DROUGHT-INDUCED CHANGES IN STRESS HORMONE CONTENT AND THEIR ASSOCIATION WITH DROUGHT TOLERANCE LEVEL IN BARLEY (*HORDEUM VULGARE* L.)

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INTRODUCTION

Summer drought occurring in the so-called critical period, i.e. at heading, blooming and seed setting, is one of the major factors causing a substantial reduction of yield and a decrease in its quality. As water scarcity is considered a key threat for the 21st century, the understanding of drought tolerance mechanisms and the production of improved cereal crops with significantly enhanced water use efficiency is one of the most important challenges facing scientists and breeders. Many studies have shown that phytohormones play an important role in abiotic stress acclimation, integrating various stress signals and controlling downstream stress responses. In the presented study, drought-induced changes in the content of abscisic acid (ABA), salicylic acid (SA) and several brassinosteroids (BRs) were studied with the use of doubled haploid (DH) lines of winter barley (*Hordeum vulgare* L.) showing significant variation in respect of drought tolerance level (Gołębiowska-Pikania, et al. 2017).

MATERIAL AND METHODS

Plant material

Ten DH lines used in the study were produced by anther culture method from F1 generation of breeding materials received from Polish breeding companies Danko HR and Strzelce HR.

Drought treatment and drought tolerance (DT) estimation

Soil drought was started when the flag leaf was fully developed. Water content in the pots was gradually reduced to 33-35% of soil water content and was maintained at that level for the next two weeks. Water content in the control pots was adjusted to 75-78%.

Leaf water content (WC) was measured in control (WC_c) and drought-treated plants (WC_d) by quantitative sampling of leaf fresh mass (L_{FM}), followed by 72-hour lyophilisation. The obtained leaf dry mass (L_{DM}) was then estimated and WC was calculated according to the following equation and expressed as a percentage:

$WC = ((L_{FM} - L_{DM})/L_{FM}) \times 100\%$.

DT was estimated on the basis of leaf water loss (LWL) calculated according to the equation: $LWL = [(WC_c - WC_d)/WC_c] \times 100\%$

Measurements of hormone content

The measurements were conducted on barley leaves with the use of ELISA (Walker-Simmons and Abrams 1991), HPLC (Balcke, et al. 2012) and UHPLC (Tarkowska, et al. 2016) procedures for ABA, SA and BR analysis, respectively. All studied parameters were measured in 3-5 biological replicates.

Table 1. Changes in leaf water content (WC) induced by drought stress and leaf water loss (LWL) characteristic for the studied DH lines of barley. Means (± Sd) within each column marked with the same letter do not differ significantly according to Duncan test (p≤ 0.05).

DH lines	control [%]	drought [%]	LWL [% of control]
DH534	84.9 ± 0.5 l	78.6 ± 1.2 g	7,4
DH561	81.3 ± 0.7 i	75.1 ± 0.7 ef	7,7
DH584	78.3 ± 0.6 g	72.0 ± 0.7 c	7,9
DH363	81.5 ± 0.5 ij	74.1 ± 0.5 de	9,1
DH65	83.5 ± 0.4 k	75.3 ± 0.8 f	9,8
DH61	83.2 ± 0.7 k	73.7 ± 0.8 d	11,4
DH158	81.7 ± 1.2 ij	72.1 ± 0.7 c	11,8
DH575	83.5 ± 0.7 k	73.3 ± 0.9 d	12,2
DH602	82.6 ± 0.6 jk	70.5 ± 1.3 b	14,7
DH435	79.7 ± 0.5 h	67.6 ± 0.6 a	15,2

Drought tolerance

RESULTS AND CONCLUSIONS

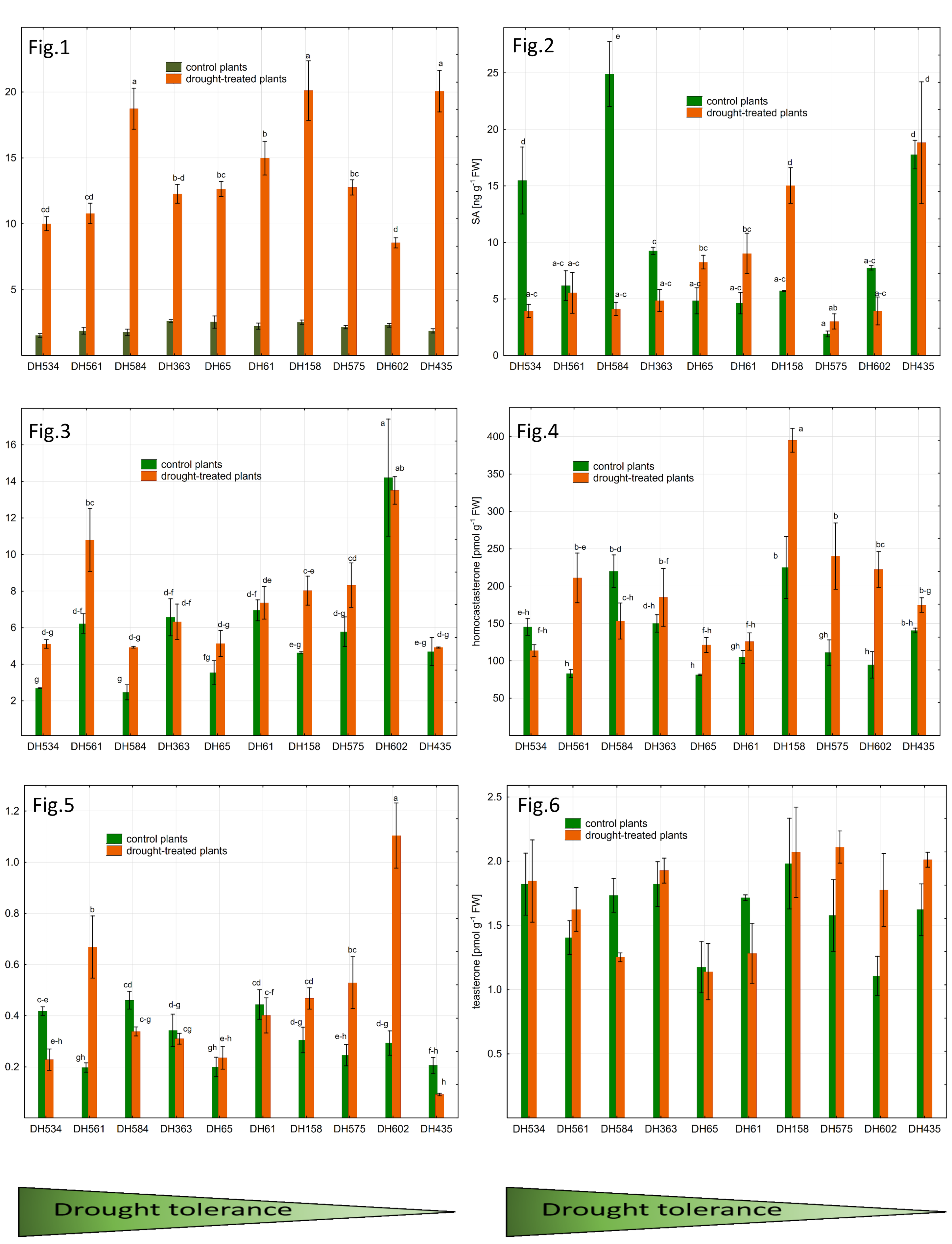
Based on the LWL parameter, drought tolerant, moderate and drought sensitive genotypes were identified among the ten, studied DH lines of barley (Table 1). Control plants of all studied DH lines optimally watered during the heading phase, showed no variation in the content of ABA and the majority of analysed BRs (Fig. 1-6). Only the level of SA in moderately drought tolerant DH lines and castasterone in drought sensitive DHs showed significant variation in comparison with two other drought tolerance groups (Table 2). In response to drought treatment (3 weeks at 35% FWC), the amount of ABA increased dramatically in all studied DH lines (Fig. 1, Table 2). A significant increase in the content of homocastasterone, dolicholide and teasterone was also observed, though only in drought sensitive DH lines. Simultaneously, higher accumulation of castasterone and a significant decrease in the content of SA were mainly restricted to drought tolerant DH lines of barley.

In conclusion, it seems that the increase in ABA content is a general stress reaction protecting plants from dehydration, whereas the variation in the level of SA and some BRs is associated with drought response specific for plants with higher adaptation ability.

Table 2. The content of ABA, SA and BRs and its changes in response to drought treatment in drought tolerant, moderate and drought sensitive lines of barley. Means (± Se) within each column marked with the same letter do not differ significantly according to Duncan test (p≤ 0.05).

DH lines	ABA [nmol g^{-1} DW]		SA [ng g^{-1} FW]	
	Control	Drought	Control	Drought
Drought sensitive	2,11± 0,12 ^{b}	13,4 ± 1,5 ^{a}	12,8 ± 2,3 ^{ab}	11,4 ±4,2 ^{ab}
Drought moderate	2,43 ± 0,11 ^{b}	14,5 ± 0,7 ^{a}	**5,27 ± 0,68 ^{c}**	7,54 ± 1,14 ^{bc}
Drought tolerant	1,70 ± 0,11 ^{b}	12,8 ± 1,0 ^{a}	15,5 ± 3,0 ^{a}	**4,52 ± 0,63 ^{c}**
	Castasterone [pmol g^{-1} FW]		Homocastasterone [pmol g^{-1} FW]	
	Control	Drought	Control	Drought
Drought sensitive	**9,45 ± 2,6 ^{a}**	10,12 ±2,5 ^{a}	12,8 ± 2,3 ^{ab}	11,4 ±4,2 ^{ab}
Drought moderate	5,49 ± 0,4 ^{bc}	7,03 ± 0,5 ^{ab}	**5,27 ± 0,68 ^{c}**	7,54 ± 1,14 ^{bc}
Drought tolerant	3,79 ± 0,7 ^{c}	6,95 ± 1,1 ^{ab}	15,5 ± 3,0 ^{a}	**4,52 ± 0,63 ^{c}**
	Dolicholide [pmol g^{-1} FW]		Teasterone [pmol g^{-1} FW]	
	Control	Drought	Control	Drought
Drought sensitive	0,25 ± 0,03 ^{b}	0,60 ± 0,23 ^{a}	1,36 ± 0,16 ^{b}	1,89 ± 0,14 ^{a}
Drought moderate	0,31 ± 0,03 ^{b}	0,38 ± 0,04 ^{ab}	1,65 ± 0,11 ^{ab}	1,71 ±0,14 ^{ab}
Drought tolerant	0,31 ± 0,04 ^{b}	0,41 ± 0,08 ^{ab}	1,65 ± 0,1 ^{ab}	1,57 ± 0,14 ^{ab}

Figs. 1-6. The content of ABA (Fig.1), SA (Fig.2), castasterone (Fig.3), homocastasterone (Fig.4), dolicholide (Fig.5) nad teasterone (Fig.6) and its changes in response to drought treatment in ten, studied DH lines of barley. Data (means ± Se) marked with the same letter do not differ significantly according to Duncan test (p≤ 0.05).



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