DOI: 10.1515/plass-2015-0030

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ANALYSIS OF YIELD AND YIELD RELATED TRAITS VARIABILITY OF WINTER WHEAT (*TRITICUM AESTIVUM* L.) CV. IZOLDA AND DOUBLE HAPLOID LINES

ABSTRACT

The yield-forming potential of winter wheat is determined by several factors, namely total number of shoots per plant and total number of spikelets per spike. The field experiments were conducted during three vegetation seasons at the Plant Breeding and Acclimatization Institute - National Research Institute (PBAI-NRI), located in Radzików, Poland. The objective of this study was a comparative analysis of the structural yield-forming factor levels, which determine grain yield per spike and per plant of the DH lines and standard Izolda cultivar. Results indicate that several DH lines showed some differences in tested morphological structures of plant, yield factor levels and in grain yield per spike and per plant in comparison to standard Izolda, regardless of the year. Mean grain yield per plant of DH lines was 26.5% lower in comparison to standard Izolda only in the second year of study. It was caused by a reduction of productive tillers number. Structural yield-forming potential of DH lines was used in 38% and 59% and in case of Izolda in 47% and 61% (the second and the third year of experiment, respectively). The mean grain yield per spike of DH lines was 14.8% lower than Izolda cultivar only in third year of experiment and it was caused by about 12% lower number of grains per spike. Structural yield-forming potential of DH spikes was used in 82.4%, 85.4% and 84.9% and in case of Izolda in 83.8%, 87% and 89.5% (the first, the second and the third year of experiment, respectively). The grain yield per winter wheat plant (both DH lines and standard Izolda) was significantly correlated with the number of productive tillers per plant (r = 0.80). The grain yield per winter wheat spike (both DH lines and Izolda cultivar) was significantly and highly correlated with the number of grains per spike (r = 0.96), number of fertile spikelets per spike (r = 0.87) and the spike length (r = 0.80). Variation of spike and plant structural yield-forming factors determining grain yield levels were also analyzed. Calculated total variation coefficients values of each analyzed trait during three-year long studies were different depending on plant material - DH lines or standard Izolda. Low variation coefficients values characterized following traits (traits ranked by increasing values for DH lines and standard Izolda, respectively): total spikelets number per spike (6.6 and 6.3%), spike length (11.1 and 12.6%), fertile spikelets number per spike (13.7 and 11.7%), single

Communicated by Grzegorz Żurek

grain weight (15.0 and 12.2%), shoot length (16.2 and 13.3%), grains number per spikelet (26.4 and 23.3%), total shoots number per plant (23.4 and 29.6%), grains number per spike (30.1 and 28.2%). Higher variation coefficients values were obtained for the following traits: grain yield per spike (40.0 and 35.7%), plant immature tillers number (35.8 and 42.6%), plant productive tillers number (42.2 and 43.2%), spike sterile spikelets number (46.6 and 44.7%) and number of grains per plant (58.3 and 60.5%). The highest values characterized grain yield per plant (66.9 and 60.8%).

Key words: winter wheat, doubled haploids, yielding, yield related traits

INTRODUCTION

Winter wheat is a major crop plant species with vast economic importance in agriculture. Literature about processes of wheat plant growth and development during season vegetation as well as duration of its several phases and structural yield-forming factor, which determine the grain yield per plant is very wide.

In terms of crop yielding physiology, including winter wheat, structural yield -forming factor, which determine the grain yield per plant is the number of shoots in the plant (overall tillering), which include the number of productive tillers (productive tillering) bearing spikes with kernels. Structural wheat yieldforming factors, as in other cereals, are created during certain stages of plant growth and development (Klepper et al., 1998) and occur in varying environmental conditions. Total number of shoots including productive tillers is formed during tillering phase (Klepper et al., 1998). Structural yield-forming factors of wheat spike are: spike length, total spikelets number including fertile spikelets, number of grains per spike and average weight of a single grain (Klepper *et al.*, 1998; Kozdój et al., 2010). The spike structure in the morphological aspect, mentioned above, is determined during stem elongation phase (Klepper et al., 1998). The processes of wheat spike morphogenesis and its structure were described by Natrova and Jokes (1974). The total number of spikelets per spike is determined during the formation of terminal spikelet from apical part of inflorescence meristem. The fertile florets number per spike is determined between booting and anthesis which prerequisite for setting grain potential (Klepper et al., 1998). The average grain weight at harvest is important yield component and is related to physiological and biochemical processes during post-anthesis period (Klepper et al., 1998). According to Hasan et al. (2011) grain length at harvest was the trait, which explained final grain weight. Moreover, carpel weight while pollination showed a positive and linear relation with final grain weight, irrespectively of the wheat cultivar and grain position within spikelet.

In agronomic aspects, grain yield depends on number of plants per unit of land area, spikes per plant, spikelets per spike, grains per spikelet, and single grain weight (Rajala *et al.*, 2009).

In wheat, two techniques are frequently used for doubled haploid production: anther culture (AC) and wheat with maize hybridization (MP) (Ma *et al.*, 1999; Guzy-Wróbelska and Szarejko, 2003). Production techniques of wheat doubled

haploids (DH) are worldwide reported (Zheng, 2003; Konieczny *et al.*, 2003; Segui-Simarro and Nuez, 2008; Cistué *et al.*, 2009; Redha and Islam, 2010; Germanà, 2011; Islam and Tuteja, 2012).

There is a need to understand plants growth and development and yieldforming factors which determine potentially high grain yield per spike and per plant of DH lines under specific environmental conditions, in long-term field experiments. One of the DH lines assessment methods is an analysis of the most important qualitative and quantitative traits variation coefficients rates. Literature gives limited information about the attributes of the DH lines produced by anther culture of pure cultivars in opposite to DH lines from the F1 hybrids of parents. Moreover, general question in this work is whether the grain yield of wheat DH lines is higher, lower or similar to pure cultivars used to obtain these DH lines. Literature data about grain yield per plant of DH lines in comparison with pure cultivars of wheat are in disagreement.

MATERIALS AND METHODS

Winter wheat (*Triticum aestivum* L.) doubled haploid (DH) lines and standard Izolda cultivar were used as a plant material in this experiment. Tested DH lines were obtained via androgenesis from isolated anthers of Izolda cv. in in vitro culture (Konieczny *et al.*, 2003; Oleszczuk *et al.*, 2011).

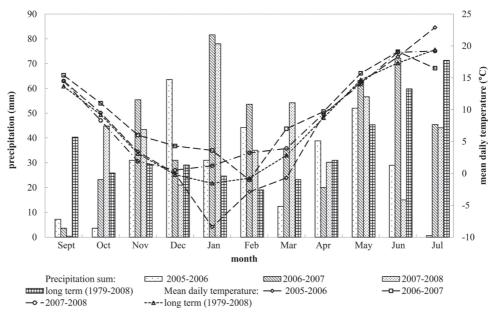
The field study were carried out at the Plant Breeding and Acclimatization Institute National Research Institute (PBAI–NRI), located in Radzików (52° 13'N, 20°38'E), Poland. The field experiments were carried out during three vegetation seasons 2005/2006, 2006/2007, 2007/2008. The experiments were performed in randomized blocks design, with black, podzolizated soil (very good rye soil complex). Subsequent generations of DH lines and Izolda cv. (100 seeds per 1 m² plot) seeds were sown in 27th of September 2005 (ten DH lines), 26th of September 2006 (two DH lines) and 26th of September 2007 (three DH lines). The number of seeds used to sow was intentionally low for better underestimated of characteristics of DH lines. Before sowing, multicomponent mineral fertilization was performed using Polifoska 6 (NPK(S) 6-20-30-(7); 300 kg/ha) and top dressing – nitrogen fertilization using ammonium nitrate (34%) in an amount of 100 kg/ha. During the tillering stage chemical weed-out was applied using Mustang agent contained active ingredients as florasulam and 2,4-D EHE (210 mg and 1000 mg in one liter at working solution, respectively).

In the full maturity stage, 15 plants were randomly sampled from each plot. The measurements of the longest plant shoot, the total number of shoots per plant, including productive tillers and immature tillers including withered (ontogenetically the youngest shoots in the plant, and strongly withered in harvesting time) were performed. The measurements of the spike length, total number of spikelets per spike including sterile and fertile spikelets and number of grains per spike were also carried out. Grains from all spikes within plant were

weighed. The average grain yield per spike was calculated as the ratio of grain yield per plant and the number of spikes within this plant. The single grain weight was calculated as the ratio of total weight of all grains from the plant and the number of grains per this plant. The average grain yield per spike and single grain weight were calculated, based on data obtained from 15 plants and three replicates for both DH lines and standard Izolda cv.

The mean values (objective average), standard deviations and coefficients of variation (CV%) for the analyzed traits of DH lines and standard Izolda cv. were estimated. The following statistical analyses were also performed:

- assessment of the significance of differences between tested objects one- and two-way analysis of variance (ANOVA) according to a fixed model (Cochran and Cox, 1992; Box *et al.*, 2005; Montgomery, 2005; Mądry *et al.*, 2010);
- assessment of the Pearson correlation coefficients between the analyzed traits (Rawlings *et al.*, 2001);
- description of the relations appearing between the analyzed traits and characteristics of the structure of these relationships a simple path analysis with designation of main effects and partial determination co-efficients (Wright, 1921, 1923, 1934; Mańkowski 2013).



Statistical analyses were performed using Statistica 12.5 (StatSoft, 2014).

Fig. 1. Mean daily temperature and sum of monthly precipitation during winter wheat vegetation in years of study in comparison to long-term averages at Radzików

The weather conditions during the experiments were described based on the average monthly air temperature and total rainfall for each month during the vegetation season, from September to July (Fig. 1). Meteorological data from the years of study were obtained from a meteorological station located in the PBAI-NRI in Radzików. The average monthly air temperatures and total rainfalls for each month were related to the monthly values of the long-term averages (Fig. 1).

Weather conditions of each vegetation season during the three-year-long field experiments were different (Fig. 1). In autumn, mean monthly temperatures were higher (October - December 2006, and December 2007), similar (October - December 2005), or lower (October and November in 2007). As for the precipitation, the lowest and the highest mean rainfall occurred in October 2005 and in October 2007, respectively. Winter months during the experiments were very cold (January - March 2006, February 2007), or very warm (January -March 2008, January 2007 and March 2007) with a significantly exceeded rainfall levels (with the exception of March 2006). In the spring months (April -June), mean monthly temperature was higher (April 2006 – 2008, May 2007, and June 2006 – 2008) or lower (May 2006, May 2008). Total monthly rainfall level in this period was significantly exceeded (with the exception of April 2007, June 2008 and June 2006) compared to long-term averages. July was characterized by a higher (2006), similar (2008) or lower (2007) mean air temperature and very low (2006) and low (2007 and 2008) rainfall level. In general, it can be concluded that the processes of plant growth and development were carried out in a wide range of thermal and rainfall conditions: humid (July 2007), the optimal (April 2006 and July 2008), quite dry (April 2008, May 2006, 2007 and 2008, June 2007), very dry (April 2007, June 2006) and even in the extremely dry (June 2008).

RESULTS

Statistical analysis of tested traits

The results of data analysis from three-year-long field experiments allowed to conclude that the tested DH lines were significantly different from the standard Izolda cv. in respect of some morphological structure features (Table 1). Plants of DH lines were significantly shorter (by 4.9 cm) than Izolda plants. The total number of tillers and number of immature tillers per plant of DH lines were higher (by 1 shoot and 0.8, respectively) in comparison to standard Izolda. Spikes of DH lines were the same length as Izolda, however they contained significantly more spikelets (by 0.3 spikelet), more (by 0.9) sterile spikelets, therefore produced significantly less grains (by 0.3) in the spikelet. Plants of DH lines and standard Izolda cv. were not significantly different in terms of other traits such as the number of fertile spikelets, number of grains per spike, number

of grains per plant, weight of single grain, grain yield per spike and grain yield per plant (Table 1).

Troit	Mean	values of	E statistic-
Trait	DH lines	Izolda cv. (control)	F statistics
Length of stem (cm)	82.745 b	87.573 a	12.00 **
Total no. of stems per plant	6.813 a	5.817 b	16.78 **
No. of productive tillers per plant	3.387 a	3.427 a	0.81 ^{NS}
No. of immature tillers per plant	3.253 a	2.500 b	11.84 **
Length of spike [cm]	9.355 a	9.634 a	0.01 ^{NS}
Spikelet no. per spike	21.090 b	21.445 a	11.43 **
Sterile spikelets no. per spike	3.500 a	2.625 b	10.09 **
Fertile spikelets no. per spike	17.591 a	18.820 a	0.28 ^{NS}
Grain no. per spike	41.031 a	49.735 a	2.23 ^{NS}
Grain no. per spikelet	1.929 b	2.295 a	5.31 *
Grain no. per plant	140.040 a	175.690 a	0.01 ^{NS}
Grain yield per plant [g]	6.045 a	7.980 a	0.01 ^{NS}
Grain yield per spike [g]	1.764 a	2.286 a	2.80 ^{NS}
Weight of single grain [(mg]	41.954 a	45.136 a	1.21 ^{NS}

One-way ANOVA results of winter wheat DH lines and standard Izolda cultivar (control) quantitative variables from 2006–2008

Homogenous groups marked with letters a and b, the same letter do not differ significantly according to Tukey test at P = 0.05; Significance: ^{NS} – not significant; ^{*} – significant at P = 0.05; ^{**} – significant at P = 0.01

Comparison of tested traits of DH lines in relation to year of study

Taking into account the average values of plant as well as spike morphological structure and grain yield per plant or per spike a significant impact of year (environmental conditions determined by rainfall and thermal factors) on differences revealing in studied traits was found (Table 2).

Table 2

Table 1

Mean values, standard deviations (SD) and coefficient of variance (CV) of traits of winter wheat DH lines and standard Izolda cultivar (control)

						20	06					
Parameter	DH 10/2/06	DH 10/7/06	DH 6/1/06	DH 6/10/06	DH 6/2/06	DH 7/10/06	DH 7/9/06	DH 8/4/06	DH 8/5/06	DH 9/13/06	Izolda	LSD*
					Lengt	h of stem [cm]					
Mean	78.7	82.1	76.5	81.3	73.4	76.7	70.3	83.7	75.9	86.6	72.9	11.6
SD	12.0	8.57	12.2	6.75	14.5	10.9	11.4	7.89	7.19	5.28	5.31	
CV [%]	15.2	10.4	16.0	8.3	19.7	14.2	16.2	9.4	9.5	6.1	7.3	

						20	06					
Parameter	DH 10/2/06	DH 10/7/06	DH 6/1/06	DH 6/10/06	DH 6/2/06	DH 7/10/06	DH 7/9/06	DH 8/4/06	DH 8/5/06	DH 9/13/06	Izolda	LSD*
				No	. of produ	ictive tiller	s per plan	t				
Mean	4.2	4.2	3.0	3.4	2.9	2.9	3.1	2.7	3.5	3.1	3.9	ns
SD	1.15	1.52	1.31	1.06	0.74	1.28	0.96	1.10	1.19	1.77	1.73	
CV [%]	27.3	36.2	43.6	31.0	25.9	43.6	31.3	40.2	34.2	56.4	44.7	
					Lengt	n of spike [cm]					
Mean	8.9	9.1	9.2	9.6	9.4	9.5	9.4	9.1	9.2	9.3	9.0	ns
SD	0.89	1.04	1.20	1.10	1.14	1.17	0.95	0.91	1.03	0.89	0.69	
CV [%]	10.0	11.4	13.0	11.5	12.1	12.3	10.1	10.0	11.2	9.6	7.7	
				F	ertile spil	kelets no. p	er spike					
Mean	16.0	15.5	17.0	17.8	16.8	17.3	16.9	17.2	17.4	17.3	16.6	ns
SD	2.12	2.51	2.37	2.08	2.27	2.79	2.62	1.88	2.38	1.83	1.63	
CV [%]	13.3	16.2	13.9	11.7	13.5	16.1	15.5	10.9	13.7	10.6	9.8	
				S	terile spil	elets no. p	er spike					
Mean	4.0	3.9	4.1	3.1	4.0	3.6	3.7	3.2	3.2	2.8	3.2	ns
SD	1.68	2.04	1.96	1.54	1.75	2.22	2.23	1.49	1.53	1.46	1.21	
CV [%]	42.0	52.3	47.8	49.7	43.8	61.7	60.3	46.6	47.8	52.1	37.8	
					Total spil	xelet no. pe	r spike					
Mean	20.0	19.4	21.1	21.0	20.8	20.9	20.6	20.5	20.6	20.1	19.8	1.17
SD	1.16	1.39	0.69	0.86	0.99	0.90	0.73	0.97	1.03	0.88	1.00	
CV [%]	5.8	7.2	3.3	4.1	4.8	4.3	3.5	4.7	5.0	4.4	5.1	
					Grain	no. per sp	ike					
Mean	32.8	31.7	39.3	42.1	39.9	39.8	38.5	41.3	38.0	34.5	34.3	ns
SD	10.8	8.79	14.1	11.1	11.6	13.2	14.0	12.1	11.3	9.07	5.71	
CV [%]	32.8	27.7	35.9	26.4	29.0	33.2	36.4	29.2	29.7	26.3	16.6	
						no. per pl						
Mean	132	129	116	147	115	115	117	113	133	104	134	ns
SD	47.8	49.7	56.1	71.3	45.5	51.5	49.4	60.1	71.8	55.3	68.5	
CV [%]	36.0	38.7	48.4	48.6	39.5	44.8	42.1	53.4	54.1	53.4	51.1	
					Grain yi	eld per pla	nt [g]					
Mean	5.15	5.42	4.67	6.57	4.50	4.62	4.78	5.01	5.26	4.72	5.33	ns
SD	2.11	2.23	2.55	3.10	2.20	2.33	2.63	2.97	3.36	2.44	2.75	
CV [%]	40.9	41.1	54.6	47.1	48.9	50.5	55.0	59.3	63.8	51.7	51.6	
					Grain yi	eld per spi	ke [g]					
Mean	1.29	1.36	1.60	1.90	1.56	1.62	1.57	1.84	1.50	1.61	1.37	ns
SD	0.58	0.48	0.78	0.51	0.66	0.76	0.85	0.67	0.66	0.54	0.33	
CV [%]	45.3	35.4	48.4	26.9	42.4	47.2	54.3	36.1	44.3	33.2	24.1	

Mean values, standard deviations (SD) and coefficient of variance (CV) of traits of winter wheat DH lines and standard Izolda cultivar (control) — continued

Table 2

Table 2

Mean values, standard deviations (SD) and coefficient of variance (CV) of traits of winter wheat DH lines and standard Izolda cultivar (control) — continued

						20	06					
Parameter	DH 10/2/06	DH 10/7/06	DH 6/1/06	DH 6/10/06	DH 6/2/06	DH 7/10/06	DH 7/9/06	DH 8/4/06	DH 8/5/06	DH 9/13/06	Izolda	LSD*
					Weight of	single gra	in [mg]					
Mean	38.5	42.1	39.0	45.3	37.7	39.3	38.7	43.9	38.4	46.1	39.8	7.25
SD	5.04	5.83	5.57	4.83	6.82	7.12	8.50	4.37	7.45	5.48	4.24	
CV [%]	13.1	13.9	14.3	10.7	18.1	18.1	21.9	10.0	19.4	11.9	10.6	
					Grain 1	10. per spil	kelet					
Mean	1.63	1.63	1.86	2.00	1.91	1.89	1.85	2.01	1.83	1.71	1.73	ns
SD	0.54	0.41	0.64	0.49	0.53	0.58	0.65	0.56	0.50	0.43	0.25	
CV [%]	32.8	25.0	34.4	24.4	27.7	30.7	35.0	27.7	27.0	25.3	14.6	
		20	07					20	008			
Parameter	DH 5/5/07	DH 9/14/07	Izolda	LSD*	DH 11/10/08	DH 9/13/08	DH 9/5/08	Izolda	LSD *			
					Length	n of stem [cm]					
Mean	74.0	75.5	75.9	ns	103	102	102	96.5	4.1			
SD	4.09	3.09	4.12		4.67	4.28	4.33	3.52				
CV [%]	5.5	4.1	5.4		4.5	4.2	4.3	3.6				
				No	o. of produ	ctive tiller	s per plant	t				
Mean	2.4	1.9	2.9	0.67	4.3	4.4	4.7	3.5	1.3			
SD	0.83	0.70	0.74		1.40	1.50	1.39	1.55				
CV [%]	34.5	36.4	25.5		32.2	34.1	29.3	44.6				
				N	o. of imma	ature tillers	per plant					
Mean	3.3	3.7	3.3	ns	3.3	3.0	2.9	2.2	1.0			
SD	1.05	1.17	1.35		1.17	1.31	1.10	0.79				
CV [%]	31.4	32.0	40.4		35.3	43.6	37.5	35.7				
				Te	otal numb	er of stems	per plant					
Mean	5.7	5.6	6.2	ns	7.7	7.4	7.7	5.7	1.2			
SD	1.16	1.72	1.26		1.23	1.40	0.90	1.84				
CV [%]	20.4	30.8	20.4		16.0	18.9	11.7	32.4				
					Length	of spike [cm]					
Mean	9.3	8.7	8.8	ns	9.4	10.6	9.7	10.0	0.9			
SD	1.07	0.71	0.90		0.88	0.84	0.71	1.25				
CV [%]	11.5	8.2	10.2		9.4	7.9	7.3	12.5				
]	Fertile spik	elets no. p	er spike					
Mean	19.1	18.2	18.8	ns	17.5	20.8	18.9	19.2	1.7			
SD	2.04	1.74	1.78		1.45	1.71	1.53	2.09				
CV [%]	10.7	9.6	9.5		8.3	8.2	8.1	10.9				
				2	Sterile spik	elets no. p	er spike					
Mean	3.5	3.0	2.8	ns	4.1	3.0	3.1	2.7	1.1			
SD	1.40	1.11	1.10		1.18	1.10	0.97	1.30				
CV [%]	40.0	37.0	39.3		28.8	36.7	31.3	48.1				
- L- J												

		20	07					20	008	
Parameter	DH 5/5/07	DH 9/14/07	Izolda	LSD*	DH 11/10/08	DH 9/13/08	DH 9/5/08	Izolda	LSD *	
					Total spik	elet no. p	er spike			
Mean	22.6	21.2	21.6	0.75	21.6	23.9	22.0	21.9	0.9	
SD	0.73	0.93	0.84		0.82	0.78	0.90	1.12		
CV [%]	3.2	4.4	3.9		3.8	3.3	4.1	5.1		
					Grain	no. per sp	vike			
Mean	46.1	43.6	45.6	ns	43.0	57.8	47.2	52.7	10	
SD	9.45	8.07	10.3		9.45	12.4	7.50	12.4		
CV [%]	20.5	18.5	22.5		22.0	21.4	15.9	23.4		
					Grain	no. per pl	lant			
Mean	111	84.6	129.0	37.0	191	264	230	175	ns	
SD	49.3	33.3	41.1		94.6	124	98.8	97.7		
CV [%]	44.5	39.3	31.7		49.4	47.2	43.1	55.7		
					Grain yi	eld per pla	ant [g]			
Mean	4.67	3.60	5.63	1.79	8.84	12.7	10.2	8.40	ns	
SD	2.48	1.61	1.87		5.54	5.92	4.76	4.67		
CV [%]	53.1	44.7	33.1		62.6	46.6	46.9	55.6		
					Grain yi	eld per spi	ike [g]			
Mean	1.93	1.83	2.00	ns	1.97	2.79	2.10	2.53	0.6	
SD	0.53	0.44	0.60		0.71	0.64	0.47	0.69		
CV [%]	27.4	23.9	30.2		36.2	22.9	22.5	27.4		
					Weight of	single gra	ain [mg]			
Mean	41.5	41.8	43.4	ns	4.0	48.2	44.1	47.8	ns	
SD	4.19	3.51	3.71		5.07	2.55	4.63	4.87		
CV [%]	10.1	8.4	8.5		11.3	5.3	10.5	10.2		
					Grain r	10. per spi	kelet			
Mean	2.03	2.05	2.10	ns	1.98	2.41	2.14	2.39	0.4	
SD	0.36	0.32	0.41		0.40	0.46	0.29	0.47		
CV [%]	17.8	15.8	19.3		20.2	19.2	13.4	19.7		

Mean values, standard deviations (SD) and coefficient of variance (CV) of traits of winter wheat DH lines and standard Izolda cultivar (control) — continued

Table 2

* — Tukey's LSD (HSD) at P = 0.05

In the first year of the study, significant differences in the stem length were observed between: DH line 10/7/06 vs. DH line 7/9/06, DH line 6/2/06 vs. DH line 9/13/06, DH line 7/9/06 vs. DH line 8/4/06, DH line 7/9/06 vs. DH line 9/13/06. Significant differences in the total number of spikelets per spike was found between lines DH 6/1/06, DH 6/10/06, DH 6/2/06, 7/9/06, DH 7/10/06, 8/5/06. In addition, there were significant differences in the single grain weight between the lines: DH 9/13/06 vs. DH 10/2/06, DH 6/2/06, DH 7/9/06, DH 8/5/06, and also between DH line 6/2/06 vs. DH line 6/10/06. Between DH lines tested in the second year of study (year 2007) significant differences in tested

traits were found only in total spikelet number per spike. Spikes of DH line 5/5/07 had significantly more formed spikelets (6.6%) than spikes of DH line 9/14/07. Between DH lines tested in the third year of experiment (year 2008) some significant differences were found in several traits, too. Spikes of DH line 9/13/08 were longer, had higher total spikelet number, included higher fertile spikelet number, higher grains number, yielded better than spikes of remaining two DH lines — DH line 11/10/08 and DH line 9/13/08.

Comparison of tested traits between individual DH lines and the Izolda cultivar in relation to year of study

Detailed comparative analysis of all the structural yield-forming factors in each year of the study allowed to demonstrate significant differences between several DH lines and the standard Izolda (Table 2). In the first year of the study, DH line 9/13/06 had longer stems (19%), whereas two lines - DH line 6/1/06 and DH line $\frac{6}{10}$ had more developed spikelets per spike (6.6 and 6.1%, respectively) than Izolda plants. In the second year of study (year 2007) DH line 9/14/07 was characterized by significantly lower values of following traits: number of productive tillers per plant (34%), grains number per plant (35%) and grain yield per plant (36%). Moreover, DH line 5/5/07 was characterized by significantly higher values of total spikelets number per spike (by 4.6%). In the third year of experiment (year 2008) following DH lines: DH 11/10/08, DH 9/13/08, DH 9/5/08 were longer from 5.4 to 6.6% and had higher total number of tillers per plant (35–40%). DH line 9/5/08 had more (47%) productive tillers per plant. Spikes of DH line 9/13/08 formed more spikelets (9%) whereas DH line 11/10/08 had less fertile spikelets (about 9%) but more sterile spikelets (52%) than standard Izolda cultivar.

Based on data presented in Table 2 we can conclude that variation in grain yield per plant between DH lines and standard Izolda in each year of study was observed. Among studied DH lines, following lines: DH 6/10/06 and DH 10/7/06, DH 9/5/08 and DH 9/13/08 tended to have higher values of yield-forming factors and higher grain yield per plant compared to standard Izolda. Other DH lines yielded insignificantly lower than standard Izolda, regardless of the year of study (Table 2).

Variation of analyzed traits

Diversified vegetation conditions (rainfall and thermal factors) determined variation coefficients values of traits both DH lines and standard Izolda, which were different depending on the year (Table 2). Calculated three-year-long total variation coefficients values of each analyzed trait were different depending on plant material – DH line and standard Izolda. Low variation coefficients values were obtained for the following traits (traits ranked by increasing values for DH lines and standard Izolda, respectively): total spikelets number per spike (7 and

6%), spike length (11 and 13%), fertile spikelets number per spike (14 and 12%), single grain weight (15 and 12%), stem length (16 and 13%), grains number per spikelet (26 and 23%), total stems number per plant (23 and 30%), grains number per spike (30 and 28%). Higher variation coefficients values were obtained for following traits: grain yield per spike (40 and 36%), immature tillers number per plant (36 and 43%), productive tillers number per plant (42 and 43%), sterile spikelets number per spike (47 and 45%), grains number per plant (58 and 61%), and the highest variation coefficient values were obtained in case of grain yield per plant (67 and 61%) (Table 2). Similar values of variation coefficients, regardless of the year, were obtained only in DH lines for following traits: grains number per plant, grain yield per plant, productive tillers number per plant, immature tillers number per plant, spike length, total spikelets number per spike. Values of variation coefficients for remaining traits in DH lines and for all analyzed traits in standard Izolda were unstable in years of experiment (Table 2).

Correlations analysis

A significant correlations between tested traits in DH lines and standard Izolda were noticed during the three years of experiment (Table 3). Significant linear correlations between the grain yield per plant and: grains number per plant (r = 0.97), grain yield per spike (r = 0.71), productive tillers number per plant (r = 0.71), spike length (r = 0.71), grains number per spike (r = 0.71), fertile spikelets number per spike (r = 0.66), grains number per spikelet (r = 0.65), total spikelets number per spike (r = 0.63), total tillers number per plant (r = 0.58), stem length (r = 0.54), single grain weight (r = 0.46), was found. Correlation was also noted between grain yield per spike and following traits: grains number per spike (r = 0.96), grains number per spikelet (r = 0.95), fertile spikelets number per spike (r = 0.87), spike length (r = 0.80), single grain weight (r = 0.75), total spikelets number per spike (r = 0.64), stem length (r = 0.56). The number of grains per spike was significantly correlated with: grains number per spikelet (r = 0.98), fertile spikelets number per spike (r = 0.90), spike length (r = 0.83), total spikelets number per spike (r = 0.69)and stem length (r = 0.51). Taking into account the morphological structure of spike it was found that spikelet fertility expressed as grains number per spikelet was highly correlated with spike length (r = 0.80), total spikelets number per spike (r = 0.55), fertile spikelets number per spike (r = 0.85) and single grain weight (r = 0.57). All these interactions have a strong or very strong character.

	Trait	1	0	ю	4	5	9	٢	8	6	10	11	12	13	14
_	Length of stem	-													
0	2 No. of productive tillers per plant	0.29**	1												
3	No. of immature tillers per plant	-0.24	-0.23**	1											
4	Total no. of stems per plant	0.29^{**}	0.74^{**}	0.48^{**}	1										
5	Length of spike	0.46**	0.29^{**}	-0.32^{**}	0.27^{**}	1									
9	Total spikelets no. per spike	0.41^{**}	0.27**	-0.15 ^{NS}	0.40^{**}	0.65**	-								
2	Sterile spikelets no. per spike	-0.34	0.02^{NS}	0.21^{*}	0.06 ^{NS}	-0.70**	-0.33**	-							
8	Fertile spikelets no. per spike	0.46^{**}	0.14^{*}	-0.22*	0.20^{*}	0.83**	0.79**	-0.84	1						
6	Grain no. per spike	0.51**	0.12^{*}	-0.29^{**}	0.15^{NS}	0.83**	0.69^{**}	-0.77**	0.90^{**}	-					
10	Grain no. per plant	0.49**	0.80^{**}	-0.28**	0.64**	0.67**	0.61^{**}	-0.40	0.61^{**}	0.65**	1				
-	Grain weight per plant	0.54**	0.71**	-0.29**	0.58**	0.71**	0.63^{**}	-0.46	0.66^{**}	0.71**	0.97**	1			
12	Grain weight per spike	0.56**	0.09 ^{NS}	-0.30^{**}	0.09 ^{NS}	0.80^{**}	0.64**	-0.76**	0.87**	0.96^{**}	0.60**	0.71^{**}	1		
13	Weight of single grain	0.57**	-0.01 ^{NS}	-0.23**	-0.08 ^{NS}	0.48^{**}	0.33**	-0.55**	0.55**	0.56**	0.28**	0.46^{**}	0.75**	1	
14	Grain no. per spikelet	0.50^{**}	0.05^{NS}	-0.29^{**}	0.07 ^{NS}	0.80	0.55**	-0.82	0.85^{**}	0.98**	0.58**	0.65**	0.95**	0.57^{**}	.

Path analysis

In order to characterize the influence of analyzed traits of morphological structure on winter wheat yield-forming factors path analysis was performed. At first, from the analysis the following traits were excluded: total shoots number per plant and spikelets number per spike, because they were a direct, linear combination of the other analyzed traits. In case of taking them into account in the analysis, model parameters could not be determined, since there would be no solution to the normal equations in the least squares method (Rawlings *et al.*, 2001).

Table 4

Direct effects among some grain weight per plant and examined trait	s (parameters) of winter wheat
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Trait (parameter)	Direct effect	t statistics
Length of stem	-0.00108	-0.09 ^{NS}
Total no. of stems per plant ¹	_	_
No. of productive tillers per plant	-0.17073	-3.65 **
No. of immature tillers per plant	0.00742	0.94 ^{NS}
Length of spike	-0.02389	-0.86 ^{NS}
Spikelet no. per spike ¹	_	_
Sterile spikelets no. per spike	-0.01034	-0.55 ^{NS}
Fertile spikelets no. per spike	0.12828	2.59 *
Grain no. per spike	-1.32123	-5.12 **
Grain no. per spikelet	0.39799	2.18 *
Grain no. per plant	1.15488	19.72 **
Grain weight per spike	0.99297	11.3 **
Weight of single grain	-0.15093	-4.51 **

Significance: ^{NS} – not significant; ^{*} – significant at P = 0.05; ^{**} – significant at $P = 0.01^{-1}$ – the parameters that are linear combination of other parameters in the model

Paths analysis for the full model was performed without removing the causal variables with insignificant Student's t-statidtics (Table 4). This approach also allows for the expression of the characteristics that make a very small contribution to observed relationships of cause and effect, but from the substantive point of view, they have influence on the dependent variable, i.e. grain yield per plant (Mańkowski, 2013). The analysis allowed to present occurring relationships using cause path model, which described observed variability of grain yield per plant in 99.3% (\mathbb{R}^2). In order to determine the impact of morphological structure individual traits on the observed grain yield per plant path coefficients were marked out (Table 4). Direct effects analysis allowed to make a conclusion that the most important role in the direct formation of the final winter wheat grain yield were: grains number per spike (-1.321), grains number per plant (1.155) and grains weight per spike (0.993). The smallest direct influence on grain yield had the following traits: shoot length (0.001), immature tillers number per plans (0.007), sterile spikelets number per spike (-0.010) and spike length (-0.024).

DISCUSSION

The three-year study showed that DH lines yielded similarly to standard Izolda cv. and that differences found in grain yield per plant were insignificant. The average grain yield per plant of DH lines as well as Izolda cv. was determined by the same average productive tillers number per plant. However, the average grain yield per spike in DH lines was significantly different in comparison with standard Izolda cv. The grain yield per spike, in both DH lines and standard Izolda cv. was determined by the following yield-forming factors: fertile spikelets number per spike, grains number per spike and single grain weight. Among the studied spike yield-forming traits - spikelets number per spike, sterile spikelets number per spike and grains number per spikelet were significantly different for DH lines and standard Izolda cv. Other traits - fertile spikelets number per spike, grains number per spike and single grain weight – had similar values, though differences were insignificant between DH lines and standard Izolda cv. This results are opposite to that of Kozdój et al. (2010) in spring barley when grain yield per plant in androgenic DH lines derived from pure cultivar "Scarlett" were compared.

According to literature, winter wheat DH lines derived from wheat anther culture (AC lines) yielded lower or higher in relation to parents used to hybridization (Ma *et al.*, 1999; Guzy-Wróbelska and Szarejko, 2003). AC lines compared with MP lines, derived from wheat with maize crosses, yielded lower (Ma *et al.*, 1999) or higher (Guzy-Wróbelska and Szarejko, 2003). Literature data shows that wheat DH lines (MP and AC lines) differs in grain yield and some other yield parameters – yield-forming factors (Inagaki *et al.*, 1998; Guzy-Wróbelska and Szarejko, 2003; Laurie and Snape, 1990). The variation in grain yield between DH lines and parent cultivars could be related to the origin of AC lines (DH androgenic genotypes) as well as MP lines derived from hybrids of F1, and parental cultivars used to hybridization in these experiments (Ma *et al.*, 1999; Guzy-Wróbelska and Szarejko, 2003).

On the agronomic point of view, grain yield of wheat depends on number of plants per unit area, spikes per plant, spikelets per spike, grains per spikelet, and single grain weight (Rajala *et al.*, 2009). Grain yield of winter wheat homozygous DH lines and standard Izolda cv. was the result of individual organs morphogenetic processes, which are a yield-forming factors of plant (total and productive number of tillers per plant) and spike (spike length, spikelets number, florets and grains number per spike) occurring during plant growth and development phases in vegetation season (Kirby and Appleyard, 1984; Klepper *et al.*, 1998; Porter and Gawith, 1999). In other papers, the number of spikes per square meter is mentioned as the yield component in the grain yield formation of winter wheat (Golba *et al.*, 2013). Total and productive tillers number per plant forms during the tillering phase, whereas spike morphogenesis, which is the basic unit of wheat yielding, as well as in other cereals, occurs during the

shooting to heading-pollination phase. Grains development processes start from complex embryological processes (embryo, endosperm initiation and development, and seed coat development, which are seed key elements), which occur from the pollination to milk stage and then during the grain growth and development phases lasting up to physiological maturity. In literature this period is known as a grain filling phase and it includes the following stages: grain formation, milk development, dough development and ripening (Zadoks *et al.*, 1974; Klepper *et al.*, 1998; Kirby and Appleyard, 1984). The single grain weight is very important yield-forming factor determining the grain yield of individual spike within plant (e.g. Modarresi *et al.*, 2010, Talukder *et al.*, 2013). Hasan *et al.* (2011) showed that final grain weight had a positive and linear relation with carpel weight at pollination stage, irrespectively of the cultivar and grain position in spikelet. Interestingly, among grain dimensions tested by Hasan *et al.* (2011), grain length was the trait, which explained final grain weight.

Weather conditions i.e. thermal and rainfall factors, influence crop plants growth and development, causing quantitative modifications of plants morphological structure, durations of developmental phases and yield-forming factors levels, which determine grain yield per spike and per plant are presented widely in literature (Gut *et al.*, 1996; Kaur and Behl, 2010; Porter and Gawith, 1999; Barnabás et. al., 2008; Modarresi *et al.*, 2010; Orzech *et al.*, 2009; Rajala *et al.*, 2009; Spiertz *et al.*, 2006; Ugarte *et al.*, 2007; Balla *et al.*, 2012, Golba *et al.*, 2013, Talukder *et al.*, 2013). Our results presented in this publication, concerning the impact of variable weather conditions on plant or spike morphological structure, yield-forming factors level as an equivalent to yield components and grain yield per spike and per plant, showed similarities with literature data presented above. Moreover, the effects of high temperature and drought on wheat grain structure in anatomical aspects and on grain mass are also presented in literature (Kaur *et al.*, 2011).

Standard Izolda cv. generated approximately 6 shoots (the average of three years – 5.9) and DH lines generated – 7 (the average of three years – 6.8) during the tillering phase. However, only some of them were productive tillers therefore their amounts were similar (the average of three years – 3.4) to both standard Izolda cv. and DH lines. Laurie and Snape (1990) showed that fertile shoots number in DH plant depends on cultivar donors used in obtaining DH plants and is higher in DH lines derived from Chinese Spring. In case of DH lines derived from Hope cv. the similarities with initial variety was observed. In addition, other literature sources suggest genotypic differences (specific) in wheat shoot number (Rahman *et al.*, 2009). Sakamoto and Matsouka (2004) found that plant shoots number is created by its morphological structure (architecture) and moreover regulation of plant shoots number is possible with genetic manipulation methods.

Our results indicated that variable weather conditions in particular years modified tillers number per plant. High values of coefficient of variation for the productive and immature tillers indicated a strong influence of weather conditions on both plant structure traits. Lower average daily temperatures in combination with lower rainfall levels during October and November of 2007/2008 season, when autumn tillering occurs, were more favourable to generate higher plant shoots number comparing to higher temperature and rainfall levels in the same period of 2006/2007 season. According to the authors cited by Nowicka (1993), in central Poland, the main factor determining winter wheat tillering degree is thermal conditions during autumn vegetation season. According to Nowicka (1993), more favorable for winter wheat yields on rye soils (which were used in the research) are high average daily temperatures in two phases: from emergence to the autumn growth inhibition and from tillering to wax maturity. In central Poland, on rye soils temperature changes from sowing to emergence and from emergence to autumn growth inhibition have a small impact on winter wheat yield. Temperature changes from the beginning of spring growing season to tillering have the highest impact on yield (Nowicka, 1993). Winter wheat yield is also determined by rainfalls (Nowicka, 1993; Panek, 1993). Water deficiency or excess affect the wheat yield in a different way, modifying growth and development, especially yield-forming traits (Panek, 1993; Gut et al., 1996). According to Dzieżyc (1988) the time course from shooting to grain milk maturity phase and duration of tillering phase (Panek, 1993) are critical for crop growth and development, when unfavorable water conditions have an impact on vield.

The plant yield-forming potential is expressed by plant shoots total number. However, the difference between the plant shoots total number and productive tillers number, indicate a structural level of plant yield-forming potential. In this study, higher structural level of yield-forming potential in Izolda cv. (58%) compared to DH lines (50%) was found. Other shoots in both DH lines and Izolda cv. were ontogenetically younger than productive tillers, stopped at various developmental stages and they were recognized as immature tillers in the full maturity phase.

Data presented in this study showed that the winter wheat productive tillers number per plant was significantly and stronger correlated with grain yield (r = 0.71) than plant shoots total number (r = 0.58). Other researchers showed similar relationships in wheat (Aycicek and Yildirim, 2006; Nauroka *et al.*, 2011; Zheng *et al.*, 2011; Gonzales *et al.*, 2011).

Spike length, spikelets total number, fertile spikelets number per spike, spikelets fertility level (expressed as the average grains number per spike), grains number per spike and single grains number are considered as yield-forming factors. In case of DH lines as well as Izolda cv. morphological traits such as spike length, spikelets total number and fertile spikelets number per spike were formed during the spikes and plants development named as stem elongation phase, prior to the heading phase, as shown in the literature (Kirby and Appleyard, 1984; Klepper *et al.*, 1998). Grains number per spikelet and

spike was determined by florets number at pollination phase capable to grain setting, while grain weight – by physiological and biochemical processes occurring while development and maturation phase, which ended in full maturity phase (Kirby and Appleyard, 1984; Klepper *et al.*, 1998). In terms of quantity, weather conditions had an impact on formation of spike yield-forming factors and grain weight. Especially thermal conditions influenced plant growth and development phase duration of both DH lines and standard variety Izolda. Variations coefficients values of described traits were different in research years and they characterized a particular trait in response to complex environmental factors influence. In addition, our study showed that mentioned yield-forming factors were strongly and significantly correlated with grain yield per spike, which is confirmed by high linear correlation coefficients. Similar relationships between grain yield per spike and yield-forming factors in other wheat varieties were shown by other researchers (Aycicek and Yildirim, 2006).

In this paper, a spike yield-forming potential of spikelets per spike total number was considered. Fertile spikelets per spike number, lower than total number of spikelets per spike, indicated used spike yield-forming potential level. The study showed that structural yield-forming potential use level was slightly higher in Izolda (88%) compared to DH lines (83%).

Literature data indicates that temperature increase (heat stress) leads to reduction of grain yield resulting in a smaller number of individuals and different yield-forming factors taking into account by researchers (Balla *et al.*, 2012; Rahman *et al.*, 2009; Modarresi *et al.*, 2010; Spiertz *et al.*, 2006; Ugarte *et al.*, 2007; Sharma et. al., 2008; Hossain *et al.*, 2013, Talukder *et al.*, 2013). Cited authors emphasize the cultivar/genotype differences in terms of grain yield.

High temperature had an impact on earlier development phase, shortening further phases, smaller grain number per spike, less single grain weight and lower grain yield per plant of 10 spring wheat genotypes compared to the controls growing under optimal conditions (Rahman *et al.*, 2009). Average daily temperature impact during the different winter wheat developmental phases has been showed by Orzech *et al.* (2009). Author showed that the correlation coefficients between winter wheat yield and average daily temperature values is highest at the stage of shooting to heading.

Rainfall deficiency (drought) causes the wheat grain yield reduction during the vegetation (Gut *et al.*, 1996; Orzech *et al.*, 2009). Results of field experiments also indicate the diverse response of wheat genotypes to rainfall deficiency (drought) during vegetation expressed by yield reduction per unit of area (Gut *et al.*, 1996; Orzech *et al.*, 2009). Correlation coefficients values calculated by Orzech (2009) between grain yield and rainfall level in each phenophase showed that rainfalls significantly reduced winter wheat yield in only sowing-emergence interphase. The results of experimental studies suggest that drought applied during the plant developmental stage is important factor in yield-forming factors and yield creation (Rajala *et al.*, 2009; Ugarte *et al.*, 2007).

Drought stress application during the intensive floret formation in spikes before pollination (grain potential number determinant), which corresponds to the shooting, resulted in a drastic decrease in fertile floret and then grain per spike numbers, but grain yield per spike was the same as control variant, which was caused by a larger single grain weight (the compensation effect). However drought application during grain filling period resulted in grain yield per spike reduction by reducing only the number of grains (Rajala *et al.*, 2009).

Experimental studies, in which higher temperature and drought were applied together in post-anthesis stage showed a greater reduction of: grain weight per spike, average single grain weight, number of grains per spike, thousand grain weight, shortening of grain filling period and development than each of the two factors applied separately (Kaur and Behl, 2010). Cited authors showed varietal differences in wheat response to applied factors.

CONCLUSION

- The potential structural yield-forming factors, such as number of shoots including productive tillers number, and the total spikelets number including fertile spikelets, number of setting grains per spike and average weight of a single grain of tested DH lines as well as Izolda cv. are created during certain stages of plant growth and development. Obtained values of above mentioned traits were related with varying environmental conditions (air temperature and precipitation) during the whole experiment. In general, plants of DH lines were significantly shorter and they had higher total number of tillers per plant than Izolda cv. Spikes of DH lines were the same length as Izolda, however they contained significantly more sterile spikelets, therefore produced significantly less grains per spikelet. Other traits were comparable between DH and Izolda plants. Based on obtained data we can conclude that variation in analyzed traits between DH lines and standard Izolda was observed every year of experiment. Among studied DH lines, following lines: DH 6/10/06 and DH 10/7/06, DH 9/5/08 and DH 9/13/08 tended to have higher values of yield-forming factors and higher grain yield per plant in comparison with Izolda cv.
- The variation coefficients values of grains number, grain yield, productive and immature tillers number per plant, as well as spike length and total spikelets number per spike were similar for both, DH lines and Izolda cv. Remaining traits values of variation coefficients were unstable in each year of experiment, thus they are hard to interpret.
- Paths analysis for the full model allowed to present occurring relationships using cause path model, which described observed variability of grain yield per plant in R² = 99.3%. In order to determine the impact of morphological structure individual traits on the observed grain yield

per plant path coefficients were marked out. Direct effects analysis allowed to make a conclusion that the most important role in the direct formation of the final winter wheat grain yield play: grains number per spike, grains number per plant and grains weight per spike. Shoot length, immature tillers number per plant, sterile spikelets number per spike and spike length were traits with least influence on grain yield.

- Yield forming potential is expressed by plant shoots total number. In this study, higher structural level of yield forming potential of Izolda cv. comparing to DH lines was found. Spike length, spikelets total number, fertile spikelets number per spike, spikelets fertility level (expressed as the average grains number per spike), grains number per spike and single grain weight are considered as yield-forming factors. In this paper, a spike yield-forming potential of spikelets per spike to-tal number was considered. Fertile spikelets per spike number, lower than total number of spikelets per spike, indicate used spike yield-forming potential level. The study showed that structural yield-forming potential level of use was slightly higher in Izolda cv. comparing to DH lines.
- We also showed that homozygous winter wheat DH lines derived from anthers via androgenesis, analyzed in three years (generations) were insignificantly different from standard Izolda variety, with few exceptions, in terms of plant morphological structure (plants height, plant shoots number, the share of productive tillers in shoots total number), spike (spike length, total spikelets number including fertile spikelets) and grain yield per spike or plant. DH lines studied throughout three years were characterized by a diverse range of variation coefficients for various morphological and yield-forming traits, which determined the yield grain per spike and plant. The observed differences in the above-mentioned traits and final grain yield from spike and plant in winter wheat DH lines between research years resulted from interaction of genetic and environmental (epigenetic effect) factors.
- Conducted analyses showed, that tested traits, yield level and morphological structures of plant and spike of androgenic double haploid lines in comparison with cv. Izolda were diverse. Moreover, obtained results indicate the necessity of research on DH lines selection according to its yield-factor traits and yield level stability. Selected DH lines with desirable traits become objects of further actions in new winter wheat cultivars creation.

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