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Full Length Research Paper

Performance of durum wheat (*Triticum durum* L.) doubled haploids derived from durum wheat x maize crosses

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Performance of durum wheat doubled haploids derived from durum wheat x maize crosses and their parents (Jenah khotifa, Biskri, Karim and Razzek) was evaluated on the basis of germination percentage and six morphological traits such as plant height, spikeless tillers/plant, spike tillers/plant, days from sowing to 50% ear emergence, kernels/spike and 1000-kernel weight. The results indicated that the doubled haploid (DH) lines Jenah Khotifa and Biskri showed greater germination percentage with 70% compared to their parent with 60%. However, DH lines derived from crosses of Rezzak and Karim genotypes hybrid with maize had the same behaviour as their parent respectively. Also, the results showed that the DH lines equalled to the control for average height of plants and spikeless tillers/plant. The difference in average days from sowing to 50% ear emergence between DH_1 lines from Biskri and Rezzak and the control was reduced to 5 and 9 days DHs lines showed 1000-kernel weight and spike tillers/plant greater in DH_1 lines than in the control. However, kernels/spike was significantly greater in control compared with DH lines.

Key words: Hybridization, germination percentage, agronomical trait.

INTRODUCTION

The production of doubled haploid (DH) is a highly valuable tool for autogamous species breeding since completely homozygous lines from F1 crosses are obtained in a single generation (Snape and Simpson, 1984). Another advantage is a substantial reduction in the cost and the time required to produce breeding lines by conventional system (Liu et al., 2002).

In wheat (*Triticum aestivum* L.), anther/microspore culture, *Hordeum bulbosum* method and wheat x maize (*Zea mays* L.) hybridization are three frequently used techniques for doubled haploid production (Raina, 1997). However, haploids generated through anther culture occasionally face problems of being aneuploids and albino plants (Wehr and Zeller, 1990). Likewise, the crossability of *Triticum aestivum* × *Hordeum bulbosum* depends on the wheat allelic composition for the *Kr* genes responsible for the incompatibility between these two species (Sitch and Snape, 1987).

Alternatively production of haploid in wheat through its crossing with wheat x maize was reported successful without the development of albino plants (Sadasivaiah et al., 1999; Ushiyama et al., 2007) and insensitivity of maize to the action of crossability inhibitor genes *Kr*, which express in the style of many wheat varieties (Laurie and Bennett, 1988; Lizarazu and Kazi, 1993). In this crossing, wheat is used as female parent, and maize are used as male parents. Several reports demonstrate the success of doubled haploid plant production using maize pollen on hexaploid wheat (Laurie and Reymondie, 1991; Amrani et al., 1993; Bidmeshkipour et al., 2007) but relatively few durum wheat genotypes show such crossability with maize (Savaskan et al., 1997; Almouslem et al., 1998; David et al., 1999; Garcia-Lilamas et al., 2004).

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Poor crossability of durum wheat could perhaps be attributed to its lack of the D genome (Doğamacı-Altuntepe and Jauhar, 2001).

During the development of embryos in crosses only wheat chromosomes are retained and the chromosomes of the male parents are eliminated during the first three divisions of hybrid embryos (Laurie and Bennett, 1988). The viability of these zygotes is low, and most of them abort during the initial stages of development (Laurie and Bennett, 1988). Furthermore, one of the biggest barriers in the wheat × maize crosses is the absence of endosperm (Laurie and Bennett, 1989). Thus, it was demonstrated that haploid wheat plants were regenerated following embryo rescue (Laurie and Bennett, 1986). Spontaneous chromosome duplication has been observed at a low frequency thus artificial chromosome doubling is essential to enhance efficiency and it results in obtaining doubled haploid lines (Laurie and Reymondie, 1991; Riera-Lizarazu et al., 1992; Ahmad and Chowdhry, 2005).

DH production using maize has been studied in detail at several levels: the influence of the genotype (Laurie and Reymondie, 1991; Bitsch et al., 1998; Ayed et al., 2011), pollination techniques (Laurie and Reymondie, 1991; Ayed et al., 2011), and agronomic performance (Laurie and Snape, 1990; Inagaki and Tahir, 1992).

Information on the field performance of lines derived from durum wheat × maize cross is limited. This study was conducted to compare the performance of lines obtained by maize (*Zea mays* L.) pollination and their parent. The DHs and their parent were evaluated by germination test and six morphological characters: plant height, spikeless tillers/plant, spike tillers/plant, days from sowing to 50% ear emergence, kernels/spike and the 1000-kernel weight.

MATERIALS AND METHODS

Donor plants

Four durum wheat genotypes (Jenah khotifa, Biskri, Karim and Razzek) were used as female parents. Durum wheat was planted every 2 weeks in the field under natural growing conditions. A maize (*Zea mays* 2n = 2× = 20) genotype ('Pioneer 37Y15') was used as male parent. Maize plants were grown in pots in an unconditioned greenhouse at temperatures slightly warmer than those outside.

Crosses and embryo rescue

Two or three days before anthesis, anthers from a single wheat were submerged in aceto-carmine (0.1%) solution and observed by photonic microscopy in order to determine the optimal stage of the microspore based on the location of nucleus relative to the microspore pore. Then the apical and basal spikelets of wheat, all florets (except for the two outermost florets) were emasculated and receptive stigmas were pollinated with freshly collected viable maize pollen (Ayed et al., 2011a). The viability of maize pollen was tested before pollination from maize observed by photonic microscopy in aceto-carmine (0.1%). Then the pollinated spikes were covered with a paper bag to maintain humidity after emasculation.

Eighteen days after pollination, the embryos were isolated from caryopses that grew over two thirds of the glume length and were sterilized with 12% bleach for 10 min and washed 3 times with sterilized water. Embryos were placed into Petri dishes with B5 medium containing 20 g/l sucrose and 7.5 g/l agar (Gamborg and Eveleigh, 1968). Embryos were kept in the dark at about 25°C in a growth room until germinated. After further growth, haploid wheat seedlings at the three-leaf stage (1 to 2 cm) were transferred to a bottle with the same medium at a light intensity of 350 µE m^{-2} s^{-1} under a 16-h photoperiod (Ayed et al., 2011b).

Chromosome studies

In our work, we determined the ploidy level using chromosome counts protocol by Jahier et al. (1992) from mitotic cells of root tips grown on development medium. At least two root tips from each seedling were cut to a length of 1 cm, kept in distilled water at 0°C for 24 h, fixed in 3:1 absolute ethanol/glacial acetic acid, then hydrolysed in 1 HCl for 12 min à 60°C. Root tips were stained with 1% aceto-carmine solution and chromosomes were observed after squashing.

Ploidy level was determined before and after submerged of all regenerated plantlets into a solution of 0.1% colchicine treatment for 4 h at 25°C. The treated seedlings were rinsed overnight in running tap water and then placed in pots of soil for growth to maturity.

Transfer of plants to soil

Plants were transferred to sterile peat moss in small pots. They were kept in a lighted growth room at 25°C in a 16 h photoperiod and irrigated daily with Hoagland solution (FAO, 1984) for hardening roots and shoots for 4 to 6 weeks. The number of plants developed was counted one month after embryo rescue.

Germination test

DH_0 seeds collected from the spikes obtained were used to determine the percentage of germination of DHs lines and compared to the control (parent). Germination counts were made daily and were considered to have germinated when the radicle emerged. At the end of the germination period, the germination percentage was calculated using the equation:

Final germination percentage = Number of germinated seeds/ total number of seeds planted

Agronomical traits

DH_1 seeds collected from spikes of DH_0 plants of Jeneh khotifa, Biskri, Rezzak and Karim grown in the growth room were sown in the field. Six morphological traits were evaluated such as plant height, spikeless tillers/plant, spike tillers/plant, days from sowing to 50% ear emergence, kernels/spike and the 1000-kernel weight.

Data analyses

In this study a complete randomized block design was used. Data were analysed by analysis of variance (ANOVA) for each trait measured, using SPSS 10.0 statistical software.

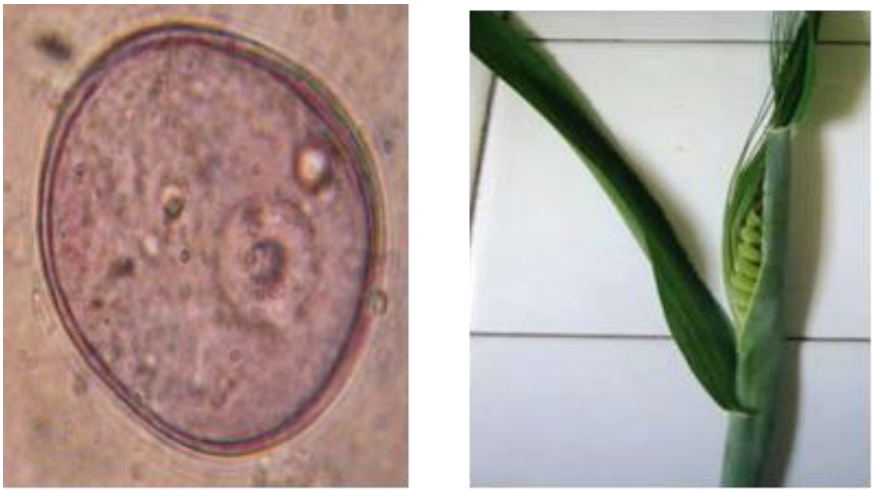

Figure 1. (a) Microspore at binucleate stage; (b) Spike at the corresponding morphological stage of emasculation of durum wheat spike (scale 1/5).

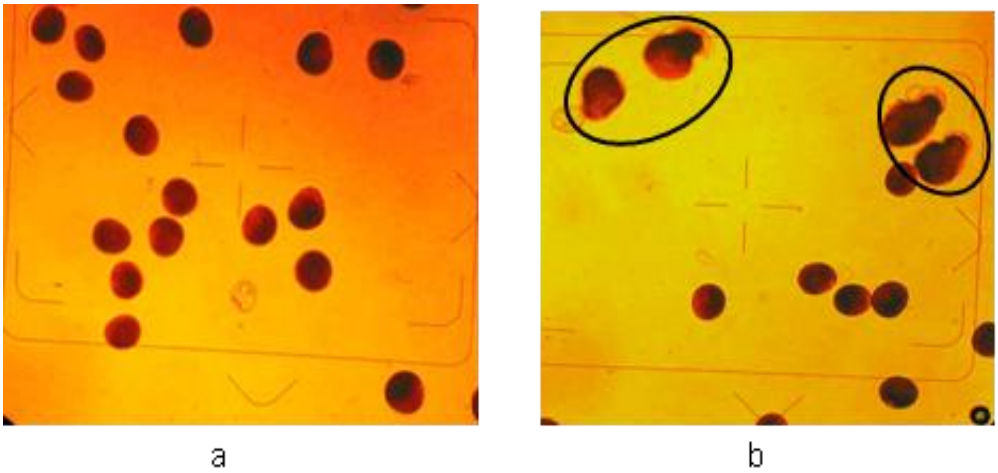

Figure 2. Viability test of maize pollen grain (Gx 200) (a) viable pollen (b) non viable pollen.

RESULTS AND DISCUSSION

The emasculation stage of durum wheat spike was determined by the development stage of the microspore. The staining of microspores from the oldest anthers on a single spike in aceto-carmine has been used to determine the stage of the microspore based on the location of nucleus relative to the microspore pore (Kasha et al., 2001). Spikes were emasculated when microspores were at the binucleate stage (Figure 1a).Subsequently, tillers containing spikes at the desired development stage could be pre-selected on the basis of their morphology (Figure 1b). The viability of maize pollen grain was also tested before pollination. Figure 2a showed spherical form of pollen grain but non viable pollen changed it form to prismatic form (Figure 2b). The deformation observed on the level of the grains of non viable pollen could be explained by the sensitivity of pollen to the dehydration marked by a change of pollen grains shape (Buitink et al., 1996). Aylor (2003) showed that after the release of pollen, its water content decreases with time and that pollen with 10 to 15% of water are not viable.

About 7 days after culture in B5 medium in the dark, the embryo responded to culture with increased volume (Figure 3a). After their exposure to light these embryos showed an abundance of shoots (Figures 3b and c). The developed haploid plantlets were obtained after 4 weeks (Figure 3d). Doubled haploid plants fertile were obtained after treatment with colchicine 0.1% (Figures 3e and f). Ploidy level was tested for haploid plants (Figure 3e) and for colchicine treated plants (Figure 3h). Plantlets survived, were all fertile. None showed any type of abnormality in

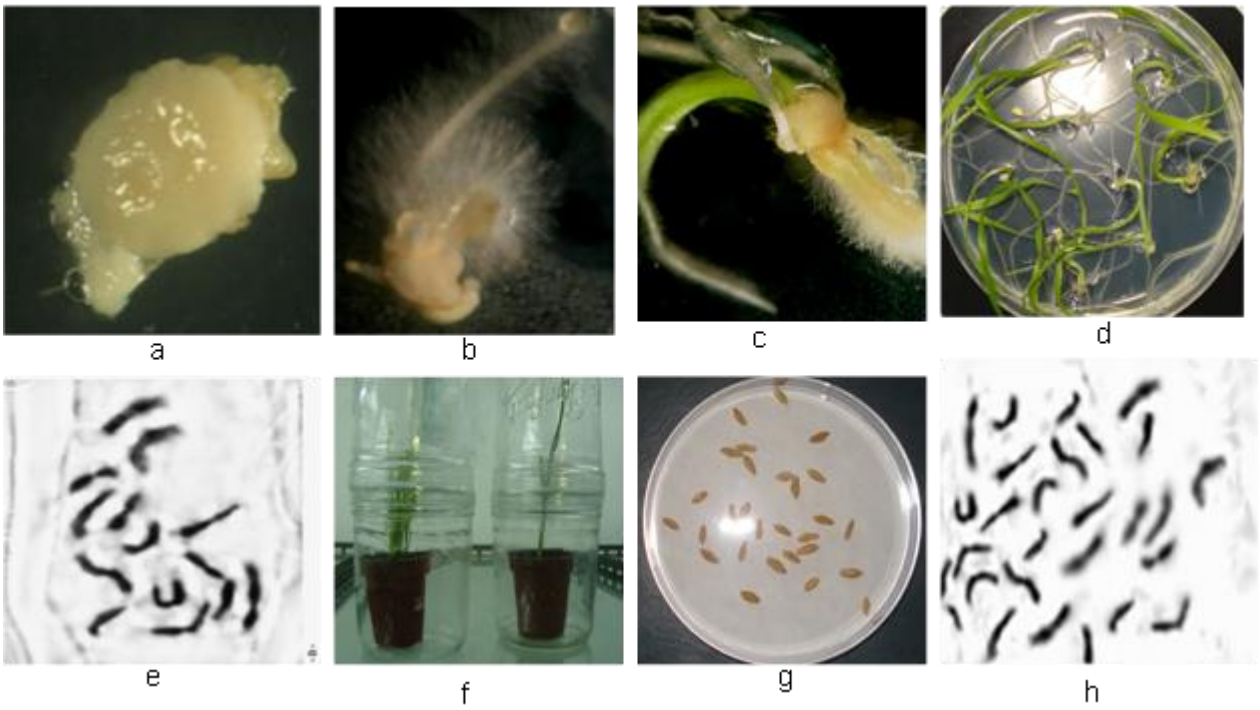

Figure 3. Different stages in durum wheat × maize cross and production of fertile doubled haploids plant (a) embryo after one week of germination (b) two weeks (c) three weeks (d) doubled haploid (e) Test for ploidy level of haploid plants (n=14) (f) doubled haploïd plants (g) seeds obtained by fertile doubled haploids (h) Test for ploidy level of doubled haploid plants (2n=28).

the chromosome number or in the morphology of the plant or of the spike. These plantlets gave spikelet tillers and set seeds (Figure 3g).

Germination percentage

DH_1 seeds collected from separate spikes of DH_0 plants were used to determine germination percentage. After 3 days of germination, the lines Jenah Khotifa showed more significant germination percentage (70%) compared to their parent (60%) as seen in Figure 4a. After 4 days of germination, these lines showed same germination percentage (100%) compared to their parent. Similar results were obtained for Biskri lines which showed percentage of germination about 100% after 4 days of germination against a rate of 90% for the parent Biskri (Figure 4b). After 5 days of germination, these lines showed same germination percentage (100%) compared to their parent. However, DHs lines derived from durum wheat × maize cross and their wheat parent had the same behaviour for Rezzak and Karim genotypes (Figures 4c to d). After 3 days, the (%) of germination for the parents Rezzak and Karim were respectively 80 and 70%. After 4 days, the percentage of germination reached 100% for the two genotypes. These results indicated that DHs plants perform approximately the same behaviour as their wheat parent.

Morphological variations among DHs plants

DH_1 seeds collected from spikes of DH_0 plants of Jeneh khotifa, Biskri, Rezzak and Karim grown in the growth room were sown in the field. The traits measured in this second generation of doubled haploids derived from durum wheat x maize cross are shown in Figure 5.

The comparison of DHs lines and their wheat parent (Jenah khotifa, Biskri, Rezzak and Karim) showed that DHs lines equalled the control for average height of plants (Figure 5b). However, results obtained by Hu and Kasha (1997) showed that the average height of plants was lower in isolated microspore-derived doubled haploids of wheat than that in the seed-derived control.

The difference in average days from sowing to 50% ear emergence between DH1 lines and the control was reduced to 5 and 9 days for DHs Biskri and Rezzak (Figure 5c). Compare these results with data's control. Spike tillers/plant was greater in DH1 lines than in the control (Figure 5f). The DHs lines of Jeneh khotifa showed the best spike tillers/ plant (6) than the parent (4). Figure 5a showed that the spikeless tillers/plant were the same among DHs lines derived from durum wheat × maize cross and their parent. For Jeneh khotifa, DHs lines were showed a 1000-kernel weight significantly better than the control (Table 1). For example, an average of 43.2 g was noted for DHs lines of Jeneh khotifa and 35.1 g for their parent (Figure 5d). However, according Hu and Kasha (1997)

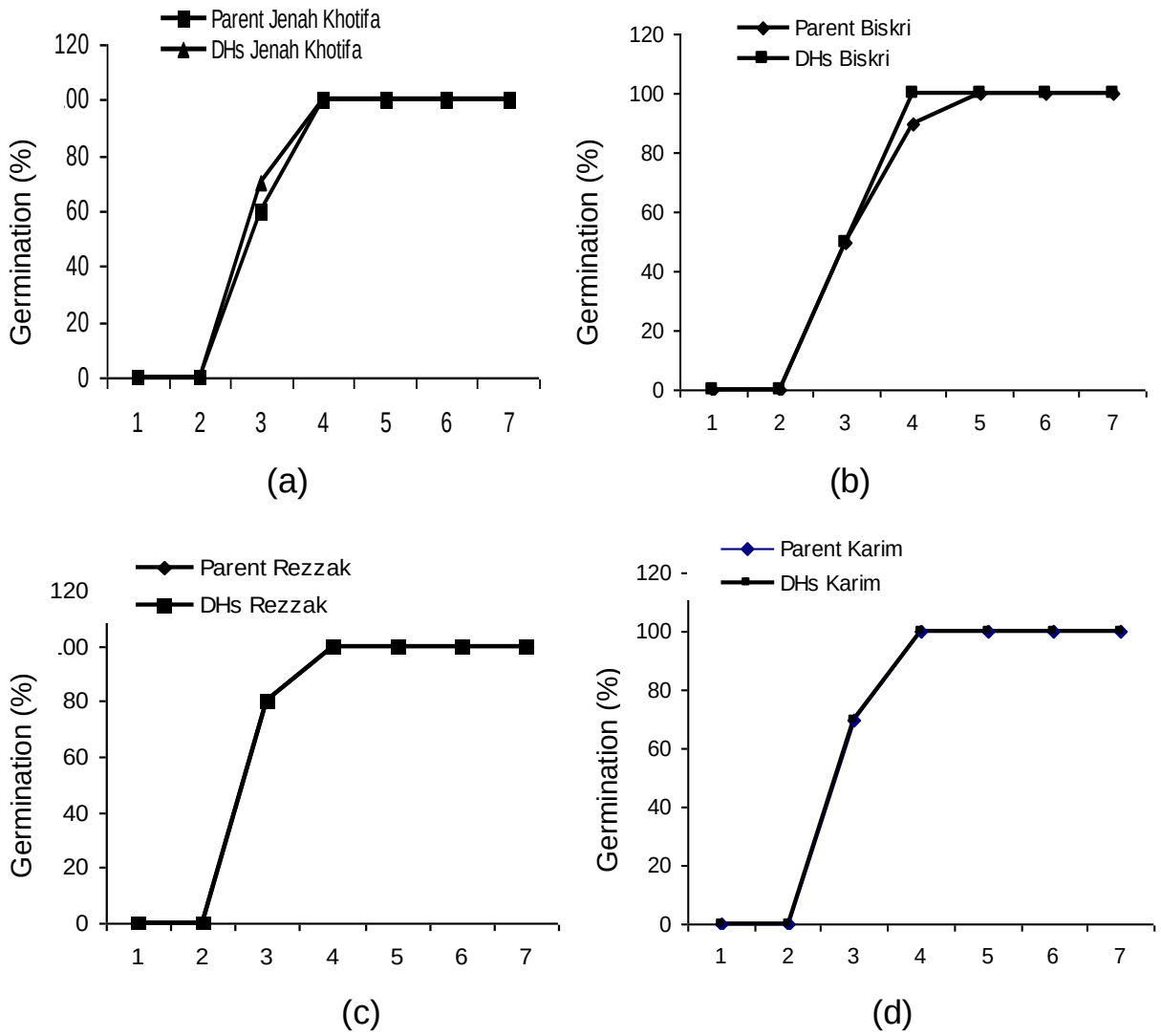

Figure 4. Germination percentage of DHs lines derived from durum wheat x maize cross and their parent (a) Jeneh khotifa (b) Biskri (c) Rezzak (d) Karim.

the 1000-kernel weight was lower in DH1 lines than in the seed-derived control.

Statistics analysis showed also that Kernels/spike was significantly greater in control compared with DHs lines for Jeneh khotifa, Biskri and Razzek (Figure 5e and Table 1). Fedak (1976) evaluated 61 DH lines of barley against three control cultivars and found that 17 DH lines equalled or exceeded the best control for yield. Kasha et al. (1977) found marked heterosis for yield in three barley crosses and some DH lines produced from each cross yielded as high as the F_1 hybrids. Yields of DH lines are mainly dependent on the kernel weight, tiller number and fertility. One possible reason for lower yield in our study was that the testing area per replication (1.2 m^2) may not be large enough to get accurate yield data. Mitchell et al. (1992) reported that DH wheat lines derived from anther culture were lower yielding than those derived from single-seed descent. Generally, anther culture-derived lines were found to be later in heading (Powell et al., 1992), lower in kernel weight (Mitchell et al., 1992) and higher in grain protein content (Bedo et al., 1996) than single-seed-descent-derived lines. Bjornstad et al. (1993) found no differences in the means of biological yield, grain yield and heading date between bulbosum and single-seed-descent-derived lines in barley, but lines from anther culture had a lower grain yield and harvest index and shorter plant height.

Conclusions

These results indicate that DH lines derived from durum wheat x maize crosses have good response to germination test and agronomical potential compared to their parent

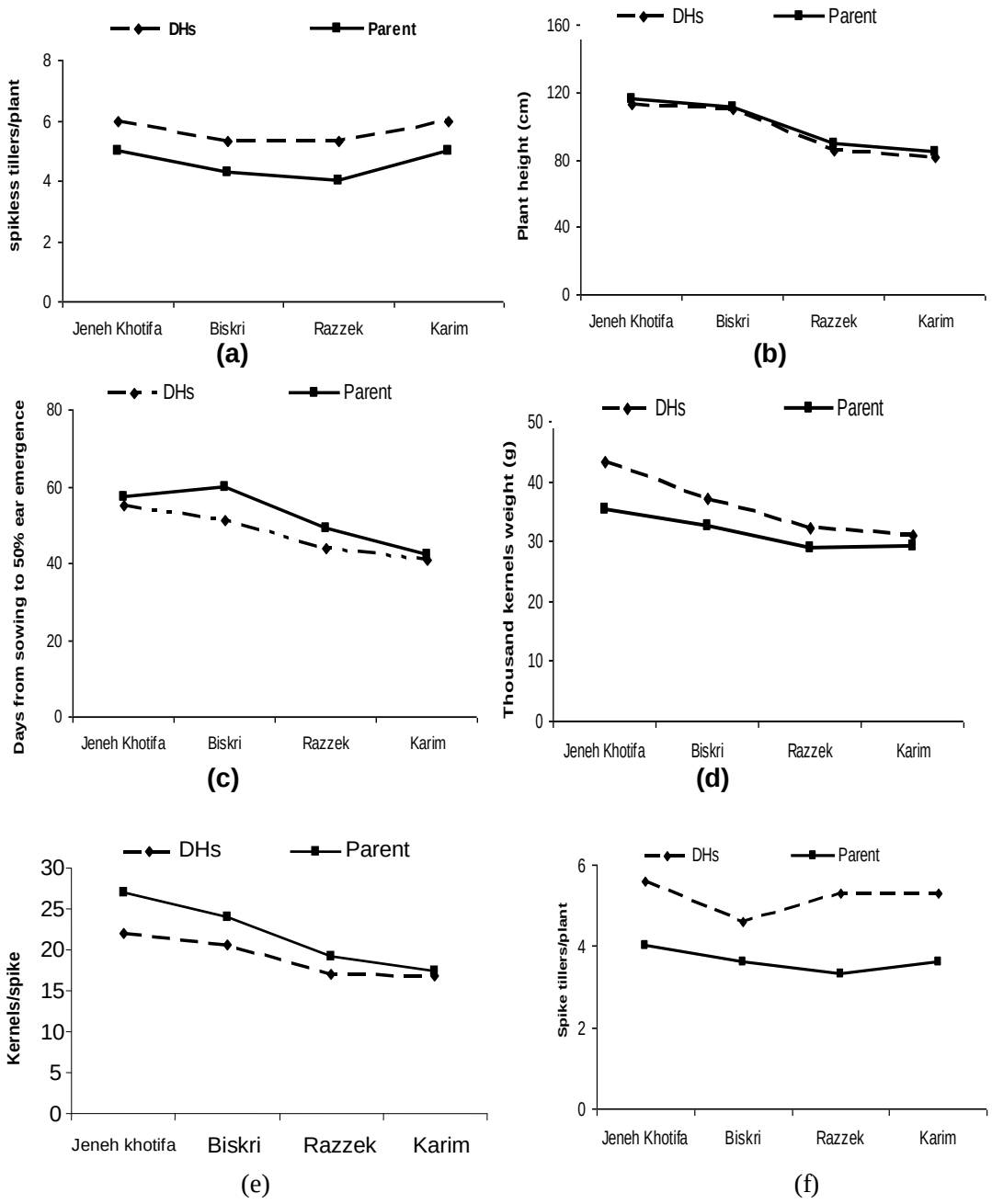

Figure 5. Trait measurements on plants derived from durum wheat x maize cross and their parent (a) Spikeless tillers/plant (b) Plant height (c) Days from sowing to 50% ear emergence (d) Thousand kernels weight (e) Kernels/spike (f) Spike tillers/plant.

Table 1. Variance analysis for six traits for durum wheat doubled haploid lines and their parents.

Variation sources	df	Days from sowing to 50% ear emergence	Spikeless tillers/plant	Kernels/ spike	Plant height	1000-kernel weight	Spike tillers/plant
Genotypes (DH Jeneh khotifa and their parent)	1	6^{ns}	$1,5^{*}$	38*	$12,9^{ns}$	$96,8^{**}$	6^{*}
Genotypes (DH Biskri and their parent)	1	121,5**	$1,5^{ns}$	18**	$1,1^{ns}$	$31,7^{ns}$	$0,1^{ns}$
Genotypes (DH Rezzak and their parent)	1	35,5*	$2,6^{ns}$	8,1*	$13,8^{ns}$	$15,3^{ns}$	6^{*}
Genotypes (DH Karim and their parent)	1	$1,5^{ns}$	$1,5^{ns}$	$0,42^{ns}$	$13,5^{ns}$	$5,04^{ns}$	$4,16^{*}$

ns: non- significant; *, Significantly at $p < 0.05$; **, Significantly at $p < 0.01$.

their parent respectively. These DH lines can be used for applications in plant breeding, *in vitro* selection, plant transformation and genetic studies.

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