible/resistant to drought stress. PCA is a procedure that utilizes orthogonal transformation to convert a set of possibly correlated variables into a set of linearly uncorrelated variables called principal components. When the angle and directions between vectors is below 90° (acute angle), it represents a positive correlation, while when the angle is higher than 90° (obtuse angle), the correlation is negative. No correlation between parameters occurs when the angle between

the vectors is 90° (perpendicular vectors). This transformation is defined in such a way that the first principal component has the largest possible variance. PCA is sensitive to the relative scaling of the original variables.

Results

Table 2 presents a linear correlation between grain dry weight (Y_{CDW}) and number (Y_{CGN}) per plant for control and grain dry weight (Y_{DDW}) and number (Y_{DGN}) per plant of drought-stressed plants as well as SI, DSI and MP. A high correlation was demonstrated between these indices and Y_{DDW} and Y_{DGN} , except for the TOL index. Yield parameters of the control plants (Y_{CDW} and Y_{CGN}) were not correlated with these indices, except for the MP index. Correlation coefficients indicated that DSI, SI and MP provided the most suitable criteria for the selection of high yielding genotypes under water stress conditions. Two-way analysis of variance for all traits in drought and control conditions indicated highly significant genotypic differences for most of the measured traits.

Gas exchange

Analysis of variance (ANOVA) for the measurements of gas exchange parameters, including water use efficiency (WUE) revealed significant differences between DH lines and the treatment (Table 3). A decrease (from 100% to even 48%) in the rate of photosynthesis (Pn), transpiration (E) and stomatal conductance (g_s) was observed in oat plants grown under drought conditions in comparison to control plants. Among the studied oat lines, a lower decrease of Pn, E and g_s values was detected for DH1, DH2 and DH3 (about 20–40%) than for other lines (about

40–50%). The lowest differences were recorded for WUE (on average 16% for all DH lines), thus it was difficult to determine which of the lines had better water use efficiency.

Chlorophyll *a* fluorescence kinetics (CF)

Chlorophyll *a* fluorescence kinetics (CF), similar to gas exchange parameters, provides rapid quantitative information on the response of photosynthetic apparatus to environmental factor changes. After drought treatment, CF values were additionally calculated as a percentage of control (shown in italics in Table 4). The F_v/F_m parameter did not differ significantly after drought treatment compared to control in all DH lines tested and their values were similar ($100 \pm 3\%$). The overall performance index of PSII photochemistry (PI) as a useful parameter of plant reaction to drought stress was higher for DH1–4 and DH8 lines by ca. 34–143% compared to the control. Moreover, these genotypes exhibited significantly lower (ca. 11–18%) energy dissipation in the form of heat from PSII (DI_o/CSm) in comparison to control. The next two parameters associated with ABS/CSm and TR_o/CSm slightly varied, however, they were not statistically different between the DH lines and drought treatment (a few percent). Energy used for electron transport (ET_o/CSm) and the number of active reaction centers (RC/CSm) were significantly higher (on average by 20%) for DH1–5 and DH8 lines grown under drought compared to control.

After effects of drought stress on yield components

Yield components were determined in oat DH lines harvested when full maturity was reached. The number and weight of grains (GN and GW) after drought treatment was

Table 2 Correlation coefficients between measured and calculated traits: yield parameters (Y_{CDW} and Y_{CGN} —grain dry weight and number per plant for control and Y_{DDW} and Y_{DGN} —grain dry weight

and number per plant for drought stress) and drought tolerance indices (SI stress index, TOL stress tolerance index, MP mean productivity index and DSI drought susceptibility index)

| Index | Y_{CDW} | Y_{DDW} | Y_{CGN} | Y_{DGN} | SI | TOL | MP | DSI |
|-----------|---------------------|---------------------|--------------------|---------------------|--------------------|---------------------|---------------------|------|
| Y_{CDW} | 1.00 | | | | | | | |
| Y_{DDW} | 0.73** | 1.00 | | | | | | |
| Y_{CGN} | 0.94*** | 0.59 ^{ns} | 1.00 | | | | | |
| Y_{DGN} | 0.78*** | 0.97*** | 0.66** | 1.00 | | | | |
| SI | 0.21 ^{ns} | 0.81*** | 0.08 ^{ns} | 0.74** | 1.00 | | | |
| TOL | 0.13 ^{ns} | −0.59 ^{ns} | 0.26 | −0.48 ^{ns} | −0.92*** | 1.00 | | |
| MP | 0.91*** | 0.94*** | 0.80** | 0.95*** | 0.58 ^{ns} | −0.29 ^{ns} | 1.00 | |
| DSI | −0.21 ^{ns} | −0.81*** | 0.08 ^{ns} | −0.74** | −1.00*** | 0.92*** | −0.58 ^{ns} | 1.00 |

ns not significant

* ** * Significant at $P \leq 0.05, 0.01, 0.001$, respectively

Table 3 Gas exchange parameters: photosynthesis rate (Pn) ($\mu\text{mol CO}_2 \text{ cm}^{-2} \text{ s}^{-1}$), transpiration (E) ($\text{mmol H}_2\text{O cm}^{-2} \text{ s}^{-1}$), water use efficiency (WUE) ($\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$) and stomatal conductance (g_s) ($\text{mmol H}_2\text{O cm}^{-2} \text{ s}^{-1}$) in control (C) and after 14 days of drought (D)

| Line | Pn | | E | | WUE | | g_s | |
|----------------------------|------|-----------|-----|-----------|-----|------------|-------|-----------|
| | C | D | C | D | C | D | C | D |
| DH1 | 12.4 | 7.4 | 3.1 | 2.3 | 4.0 | 3.2 | 113.0 | 83.7 |
| | | <i>60</i> | | <i>75</i> | | <i>80</i> | | <i>74</i> |
| DH2 | 12.1 | 8.0 | 3.6 | 3.0 | 3.3 | 2.7 | 115.3 | 63.3 |
| | | <i>66</i> | | <i>83</i> | | <i>81</i> | | <i>55</i> |
| DH3 | 15.2 | 7.9 | 4.5 | 3.1 | 3.4 | 2.6 | 103.7 | 78.3 |
| | | <i>52</i> | | <i>69</i> | | <i>75</i> | | <i>76</i> |
| DH4 | 15.0 | 7.9 | 5.4 | 2.9 | 2.8 | 2.8 | 114.3 | 84.7 |
| | | <i>52</i> | | <i>54</i> | | <i>100</i> | | <i>74</i> |
| DH5 | 14.1 | 8.1 | 4.8 | 2.9 | 3.0 | 3.1 | 125.7 | 73.0 |
| | | <i>58</i> | | <i>61</i> | | <i>103</i> | | <i>68</i> |
| DH6 | 16.4 | 7.8 | 5.1 | 2.3 | 3.3 | 3.6 | 121.7 | 60.7 |
| | | <i>48</i> | | <i>45</i> | | <i>109</i> | | <i>50</i> |
| DH7 | 14.7 | 8.0 | 5.0 | 3.3 | 3.0 | 2.4 | 114.0 | 63.3 |
| | | <i>55</i> | | <i>66</i> | | <i>81</i> | | <i>56</i> |
| DH8 | 13.3 | 6.7 | 5.2 | 3.2 | 2.6 | 2.1 | 118.7 | 58.7 |
| | | <i>50</i> | | <i>61</i> | | <i>82</i> | | <i>49</i> |
| DH9 | 14.5 | 7.9 | 5.6 | 3.2 | 2.6 | 2.5 | 113.7 | 64.3 |
| | | <i>54</i> | | <i>57</i> | | <i>96</i> | | <i>57</i> |
| DH10 | 12.4 | 7.1 | 4.9 | 3.7 | 2.5 | 1.9 | 124.0 | 70.3 |
| | | <i>57</i> | | <i>75</i> | | <i>76</i> | | <i>57</i> |
| DH11 | 13.6 | 8.4 | 5.2 | 3.5 | 2.8 | 2.4 | 118.0 | 69.0 |
| | | <i>62</i> | | <i>67</i> | | <i>91</i> | | <i>58</i> |
| Source of variance | | | Pn | E | | WUE | | g_s |
| DH line | | | *** | *** | | *** | | *** |
| Treatment | | | *** | *** | | ** | | *** |
| DH line \times treatment | | | * | ns | | ns | | ** |

In italics percentage values of control are given. $n = 3$. The results of two-way analysis of variance (ANOVA) are presented in the lower part of the table. The sources of variance for Pn, E , WUE and g_s were as follows: eleven DH lines, two treatments, and interaction between DH line and treatment

ns not significant

* ** *** Significant at $P \leq 0.05, 0.01, 0.001$, respectively

decreased by about 53–64% and 42–58%, respectively, in potentially susceptible lines (DH4, DH7, DH9, DH10 and DH11) and only by 16–36% and 4–36%, respectively, in potentially resistant lines (DH1, DH2, DH3, DH5, DH6 and DH8) in comparison to control (Table 5). Shoot biomass (SB) did not change in a similar manner, but it was reduced by 8–37% in six DH lines. In other lines, an increase of 2–34% was observed, and in consequence, a reduction of harvest index (HI) in drought-stressed plants. Reduction of above-ground biomass (AB) in all oat lines

after drought treatment ranged between 5 and 38%, while higher values of this parameter were observed for resistant DH lines. We named it an after effect of drought stress. It was observed that some of the lines were able to overcome the effects of water stress during such a long growth period when compared to others. Based on the yield components and harvest index values, we created a ranking of resistant/susceptible oat DH lines, where DH1–DH3 and DH8 were selected as the most resistant to drought stress. According to the data from breeding company Strzelce Plant Breeding Ltd. investigated by us cultivars: Szakal, Bajka, Deresz, Krezus are the most resistant to drought stress and had the highest yield in the field in such conditions. It was described in the report of the Institute of Meteorology and Water Management (in polish). For that reason these cultivars were used as a components for crossings and then for DH lines production. Our results indicated that DH lines obtained from F_1 hybrids when components for crossing were cultivars: Szakal, Bajka, Deresz, Krezus, were also resistant to drought.

Relationship between drought susceptibility index for oat grain number and weight

The relationships between the number and dry weight of grains per plant and drought sensitivity index (DSI) observed in the current study are presented in Fig. 1a, b. Although DSI did not correlate with dry weight and number of grains in the control conditions (Fig. 1a), it was possible to select and identify resistant/sensitive genotypes grown under drought stress (Fig. 1b). This way, six DH lines of oat, i.e., DH1, DH2, DH3, DH5, DH6 and DH8 (DSI values ranged from 0.1 to 0.9) were selected as more resistant to soil drought stress than the remaining five DH lines, i.e., DH4, DH7, DH9, DH10 and DH11 (where DSI values ranged from 1.3 to 1.8).

Principal component analysis (PCA)

The susceptibility index (SI) was calculated based on the data concerning the gas exchange and CF parameters in the control and drought treatment simultaneously (Fig. 2a, b). Yield components and drought susceptible indices were also included in this analysis. PCA analysis was used to identify superior genotypes for both stressed and non-stressed environments. The reason for this is that the genotypes in biplot analysis are compared for all traits at the same time. This orthogonal transformation is defined in such a way that the first principal component has the largest possible variance. PCA is sensitive to relative scaling of the original variables. In our experiment, PC1 and PC2 components explained 37.28 and 28.10% of the total variation in all the DH lines,

Table 4 Chlorophyll *a* fluorescence kinetics parameters in oat DH lines in control (*C*) and after 14 days of drought (*D*)

| Line | F_v/F_m | | PI | | DIO/CSm | | ABS/CSm | | TRo/CSm | | ETo/CSm | | RC/CSm | |
|---------------------|-----------|------------|----------|------------|----------|------------|----------|------------|----------|------------|----------|------------|----------|------------|
| | <i>C</i> | <i>D</i> | <i>C</i> | <i>D</i> | <i>C</i> | <i>D</i> | <i>C</i> | <i>D</i> | <i>C</i> | <i>D</i> | <i>C</i> | <i>D</i> | <i>C</i> | <i>D</i> |
| DH1 | 0.789 | 0.817 | 2.2 | 4.0 | 83.5 | 74.3 | 395.7 | 405.2 | 312.2 | 330.9 | 171.7 | 215.8 | 893.2 | 1037.8 |
| | | <i>103</i> | | <i>179</i> | | <i>89</i> | | <i>102</i> | | <i>106</i> | | <i>125</i> | | <i>116</i> |
| DH2 | 0.820 | 0.825 | 2.8 | 5.0 | 63.1 | 66.1 | 350.2 | 376.7 | 287.1 | 310.6 | 160.6 | 210.1 | 947.1 | 1080.2 |
| | | <i>101</i> | | <i>176</i> | | <i>105</i> | | <i>107</i> | | <i>108</i> | | <i>130</i> | | <i>114</i> |
| DH3 | 0.791 | 0.819 | 2.3 | 5.2 | 83.2 | 68.2 | 395.0 | 375.4 | 311.8 | 307.2 | 174.0 | 209.5 | 871.9 | 1100.3 |
| | | <i>104</i> | | <i>227</i> | | <i>82</i> | | <i>95</i> | | <i>98</i> | | <i>120</i> | | <i>126</i> |
| DH4 | 0.796 | 0.822 | 2.1 | 5.1 | 76.1 | 64.6 | 368.2 | 362.5 | 292.1 | 297.9 | 158.2 | 204.0 | 821.5 | 1037.9 |
| | | <i>103</i> | | <i>243</i> | | <i>85</i> | | <i>98</i> | | <i>102</i> | | <i>128</i> | | <i>126</i> |
| DH5 | 0.816 | 0.806 | 3.3 | 3.4 | 70.0 | 77.9 | 379.6 | 401.4 | 309.6 | 323.5 | 183.7 | 207.5 | 1051.9 | 928.1 |
| | | <i>99</i> | | <i>101</i> | | <i>111</i> | | <i>106</i> | | <i>104</i> | | <i>112</i> | | <i>88</i> |
| DH6 | 0.794 | 0.810 | 2.6 | 3.8 | 78.6 | 79.2 | 380.0 | 414.7 | 301.4 | 335.5 | 173.2 | 216.5 | 897.3 | 1033.3 |
| | | <i>102</i> | | <i>149</i> | | <i>101</i> | | <i>109</i> | | <i>111</i> | | <i>125</i> | | <i>115</i> |
| DH7 | 0.810 | 0.829 | 3.1 | 5.8 | 69.5 | 60.4 | 364.8 | 352.4 | 295.3 | 291.9 | 174.4 | 199.9 | 973.9 | 1113.9 |
| | | <i>102</i> | | <i>184</i> | | <i>87</i> | | <i>96</i> | | <i>98</i> | | <i>114</i> | | <i>114</i> |
| DH8 | 0.817 | 0.819 | 3.1 | 4.5 | 60.7 | 69.4 | 332.0 | 383.5 | 271.3 | 314.0 | 157.8 | 204.8 | 892.3 | 1076.9 |
| | | <i>100</i> | | <i>148</i> | | <i>114</i> | | <i>115</i> | | <i>116</i> | | <i>129</i> | | <i>120</i> |
| DH9 | 0.832 | 0.823 | 3.4 | 4.9 | 59.4 | 65.6 | 352.0 | 370.2 | 292.6 | 304.5 | 170.4 | 203.9 | 1010.8 | 1068.5 |
| | | <i>96</i> | | <i>144</i> | | <i>110</i> | | <i>105</i> | | <i>104</i> | | <i>119</i> | | <i>105</i> |
| DH10 | 0.815 | 0.812 | 3.4 | 5.2 | 75.2 | 69.9 | 403.0 | 377.0 | 327.9 | 307.1 | 194.9 | 209.5 | 1109.4 | 1083.6 |
| | | <i>101</i> | | <i>152</i> | | <i>92</i> | | <i>93</i> | | <i>93</i> | | <i>107</i> | | <i>97</i> |
| DH11 | 0.826 | 0.817 | 4.0 | 3.6 | 64.7 | 73.7 | 371.5 | 401.3 | 306.8 | 327.6 | 187.7 | 208.0 | 1143.2 | 1012.1 |
| | | <i>99</i> | | <i>90</i> | | <i>113</i> | | <i>108</i> | | <i>106</i> | | <i>110</i> | | <i>88</i> |
| Source of variance | F_v/F_m | | PI | | DIO/CSm | | ABS/CSm | | TRo/CSm | | ETo/CSm | | RC/CSm | |
| DH line | ns | | * | | ** | | * | | *** | | *** | | ns | |
| Treatment | *** | | *** | | *** | | *** | | *** | | *** | | *** | |
| DH line × treatment | ns | | *** | | ns | | ns | | ns | | ns | | ** | |

Percentage values of control are given in italics. The results of analysis of variance (ANOVA) are presented in the lower part of the table. The sources of variance for FC parameters: F_v/F_m , PI, DIO/CSm, ABS/CSm, TRo/CSm, ETo/CSm and RC/CSm were as follows: eleven lines, two treatments and interaction)

ns not significant. $n = 5$

* ** *** Significant at $P \leq 0.05, 0.01, 0.001$, respectively

respectively, and accounted for 65.38% of the total variation (Fig. 2a, b). Positive correlations were found for the following indices: DSI and TOL; Y_C and MP, Y_D , SI, $SI_{ETo/CSm}$, SI_{Pn} , SI_{gs} , SI_E (Fig. 2a). Negative correlations were observed between Y_C and DSI, TOL, SI_{F_v/F_m} , SI_{PI} , $SI_{RC/CSm}$, $SI_{ABS/CSm}$, $SI_{TRo/CSm}$, $SI_{DIO/CSm}$ and $SI_{ABS/CSm}$ parameters. Negative correlation was also recorded for gas exchange parameters: SI_{WUE} and SI_{Pn} , SI_E , SI_{gs} . There was no correlation between SI_{F_v/F_m} , SI_{PI} , $SI_{RC/CSm}$ and SI_{Pn} , since the angle between the vectors was 90° . It was possible to select three groups of DH lines (I, II and III) (Fig. 2b). Group I comprised DH1, DH2 and DH3 lines with high resistance to drought and

stability. By comparing it with yield components in Table 5, we could see that these lines demonstrated the highest yield components (stable genotypes). Group II comprised DH5, DH6 and DH8 lines, which had the highest PC1 and lowest PC2 and produced similar yield as lines in group I. Group III with the highest PC1 and PC2 consisted of DH4, DH7, DH9, DH10 and DH11 lines with the lowest values of yield components could be named as the most sensitive to drought stress. The lines in group I and II had lower DSI_{GN} (DSI of grain number) and DSI_{GW} (DSI of grain weight) (Fig. 1a, b) and higher yield components (GN, GW) (Table 5).

Table 5 Yield components per plant: grain number, grain weight (GN, GW), shoot biomass (SB), above-ground biomass (AB) mean values and harvest index (HI) in oat DH lines harvested after full maturity in control (C) and after 14 days of soil drought treatment (D), $n = 5$

| Line | Grain number (GN) | | Grain weight (g) (GW) | | Shoot biomass (g) (SB) | | Above-ground biomass (g) (AB) | | Harvest index (HI) | |
|---------------------|-------------------|----------|-----------------------|----------|------------------------|----------|-------------------------------|----------|--------------------|----------|
| | <i>C</i> | <i>D</i> | <i>C</i> | <i>D</i> | <i>C</i> | <i>D</i> | <i>C</i> | <i>D</i> | <i>C</i> | <i>D</i> |
| DH1 | 169 | 149 | 6.6 | 5.7 | 6.6 | 6.7 | 13.2 | 12.5 | 0.50 | 0.46 |
| | | 88 | | 87 | | 102 | | 94 | | 92 |
| DH2 | 185 | 144 | 6.1 | 5.9 | 4.8 | 4.4 | 10.9 | 10.3 | 0.56 | 0.57 |
| | | 77 | | 96 | | 92 | | 94 | | 101 |
| DH3 | 140 | 90 | 4.6 | 4.1 | 4.2 | 3.5 | 8.7 | 7.5 | 0.52 | 0.54 |
| | | 64 | | 88 | | 82 | | 85 | | 103 |
| DH4 | 180 | 65 | 5.6 | 2.6 | 3.8 | 4.0 | 9.4 | 6.6 | 0.60 | 0.39 |
| | | 36 | | 45 | | 106 | | 70 | | 65 |
| DH5 | 112 | 88 | 3.9 | 3.1 | 4.2 | 5.6 | 8.1 | 8.7 | 0.48 | 0.36 |
| | | 78 | | 81 | | 132 | | 88 | | 75 |
| DH6 | 177 | 114 | 6.2 | 4.0 | 6.4 | 4.0 | 12.6 | 8.0 | 0.49 | 0.50 |
| | | 64 | | 64 | | 63 | | 63 | | 102 |
| DH7 | 108 | 41 | 3.6 | 1.5 | 5.6 | 4.3 | 9.3 | 5.8 | 0.39 | 0.26 |
| | | 38 | | 42 | | 76 | | 62 | | 66 |
| DH8 | 96 | 81 | 3.6 | 3.1 | 4.3 | 5.8 | 7.9 | 8.9 | 0.45 | 0.35 |
| | | 84 | | 86 | | 134 | | 95 | | 77 |
| DH9 | 134 | 48 | 4.8 | 1.9 | 6.2 | 5.2 | 10.9 | 7.2 | 0.44 | 0.27 |
| | | 36 | | 40 | | 85 | | 65 | | 61 |
| DH10 | 77 | 37 | 2.8 | 1.6 | 6.0 | 5.3 | 8.8 | 7.0 | 0.32 | 0.23 |
| | | 47 | | 58 | | 89 | | 79 | | 71 |
| DH11 | 171 | 83 | 5.0 | 2.4 | 3.8 | 4.3 | 8.8 | 6.7 | 0.57 | 0.36 |
| | | 48 | | 48 | | 114 | | 76 | | 63 |
| Source of variance | | | GN | | GW | SB | | AB | | HI |
| DH line | | | *** | | *** | *** | | *** | | *** |
| Treatment | | | *** | | *** | ns | | *** | | *** |
| DH line × treatment | | | * | | ns | * | | ** | | ns |

Percentage values of control are given in italics. The results of two-way analysis of variance (ANOVA) are presented in the lower part of the table. The sources of variance for GN, GW, SB, AB and HI were as follows: eleven DH lines, two treatments and interaction between DH line and treatment.)

ns not significant

* ** *** Significant at $P \leq 0.05, 0.01, 0.001$, respectively

Shapiro–Wilk test of normality and one-way analysis of variance (ANOVA)

The distribution of normality was presented in Table 6. As the level of significance P was greater than 0.05 for 28 of the 32 cases examined, so there was no reason to reject the hypothesis of normality. Only the for cases, a F_v/F_m , WUE and DI_v/CSm in drought-treated plants and a F_v/F_m in control ($P < 0.05$) the null hypothesis of normality was rejected. The results were confirmed also via test of homoscedasticity and one-way analysis of variance (Table 7) where the same parameters were statistically significant.

Discussion

Considerable differences were found between the DH lines, treatments and interactions between the DH line and the treatment for the majority of gas exchange parameters, CF traits and yield components. This indicated the existence of genetic variation and the possibility of selection for favorable genotypes in both environments. The presence of SI, DSI and MP among the indices showing a high correlation with grain yield parameters of drought-stressed plants is consistent with the results reported by Talebi et al. (2009) and Farshadfar et al. (2013). Drought resistance should be based on yield stability under water deficits.

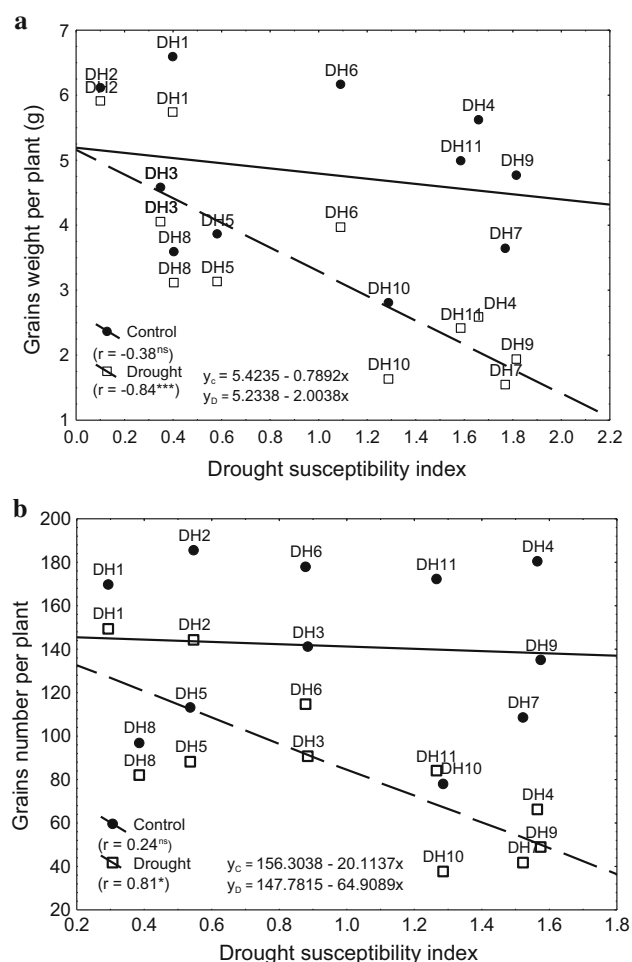


Fig. 1 Relationship between drought susceptibility indexes (DSI) for the weight of oat grains per plant (**a**) and for the grain number per plant (**b**) in oat DH lines (DH1-DH11). Linear correlation coefficient (Pearson's, r) and the significance level were indicated; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns not significant

Thus, the genotypes showing low fluctuations can be considered as a drought-resistant. The analyzed resistance/sensitivity indices provide the most suitable criteria for the selection of high yielding genotypes under water stress. This is in agreement with the results of other authors who studied wheat (Ahmadizadeh et al. 2012; Drikvand et al. 2012). They found that statistical methods, including correlation between grain yield and stress indices or biplot analysis identified the same genotypes as resistant to drought. These results were confirmed by low DSI values and elevated GN and GW parameters. Hence, these statistical methods are useful for identifying drought-tolerant genotypes. Our experiment, involving treatment of young wheat seedlings with drought stress, showed slight differences in the reduction of P_n , E and g_s compared to control. Probably, the plants in this stage of development were not sufficiently sensitive to changes in gas exchange parameters. Some authors underlined the fact that the largest

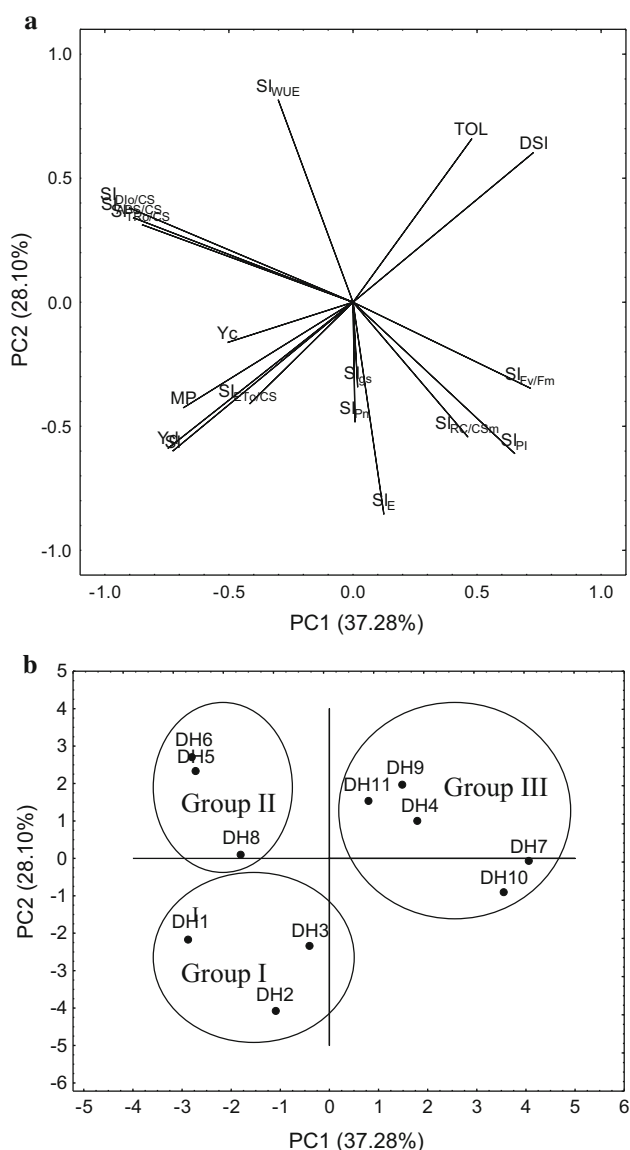


Fig. 2 Principal component analysis showing relationships between the yields under control (Y_c) and drought conditions (Y_d), and drought tolerance/resistance indices after 14 days of drought treatment (**a**). DSI sensitivity drought index; TOL tolerance index, SI susceptibility stress index, MP mean productivity; calculated for control and drought SI indices for: kinetics fluorescence parameters (F_v/F_m , PI, ABS/CSm, TR_o/CSm, DI_o/CSm, ABS/CSm, ET_o/CSm and RC/CSm, gas exchange parameters (P_n , E , WUE and g_s) and tolerance/sensitivity of DH lines to drought (**b**)

differences in these parameters in crop plants were recorded during later stages of development (anthesis or grain filling stage), which indicated that the growth period should be properly selected to improve further selection of better cultivars (Jiang et al. 2000; Reynolds et al. 2000). These authors indicated that, in addition to leaf aging, P_n rate was closely associated with chlorophyll loss. In our experiment, we observed a high decrease of P_n and g_s in comparison to control, however, differences in the reaction

Table 6 Distribution normality using Shapiro–Wilk test (*W*) of selected traits: gas exchange, chlorophyll *a* fluorescence kinetics parameters and yield components per plant in control and drought-treated DH lines of oat during 14 days

| Parameter | Treatment | Trait | <i>W</i> | <i>P</i> | | |
|----------------------------|--|----------------------|----------|-----------|-------|-------|
| Gas exchange | Control | Pn | 0.102 | >0.20 | | |
| | | <i>E</i> | 0.127 | >0.20 | | |
| | | WUE | 0.185 | >0.20 | | |
| | | <i>g_s</i> | 0.127 | >0.20 | | |
| | | Drought | Pn | 0.128 | >0.20 | |
| | | | <i>E</i> | 0.124 | >0.20 | |
| | WUE | | 0.199 | <0.15 | | |
| | <i>g_s</i> | | 0.084 | >0.20 | | |
| | Chlorophyll <i>a</i> fluorescence kinetics | | Control | F_v/F_m | 0.096 | <0.05 |
| | | | | PI | 0.140 | >0.20 |
| | | DI _o /CSm | | 0.070 | >0.20 | |
| | | ABS/CSm | | 0.075 | >0.20 | |
| TR _o /CSm | | 0.084 | | >0.20 | | |
| ET _o CSm | | 0.087 | | >0.20 | | |
| Drought | | RC/CSm | 0.094 | >0.20 | | |
| | | F_v/F_m | 0.196 | <0.05 | | |
| | | PI | 0.070 | >0.20 | | |
| | | DI _o /CSm | 0.195 | <0.05 | | |
| | | ABS/CSm | 0.144 | >0.20 | | |
| | | TR _o /CSm | 0.112 | >0.20 | | |
| Yield components per plant | Control | ET _o CSm | 0.133 | >0.20 | | |
| | | RC/CSm | 0.087 | >0.20 | | |
| | | GN | 0.078 | >0.20 | | |
| | | GW | 0.107 | >0.20 | | |
| | | SB | 0.094 | >0.20 | | |
| | | AB | 0.126 | >0.20 | | |
| | Drought | HI | 0.160 | >0.20 | | |
| | | GN | 0.123 | >0.20 | | |
| | | GW | 0.103 | >0.20 | | |
| | | SB | 0.083 | >0.20 | | |
| | | AB | 0.096 | >0.20 | | |
| | | HI | 0.091 | >0.20 | | |

Shapiro–Wilk test: data are normally distributed if probability value $P > 0.05$

W are the statistical values of the test

of the tested oat DH lines to drought stress (D) was low. A lower decrease was observed for *E* and particularly for WUE parameters in the stressed DH lines. Jiang et al. (2000) and Hisir et al. (2012) reported that higher *g_s* values were associated with higher Pn values. Under drought, stomata closure limits CO₂ fixation in the chloroplast, so that the electron flow in the light reaction exceeds the quantity required for CO₂ assimilation (Sanchez-Martín et al. 2012). This leads to an excessive reduction in photosynthetic components. Low differences between WUE parameters observed in our experiment made it difficult to determine which of the lines had a better water use efficiency and whether it was consistent with other gas exchange parameters. However, WUE is one of the most

frequently studied parameters related to drought resistance and is often calculated in a simplistic manner with drought resistance (Sanchez-Martín et al. 2012). Some authors (Condon et al. 2002; Blum 2005) suggested that the increased WUE corresponded to improved yields under stress, although they did not show a clear correlation between WUE and drought resistance.

Stomatal closure, while avoiding water loss, reduces the entrance of CO₂ inside the leaves (Flexas et al. 2002). Under drought not only the stomatal functioning is affected, since the mesophyll conductance to CO₂ is also decreased (Flexas et al. 2002), leading to the proposal that one of the major limitations to photosynthesis under drought arises from the low chloroplast CO₂ availability

Table 7 One-way analysis of variance of the traits measured after 14 days of drought treatment

| Parameters as a source of variance | Trait | <i>F</i> | <i>P</i> |
|--|---|----------|---------------------|
| Gas exchange | Pn | 195.417 | 0.000*** |
| | <i>E</i> | 35.606 | 0.000*** |
| | WUE | 3.605 | 0.072 ^{ns} |
| | <i>g</i> _s | 193.342 | 0.000*** |
| Chlorophyll <i>a</i> fluorescence kinetics | <i>F</i> _v / <i>F</i> _m | 3.000 | 0.101 ^{ns} |
| | PI | 31.037 | 0.000*** |
| | DI _o /CSm | 0.181 | 0.675 ^{ns} |
| | ABS/CSm | 1.758 | 0.199 ^{ns} |
| | TR _o /CSm | 4.353 | 0.049* |
| | ET _o /CSm | 79.727 | 0.000*** |
| | RC/CSm | 6.276 | 0.021* |
| Yield components per plant | GN | 11.744 | 0.002** |
| | GW | 6.760 | 0.017* |
| | SB | 4.237 | 0.043* |
| | AB | 4.995 | 0.036* |
| | HI | 8.6428 | 0.004** |

ns not significant

* ** *** Significant at $P \leq 0.05, 0.01, 0.001$, respectively

(Flexas and Medrano 2002). When the water stress is moderate or severe, decreases in photosynthesis are possibly due to a decreased RuBP availability and/or decreased Rubisco activity (Lawlor 1995). Water stress has been reported to lead to an accumulation of sugars and a feedback down-regulation of photosynthesis (Souza et al. 2004, Silva et al. 2012). Under mild water stress, diffusional limitations (both stomatal and mesophyll conductance to CO₂) dominate over the non-diffusional ones. When drought is moderate or severe, decreases in PSII photochemistry may contribute to the decreases in photosynthesis, in addition to the diffusional causes.

Chlorophyll *a* fluorescence kinetics (CF) allows determining whether the photosynthetic apparatus was damaged after cessation of watering. Fluorescence methods have been successfully applied in many studies related to plant drought resistance (Lichtenthaler and Babani 2004; Lichtenthaler et al. 2005; Hura et al. 2007; Rapacz et al. 2010; Yin et al. 2010). CF-modulated parameters are commonly used in the JIP-test. In our study, we assayed CF parameters to identify resistant or sensitive to drought oat DH lines. Similar to other authors, who observed only a slight decrease in *F*_v/*F*_m in wheat cultivars grown under drought stress (Zlatev 2009), *F*_v/*F*_m in our experiment did not differ significantly in all DH lines tested. Other authors suggested that this was caused by the fact that a large proportion of absorbed light energy was not utilized by the plants in photosynthesis. Other studies demonstrated that the dry mass accumulation and increased yield traits were associated with an increase in *F*_v/*F*_m (Liang et al. 2010). In our

previous experiments, based on the higher values of ABS/CSm, TR_o/CSm, ET_o/CSm, RC/CSm and PI, we have selected some wheat genotypes with a better functioning photosynthetic apparatus (Czyczyło-Mysza et al. 2013). Most of the genotypes tested have exhibited similar energy dissipation as heat from PSII (DI_o/CSm). The results obtained in our present experiment allowed to select several oat DH lines with higher PI and RC/CSm (calculated as a percentage of control), grown under drought conditions, and include them to resistant lines (DH1–4 and DH8). These genotypes also showed higher yield components, in comparison to other lines.

Severe water stress not only causes loosing of amount of photosynthetic pigments, but also the disruption and loss of thylakoid membranes (Wright et al. 2009; Zhang et al. 2009). Under conditions of stress take place, a deficit of mineral components which often causes a decrease in the content of pigments (Starck 2002). Decreases among other magnesium contents cause a decrease in oxygen production. During the moderate drought, compared to severe ones, the centers of PSII photosynthetic apparatus effectively capture excitation energy and trigger further photochemical reactions. In studies of Souza et al. (2004), the authors concluded that photosystem PSII is more resistant to water deficit, compared with PSI, and the effect of stress on the course of the photochemical reaction is manifested only in the prolonged and deep drought stress. Due to the osmotic adjustment of cells, a relatively large volume of protoplasts maintains and reduces the inhibition of photosynthesis in a low water potential of leaves (Shangguan and

Shao 1999). The plants in response to drought exhibit uncontrolled generation of reactive oxygen species (ROS) in cells and disturbances in the electron transport in the respiratory chain and in the light phase of photosynthesis (Starck 2005). It has been shown that the drought most of all affects the flow of energy between the centers PSII reaction of a quinone Q_A that was visible in the changes of parameters of JIP-test, particularly such as the overall index performance (PI) of PSII system and the number of the active centers of reaction (RC/CSm). The most sensitive to water deficit in the soil was parameter F_v/F_m which determines the quantum yield of PSII but does not give complete information on its photochemical properties. In our studies, in agreement of the results of Qiu and Lu (2003) and Lu and Zhang (1998), it was also observed no reduction of the F_v/F_m under drought stress conditions. The authors indicate that the stabilization of the PSII complex depends on increasing concentration of osmotically active substances.

Yield components, such as GN, GW, SB, AB and HI were determined in oat DH lines harvested after re-watering and reaching full maturity in the soil. It was interesting to study the consequent effect of a two-week drought treatment on 17-day seedlings many weeks later, when plants reached full maturity. It was observed that the plants “remembered” the stress treatment and some of the DH lines were more efficient in overcoming the effects of a distant short water stress compared to others. Although yield components did not change in a similar manner, it was possible to create a ranking of resistant/sensitive to drought stress oat DH lines. Similar effect was obtained in our previous study on wheat, where yield components values were reduced. Wheat plants in that study were harvested after reaching full maturity in the soil, but first they underwent 7-day hydroponic cultures supplemented with three different PEG concentrations, causing osmotic stress for young seedlings, before transfer to the soil (Marcinińska et al. 2013b). We called it an after effect of osmotic stress. In the present experiment, we created a ranking of resistant/sensitive oat DH lines. Other authors, in many studies related to the evaluation of drought tolerance in cereals, created similar rankings of resistant/sensitive genotypes, for example, in oat (Akcura and Ceri 2011; Hisir et al. 2012; Rabiei et al. 2012; Zaheri and Bahraminejad 2012), in wheat ((Talebi et al. 2009; Geravandi et al. 2011; Ahmadizadeh et al. 2012), in corn (Khayatnezhad et al. 2011), and in wheat-rye lines (Farsadfar et al. 2013). Many authors used sensitivity indices to environmental stresses to determine genetic variation of different seedling traits. To establish the variability of resistance to drought, breeders using qualitative physiological tests require quantitative indicators, for example, the DSI index. There are formulas available with respect to

the drought stress in a couple of published reports (Fischer and Maurer 1978; Golbashy et al. 2010; Nouraein et al. 2013). More frequently, grain yield and dry weight of shoots or plant height are used for this purpose in the experiments. In our previous studies, we showed that the DSI index can be used as a good criterion for the selection of resistant/sensitive triticale and maize genotypes (Grzeziak et al. 2012, 2013). We found that when the height of the plant and leaf area was lower, yield component values also tended to be reduced. DSI index, calculated on the basis of yield parameters, was low for resistant genotypes, whereas much higher for sensitive ones.

Sensitivity index (SI) was calculated based on the data concerning gas exchange and CF parameters in the control and drought treatment simultaneously. The biplot analysis can be a better approach than a simple correlation analysis in the identification of suitable genotypes for stressed and non-stressed environments. PCA analysis is often used to select resistant/sensitive genotypes in crops (Talebi et al. 2009; Zhang et al. 2011; Zaheri and Bahraminejad 2012; Parihar et al. 2012; Nouraein et al. 2013).

In our experiment, it was possible to distinguish three groups of DH lines (I, II and III) with a low, moderate and strong sensitivity to drought. By comparing it with yield components, we found that the lines from group I produced the highest grain yield (stable genotypes), group II produced lower grain yields (semi-stable genotypes) under both conditions, and group III had the lowest values of yield components. This suggested that the last group of lines had the highest sensitivity to drought stress. It was also observed that the lines in group I and II had a lower DSI index than group III. This could be the reason for increased drought tolerance of these lines than those in group III. Therefore, it is interesting that the PCA analysis confirmed the results of linear correlation between DSI and the number and dry weight of grain as well as production of yield components. Lastly, this analysis was allowed to select groups of lines differing in resistance/sensitivity to drought stress. These findings are consistent with the studies (Golabadi et al. 2006; Mohammadi et al. 2010) conducted on wheat and mung bean (Zabet et al. 2003). Moreover, the indices of stress susceptibility/resistance can also be used for genotype screening to determine their tolerance to stress.

Conclusions

DH lines, potentially more tolerant to drought stress, selected based on the measurements of different physiological factors, such as gas exchange and chlorophyll *a* fluorescence kinetics parameters, specific yield components or the drought tolerance indices, largely overlap.

Thus, it can be assumed that the measurements performed in this work may serve as useful tools in estimating the degree of tolerance to drought stress in oat. It is a quite remarkable and novel finding of this experiment that although water stress was imposed in the initial stage of growth, and only for 14 days, crop yield was affected in the maturity stage. This study showed that the yield of drought-stressed lines of certain genotypes was reduced more than in other lines, suggesting genetic diversity of drought tolerance in these plants. Breeders are interested in improving drought resistance, while maintaining high quantity and quality of yield. Fluorescence and gas exchange techniques are simple and non-invasive tools, which are very useful in physiological analyses and can be applied to assess plant responses to various environmental stresses in early phases of development. In our recent studies, we wanted to point out that these techniques offer the possibility of an early evaluation of genotype potential in terms of water stress tolerance/sensitivity. Analyzing data using statistical PCA components can be a suitable method for studying the complex structure of traits and determination of their relative importance in conjunction with the yield, which can be further used in breeding programs to increase yield efficiency per unit area.

Author contribution statement IM, IC-M and ES designed the research; IM, IC-M, ES, MTG, MPK, MW and SG conducted the research; IM, IC-M, MTG and ES analyzed the data; IM, MTG, MW and SG wrote the paper; IM had primary responsibility for the final content. All authors have read and approved the final manuscript.

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