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Evaluation of DH lines produced from superior F_3 plants and corresponding breeding lines in barley

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Abstract

This study was undertaken to compare doubled haploid (DH) lines produced from high yielding F_3 barley plants selected at two plant densities {i.e., 1.15 plants m^{-2} (PD_1) and 4.61 plants m^{-2} (PD_2)} for two generations (F_2 and F_3) to the F_6 lines produced from the same cross (Niki × Karina) after phenotypic pedigree selection for five generations. These lines were evaluated for three years at farmers' plant density. During the first season (2005-2006) 178 F_6 and 17 DH lines were evaluated in rows using adjacent control. The two parents (Niki and Karina) of the F_1 barley cross were used as controls. Mid- parent heterosis (MP) (% yield as compared to the mean of the two controls) was estimated, and finally 26 pedigree and 6 DH lines exhibiting 45% and 26% or higher MP heterosis, respectively, were selected. In the next growing season (2006-2007) a randomized complete block design was established to evaluate these lines. From the 32 genotypes studied the 29 were superior to the mean of the two parents in grain yield, whereas 18 of them (i.e. 10 from PD_1, 5 from PD_2 and 3 DH lines) exhibited 30% or higher MP heterosis. These 18 genotypes were further evaluated during the third growing season (2007-2008). Finally 9 lines (i.e. 4 PD_1, 3 PD_2, and 2 DH) yielded significantly higher than both of the controls. However, four advanced pedigree lines (2 PD_1 and 2PD_2) yielded significantly higher than the best DH line. It was concluded that a combination of honeycomb early generation selection for two generations (F_2 and F_3) and the production of DH lines from high yielding F_3 plants could be considered as a beneficial alternative approach only in the case where a comparable number of DH lines are produced and evaluated.

Keywords: Early generation, Pedigree selection, F_3 doubled haploid, Grain yield.
Abbreviations: DH-doubled haploid, MP heterosis-% yield as compared to the mean of the two controls, PD_1-Plant Density 1.15 plants/m^2, PD_2-Plant Density 4.61 plants/m^2.

Introduction

The production of pure high yielding lines, by crossing of parent lines and subsequent inbreeding generations, is a difficult and long procedure. However, DH (doubled haploid) production via anther culture technique accelerates the breeding cycle by shortening the time required to attain homozygosity (Jain et al., 1996; Thiemt and Oettler, 2008). Indeed, the production of doubled haploids through androgenesis is a very useful tool for producing high yielding homozygous cereal lines in a relatively short time (Hennawy et al., 2011). In addition, the androgenic barley protocol has been widely improved for years leading to the production of many doubled haploid lines (Forster and Thomas, 2005; Lazaridou et al., 2005, 2011). The question that is open is whether the material produced by doubled haploidy is equivalent to the ones produced by conventional breeding of the same material. In the past decades several studies were conducted comparing the pure lines produced by the conventional breeding methods and doubled haploid method for a range of agronomic characters and reported a comparable outcome in barley (Powell et al., 1986; Caligari et al., 1987: Bjornstad et al., 1993), in wheat (Winzeler et al., 1987) and triticale (Arzani and Darvey, 2002). Furthermore, Arabi and Jawhar (2005) reported that some of the barley DH lines are equipped with resistance to scald, high potential for grain yield and earliness as compared to others. In contrast, the main disadvantage of the doubled haploids produced from F_1 hybrids (F_1DH) is that recombination between loci is limited to a single meiotic event. Hence, Snape and Simpson (1981) recommended the production of DH from later generations. This strategy could be more efficient if selected F_2 or F_3 high yielding plants are used for DH production. Given that honeycomb selection at low plant density can be used effectively for early generation selection of high yielding individual plants in many species (Roupakias et al., 1997; Ntanos and Roupakias, 2001; Batzios et al., 2001; Kotzamanidis and Roupakias, 2004), it could be useful to combine early generation honeycomb selection with DH production. The objective of this study was to compare the DH lines produced from superior F_3 plants selected at low plant density for two generations (F_2 and F_3) with the lines produced from the same material after phenotypic pedigree selection.

Table 1. Yield range of seven families expressed as % MP heterosis, number of progenies evaluated per family, number of progenies selected, and percentage of the progenies selected from each of seven barley families evaluated during the season 2005-06.

Families evaluated*	% MP heterosis range***	No of progenies evaluated	No of progenies selected	Percentage of progenies selected (%)
PD1 (1.4%)**	-43 to +74 (19)****	30	6	20
PD1 (3.2%)	-56 to +66 (16)	29	6	20
PD1 (5.3%)	-45 to +86 (10)	30	2	7
PD2 (1.4%)	-71 to +87 (14)	30	4	13
PD2 (3.2%)	-51 to +64 (21)	30	4	13
PD2 (5.3%)	-45 to +98 (16)	29	4	13
DH	-48 to +34 (7)	17	6	35

*Families derived from PD1 (density 1.15 plant m^{-2}), PD2 (density 4.61 plant m^{-2}) inbred plants, or DH (doubled haploid) plants.
**Numbers in brackets indicate the selection pressure applied for the selection of the best plant family from each plant density.
***MP Heterosis (%) as compared to mean of the two controls (Niki and Karina). ****Numbers in brackets indicate the number of lines evaluated that exceeded in yield the MP value.

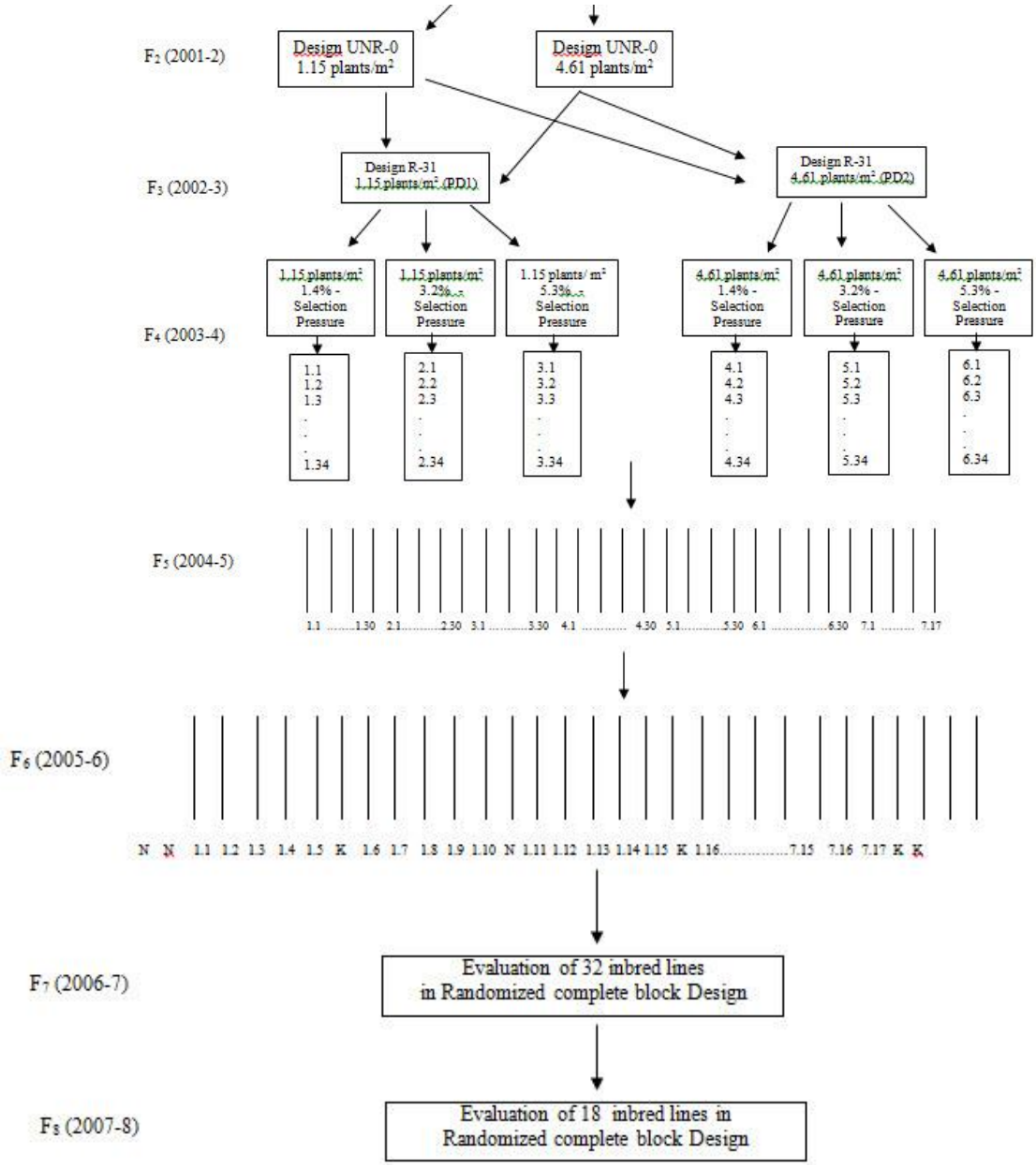

Fig 1. Flow diagram illustrating the handling of the genetic material.

Results

During the season 2005-06 the yield of the 178 F_6 lines ranged from -71 to +98 % as compared to the mean yield of the controls, whereas the corresponding heterosis of the 17 DH lines ranged from -48 to +34% (Table 1). In particular, most (103 vs. 92) of the genotypes studied (i.e., 45 PD_1, 51 PD_2 and 7 DH lines) produced higher yield compared to the mean yield of the two controls. The 26 pedigree lines that exhibited higher than 45% MP heterosis and 6 DH lines with higher than 26% MP heterosis were further evaluated the following growing season. From these lines 14 (+45 to +86% heterosis) were derived from PD_1, 12 (+45 to +98% heterosis) from PD_2, and six (+26 to +32% heterosis) from DH lines (Table 2). Twelve of the 14 superior PD_1 lines were derived from the 1.4% (6 lines) and 3.2% (6 lines) selection pressure, whereas only 2 lines were derived from the 5.3% selection pressure (Tables 1 and 2). On the contrary 4 of the 12 superior PD_2 lines were derived from the 1.4%, 4 lines from the 3.2% and 4 lines from the 5.3% selection pressure (Tables 1 and 2). Finally, the percentage of the pedigree lines selected from each family (or superior F_3 plant originated from each plant density and selection pressure) ranged from 7% to 20% (Table 1). Next growing season (2006-07) the yield of the 32 lines evaluated ranged from -16 to +66% as compared to the mean yield of the controls (Table 2). The 29 of the 32 genotypes evaluated in 2006-07 yielded higher than the two controls (Table 2). The 18 lines (10 derived from PD_1, 5 from PD_2 and 3 from DH lines) that provided higher than 30% MP heterosis were further evaluated the following growing season. Nine out of the 10 PD_1 lines selected in 2006-07 were derived from the 1.4% (4 lines) and 3.2% (5 lines) selection pressure, whereas only one line was derived from the 5.3% selection pressure (Table 2). On the contrary, one of the 5 superior PD_2 lines were derived from the 1.4%, 2 lines from the 3.2%, and 2 lines from the 5.3% selection pressure (Table 2).

During the season 2007-08, the yield of the lines ranged from -10 to +38% as compared to MP yield (Table 3). The 10 of the 18 lines exhibited higher yield than 4% of the mean yield of the two controls. Moreover, 9 of them (i.e., 4 PD_1, 3 PD_2, and 2 DH lines) produced statistically higher yield than the best parent (control). Finally all these lines were early heading. The lines evaluated in 2006-07 and 2007-08 exhibited no significant differences with respect to their plant height, the spike's length and the number of grains per spike. The values of these traits ranged from 95-108 cm in height, from 9.3-11.4 cm in spike's length and from 26-30 grains per spike in 2006-07 and from 98-109 cm in height, from 9.1-11.1 cm in spike's length and from 26-29 grains per spike in 2007-08. Lines exhibited significant differences, however, in both years with respect to their number of fertile tillers per m^2. This number ranged from 296-548 in 2006-07 and from 308-570 in 2007-08 (Tables 2, 3).

Discussion

Using conventional breeding methods, the production of homozygous lines is a long procedure. In contrast, the production of DH lines from an F_1 cross leads rapidly to homozygous lines. Therefore, the genetic variation of a segregating population can be exploited more rapidly, via DH production, than by classical breeding methods such as pedigree selection or single seed descent. However, the production of doubled haploids from an F_1 hybrid limits the opportunity for recombination between loci to a single meiotic event, whereas in pedigree method several rounds of recombination occur (Arzani and Darvey, 2002). Hence, derivation of DH lines from later generation has been recommended (Snape and Simpson, 1981). In other words, one could carry out the selection for two generations (F_2 and F_3) and produce DH lines from high yielding F_3 plants. Then, the breeder could proceed with selection between these lines. Furthermore, Roupakias et al. (1997) concluded that evaluation of the F_1 and F_2 under low plant density and in comparison with the best cultivars in the area, could identify promising faba bean populations in an early generation. These populations could be further advanced successfully by early generation selection of individual plants under low plant density for at least two generations (F_2 and F_3). The same authors suggested that the breeder may further advance his high yielding F_3 material by any effective breeding methodology. Indeed, Kotzamanidis and Roupakias (2004) working with barley reached the conclusion that the combined yield of the two generations (F_1 and F_2) in a honeycomb design at low plant density was effective in predicting yield performance of the F_3 populations in barley. These researchers reported that Niki × Karina was the most promising cross from the six crosses evaluated. The question that is raised at this point is whether, after a two-year-honeycomb selection in segregating populations (F_2 and F_3 generations), it is more productive for the breeder to proceed via phenotypic pedigree selection or via the production of DH lines from the superior F_3 plants.

In the process of our study we ended with 178 F_6 lines originating from six high yielding F_3 plants (six families) (30 progeny lines from each F_3 selected plant, two of the lines were lost in the process) selected at low plant density, and 17 DH lines produced from high yielding F_3 plants (Fig. 1, Table 1). The six selected F_3 plants could have originated, at the most, from six high yielding F_2 plants (Fig.1). The origin of the 10 lines selected after three years of evaluation was one from each of the 5 families PD_1(1.4%), PD_1(5.3%), PD_2(1.4%), PD_2(3.2%), and PD_2(5.3%), 3 from the PD_1(3.2%) family and 2 were DH (Table 4). This indicates that from each of the 5 aforementioned families only 3.3% advanced lines were finally selected, while from the PD_1(3.2%) family 10.3% of the lines were selected and from the DH 11.8% of the lines were selected (Table 4). Overall, the percentage of the selected pedigree lines was 4.5% as compared to 11.8% of the selected DH lines.

Based on these data one could argue that by following the DH process the breeder could, proportionally, end up with more high yielding lines as compared to pedigree phenotypic selection. These results are very promising considering that Luz et al. (2009) reported only 4 out of the 120 (3.3%) barley DH lines were superior to the best parent. However, over the years the selected pedigree lines exhibited higher MP heterosis and wider range in grain yield than the DH lines (Tables 1, 2, 3 and 4). Indeed, although all of the finally selected lines exhibited significantly higher grain yield than the best parent, yet four advanced pedigree lines {i.e. PD_1 (2.24), PD_1 (3.24), PD_2 (4.29), and PD_2 (5.1)} yielded significantly higher than the best DH line (i.e. line 7.2) (Table 3). These findings are in agreement with those reported by Powel et al. (1986) and Caligari et al. (1987) who compared barley lines produced by pedigree inbreeding, doubled haploidy and single seed descent and concluded that random lines should be considered as an alternative to pedigree methods. Our data, however, are quite

Table 2. Yield, MP heterosis, fertile tillers/m^2, and date to heading of 34 barley lines originating from seven families and evaluated during 2006-07.

		2005-06	2006-07			
Line	Family*	MP Heterosis (%)***	Yield (ton ha^{-1})	MP Heterosis (%)	Fertile tillers/m^2	Date to heading
1.20	PD1 (1.4%)**	+62	3.67	+66	382	Mid
2.30	PD1 (3.2%)	+52	3.49	+58	411	Early
2.4	PD1 (3.2%)	+66	3.34	+52	382	Mid
2.24	PD1 (3.2%)	+51	3.33	+51	470	Early
6.12	PD2 (5.3%)	+98	3.27	+49	427	Mid
2.5	PD1 (3.2%)	+51	3.25	+48	454	Mid
1.7	PD1 (1.4%)	+48	3.14	+43	477	Mid
7.10	DH	+26	3.13	+42	478	Early
3.24	PD1 (5.3%)	+86	3.13	+42	430	Mid
7.2	DH	+32	3.03	+37	482	Early
5.1	PD2 (3.2%)	+46	3.02	+37	386	Early
6.13	PD2 (5.3%)	+69	2.98	+35	381	Mid
1.24	PD1 (1.4%)	+65	2.97	+35	484	Early
5.16	PD2 (3.2%)	+43	2.96	+34	520	Early
2.12	PD1 (3.2%)	+45	2.95	+34	292	Early
7.1	DH	+31	2.93	+32	341	Late
4.29	PD2 (1.4%)	+61	2.92	+32	431	Early
1.16	PD1 (1.4%)	+58	2.87	+30	437	Late
5.21	PD2 (3.2%)	+64	2.76	+25	312	Early
4.20	PD2 (1.4%)	+52	2.75	+25	428	Mid
6.22	PD2 (5.3%)	+45	2.74	+24	548	Mid
1.30	PD1 (1.4%)	+74	2.70	+22	537	Early
3.25	PD1 (5.3%)	+47	2.67	+21	372	Early
5.2	PD2 (3.2%)	+52	2.63	+19	445	Mid
6.18	PD2 (5.3%)	+69	2.62	+19	393	Mid
4.27	PD2 (1.4%)	+87	2.53	+15	414	Early
2.15	PD1 (3.2%)	+58	2.52	+14	420	Mid
7.16	DH	+28	2.36	+7	546	Early
1.23	PD1 (1.4%)	+65	2.29	+4	426	Early
Niki (control)	-	-	2.29	-	255	Mid
7.11	DH	+26	2.21	0	296	Late
	-					
Karina (control)	-	-	2.12	-	398	Mid
7.9	DH	+34	1.99	-10	315	Mid
4.10	PD2 (1.4%)	+47	1.86	-16	432	Late
$LSD_{0.05}$			0.32		50	

*Families derived from PD1 (density 1.15 plant m^{-2}), PD2 (density 4.61 plant m^{-2}) inbred plants, or DH (doubled haploid) plants. **Numbers in brackets indicate the selection pressure applied for the selection of the best plant family from each plant density. ***MP Heterosis (%) as compared to mean of the two controls (Niki and Karina).

different than those reported by Winzeler et al. (1987) and Arzani and Darvey (2002). These researchers reported that some doubled haploid lines in wheat and triticale were equal or in some cases better than the lines derived from the early pedigree selection. In addition, Arzani and Darvey (2002) reported that the DH lines exhibited wider ranges in grain yield than the field selected lines. The narrower range in grain yield and the lower productivity of the DH lines, as compared to the pedigree lines, observed in this study could be attributed to the relatively small number of DH lines evaluated. In this case a combination of honeycomb early generation selection for two generations (F_2 and F_3) and the production of a comparable number of DH lines from high yielding F_3 plants could result to a higher percentage of high yielding DH lines with a comparable productivity to the pedigree ones and therefore it could be considered as a valuable alternative approach. If, however, even after the production and evaluation of a comparable number of DH lines, the yielding ability of the pedigree lines will remain superior, then field selection alone might be more beneficial. However, further research is required to verify this issue.

Materials and Methods

Plant material

In a breeding program applied at the Department of Genetics and Plant Breeding of the Aristotle University of Thessaloniki several crosses of barley were evaluated for four years and among them the cross Niki × Karina was reported to be the superior one (Kotzamanidis and Roupakias, 2004). During the growing season 2001-02 a mixture of equal number of F_2 seeds originating from 40 F_1 Niki × Karina plants (Fig. 1) were evaluated under two plant densities (i.e., PD_1 and PD_2 plant

Table 3. Yield, MP heterosis, fertile tillers/m^2, and date to heading of 20 barley lines originating from seven families and evaluated during 2007-08.

Line	Family*	Yield (ton ha^{-1})	MP Heterosis (%)*	Fertile tillers /m^2	Date to heading
4.29	PD2 (1.4%)**	5.87	+38	492	Early
2.24	PD1 (3.2%)	5.50	+29	402	Early
3.24	PD1 (5.3%)	5.27	+24	570	Early
5.1	PD2 (3.2%)	5.18	+22	468	Early
2.30	PD1 (3.2%)	4.87	+15	502	Early
7.2	DH	4.73	+11	556	Early
2.12	PD1 (3.2%)	4.68	+10	482	Early
6.13	PD2 (5.3%)	4.63	+9	483	Early
7.10	DH	4.62	+8	516	Mid
1.24	PD1 (1.4%)	4.44	+4	340	Early
Niki	-	4.32	-	502	Early
1.16	PD1 (1.4%)	4.26	0	493	Late
Karina	-	4.20	-	308	Mid
2.4	PD1 (3.2%)	4.11	-3	537	Early
1.20	PD1 (1.4%)	4.09	-4	468	Mid
2.5	PD1 (3.2%)	4.09	-4	372	Mid
7.1	DH	4.05	-5	520	Late
6.12	PD2 (5.3%)	3.98	-6	580	Late
1.7	PD1 (1.4%)	3.88	-9	412	Late
5.16	PD2 (3.2%)	3.85	-10	484	Mid
$LSD_{0.05}$		0.29	-	71	

*Families derived from PD1 (density 1.15 plant m^{-2}), PD2 (density 4.61 plant m^{-2}) inbred plants, or DH (doubled haploid) plants. **Numbers in brackets indicate the selection pressure applied for the selection of the best plant family from each plant density. ***MP Heterosis (%) as compared to mean of the two controls (Niki and Karina).

Table 4. Lines finally selected after evaluation of 195 lines, their MP heterosis in consecutive years, number of lines evaluated, number of lines finally selected, and percentage of line selected from each family.

Lines finally selected	Family*	MP Heterosis (%)***			No of lines evaluated	No of lines finally selected	Lines selected (%)
		2005-06	2006-07	2007-08			
1.24	PD1 (1.4%)**	+65	+35	+4	30	1	3.3
2.24	PD1 (3.2%)	+51	+51	+29	29	3	10.3
2.30	PD1 (3.2%)	+52	+58	+15	29	3	
2.12	PD1 (3.2%)	+45	+34	+10	29	3	
3.24	PD1 (5.3%)	+86	+42	+24	30	1	3.3
4.29	PD2 (1.4%)	+61	+32	+38	30	1	3.3
5.1	PD2 (3.2%)	+46	+37	+22	30	1	3.3
6.13	PD2 (5.3%)	+69	+35	+9	29	1	3.4
7.2	DH	+32	+37	+11	17	2	11.8
7.10	DH	+26	+42	+8			

*Families derived from PD1 (density 1.15 plant m^{-2}), PD2 (density 4.61 plant m^{-2}) inbred plants, or DH (doubled haploid) plants. **Numbers in brackets indicate the selection pressure applied for the selection of the best plant family from each plant density. ***MP Heterosis (%) as compared to mean of the two controls (Niki and Karina).

density with 1.15 and 4.61 plants m^{-2}, respectively; two unreplicated honeycomb designs). Among them the 30 (15 from each plant density) highest yielding plants were selected (Kotzamanidis et al., 2009). Next growing season (2002-03) two replicated R-31 honeycomb designs were established to evaluate the progeny of these 30 F_2 families (F_3 generation) under the PD_1 and PD_2 plant densities and select the highest yielding plants under three selection pressures (i.e.,1.4, 3.2 and 5.3%). The best F_3 plant (F_4 seeds) from each plant density and each selection pressure was selected {totally 6 F_4 families, i.e. PD_1(1.4%), PD_1(3.2%), PD_1(5.3%), PD_2(1.4%), PD_2(3.2%), PD_2(5.3%)}(Fig. 1). These 6 F_4 families (originating from no more than six different F_2 plants and in the extreme case from only one F_2 plant) were grown in 6 blocks (one family in each block) under farmer conditions the following growing season (2003-04). In this experiment 34 rows were sown in each block and the best spike from each of the 30 middle rows per family was phenotypically selected. Therefore, 180 spikes (30 rows × 1 spike × 6 families) were collected. During the 2004-05 growing season these 180 spikes (30 F_5 lines per family) were sown each spike to one row. Finally 178 lines (F_6 seed) (two lines did not reach maturity) were derived. In addition, the seed of 17 DH plants produced by anther culture of F_3 high yielding barley plants derived from the same cross (Niki × Karina) were sown, each spike to one row, to multiply the seed. The 178 F_6 and the 17 DH lines were evaluated in this study.

Field experiment

A field experiment was conducted at the University Farm of Thessaloniki in northern Greece, in a loam (L) soil (Typic Xerorthent) with pH 7.8 organic matter content 13.4 g kg^{-1}, N-NO_3 38 mg kg^{-1} , P (Olsen) 26 mg kg^{-1} and K 156.6 mg kg^{-1} (0 to 30 cm depth) during 2005-06 growing season. Seedbed preparation included mouldboard plough, disc harrow and cultivator. Nitrogen and P_2O_5 at 80 and 40 kg ha^{-1}, respectively, were incorporated into the soil as diammonium phosphate (20-10-0) before sowing. The 178 F_6 lines and the 17 DH lines were sown within the last week of November and were evaluated by the method of adjacent control (Briggs and Shebeski, 1968). Every fifth row the two parents of the F_1 barley plants (Niki and Karina) were alternately planted as controls (Fig. 1). In particular, 235 rows 6 m long with 0.25 m row spacing were established. The seeding rate for each barley line was 160 kg ha^{-1}. The crop was kept free of weeds by hand hoeing when necessary. Barley was harvested after mid-June and grain yield was adjusted to 13% grain moisture using a grain moisture meter (Wile-35, OT-tehdas Oy Co., Helsinki, Finland). The yield of each line was estimated as % of the mean yield of the two parent controls (MP heterosis) (Mohammadi et al., 2010).

The next growing season (2006-07), 26 F_7 lines (i.e. 14 PD_1 and 12 PD_2) that exhibited higher than +45% 'MP heterosis' (heterosis as compared to the mean of check cultivars), and six DH lines higher than +26% 'MP heterosis' were further evaluated in a randomized complete block design with three replications, in the same field, but in an adjacent site to that of the previous year. Cultivars Niki and Karina were used as controls. All other cultural practices were similar to the ones used during the previous growing season. Grain yield, days to heading, plant height at maturity, number of tillers, length of spike and number of fertile grain per spike were measured. Grain yield was determined by harvesting the middle rows of each experimental plot. The yield of each line was estimated as % of the mean of the two controls.

In the next season 2007-08 15 F_8 (i.e., 10 PD_1 and 5 PD_2) and three DH lines that exhibited higher than 30% 'MP heterosis' were further evaluated in a randomized complete block design with three replications in the same area. The two parents (Niki and Karina) were used as controls. All cultural practices and measurements were similar to the ones used in the previous growing season. MSTAT program was used to conduct the analysis of variance (ANOVA) (Freed, 1994). Treatment mean differences were separated by the least significant difference (LSD) test at the 0.05 probability level.

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