Research Article

Evaluation of Various Agro-Morphological and Genetic Parameters of Single Cross Doubled Haploid Maize Hybrids

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Abstract | Maize (*Zea mays L.*) is among the most important crops in the world for its nutritional value and productivity. Skyrocketing populations demand more food to consume without expansion in agricultural land which can be achieved by utilizing hybrid vigor. A field trial during 2018 was conducted at the University of Agriculture Faisalabad to screen out the best single cross doubled haploid hybrids of maize using eleven doubled haploid single cross hybrids. A randomized complete block design was used to conduct the experiment with three replications under normal environmental conditions. Data for different growth, yield, and agro-morphological parameters were analyzed at a 5% probability of the HSD test. Significant differences were present among the doubled haploid maize hybrids for all the studied agro-morphological parameters. Grain's yield showed the highest positive and significant genotypic correlation with plant height (r = 0.784) followed by number of leaves per plant (r = 0.715). Grains yield also showed the highest positive and significant phenotypic correlation with plant height (r = 0.469) followed by total biomass (r = 0.431). Number of grain rows per cob (r = 2.662) exhibited the highest positive direct effect on grain yield per plant followed by total biomass (r = 1.993) and 100 grains weight (r = 1.194). Among the hybrids understudy, DH-8XDH-6 showed the best performance in Plant height (196.67 cm), Leaves per plant (14), total biomass per plant (440.91 g), cob length (18.169 cm), 1000 grain weight (40.47 g) and grain yield per plant (274.92 g), thus is recommended for further production and research.

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Introduction

Maize ranks 3rd among major cereals in Pakistan for its cultivation area and productivity. It is classified in family Poaceae having chromosomes (2n=20) and is extremely cross-pollinated. Maize yield has been increased in Pakistan, over time, because of hybrid seeds cultivation (Ali *et al*., 2020).

In order to meet the global demand for maize, we need to double its production by 2050. So, we need to minimize the time required for maize inbred lines production, for which doubled haploid technology have huge advantage over conventional methods. Difference between doubled haploid maize hybrids and conventional maize hybrids production is utilization of different inbred line production methodology. In doubled haploid maize hybrids, the inbred lines have been developed by doubled haploid technology within three cropping seasons but in convention hybrid, the inbred lines have been developed by selfing of each line for 7-8 generations (Khakwani *et al*., 2019). The data obtained demonstrate that hybrids derived from doubled-haploid lines, in addition to accelerating the time to obtain new cultivars, enable the development of hybrids with superior agronomic performance (Lourençoni *et al*., 2021).

It is recommended that maize doubled haploid lines should be produced. The traditional methodology of making maize hybrid involved the formation of inbred-lines by selfing till 6-7 successive generations and succeed by testcrosses to find out the finest combining ability by allelic-interaction. A speedy substitute to attain inbred lines was by induction of haploidy to produce haploid and following by chromosome doubling convert them to doubled haploids.

Popularity of this technique is owing to short duration for breeding by developing homozygous lines at pace (2-3 generations only) when compared to traditional methods for achieving 99% homozygosity which usually take 6-8 generation (Chaikam *et al*., 2019; Meng *et al*., 2021). Mostly, colchicine (chromosome doubling agent) has been used but pendimethalin herbicide (0.66 ppm) is an attractive and cost-effective alternative of colchicine (Aslam *et al*., 2017). Developing countries such Pakistan that cannot afford costly haploid inducer lines can work on Stock6 and other cheap sources available free of cost and can develop their own haploid inducer lines well adapted to their own climatic conditions (Khakwani *et al*., 2015).

Plant height (PH) and ear height (EH) are tendencies of interest when adapting germplasm as they may be intently related to flowering time, dry matter, lodging resistance, and yield (Durand *et al*., 2012; Tang *et al*., 2006). Doubled Haploid (DH) lines act as extraordinarily effective equipment in practical maize breeding and genetic research. Outcomes confirmed that the usage of DH test crosses give higher performance as compared to hybrids which are commercially available (Beyene *et al*., 2011). Among the potential uses of DH lines are the development of inbred lines, genomic selection (GS), quantitative trait loci (QTL) mapping, and unlocking new genetic variations (Maqbool *et al*., 2020). Main benefits of DH-lines are (i) increase the genetic variance from the 1st generation amongst strains for in step *per se* and testcross performance; (ii) decreased duration of breeding-cycle; (iii) Perfect contentment of distinctness, uniformity, and stability (DUS) achievement for agreeable varietal grading; (iv) decreased fees of breeding maintenance; (v) basic logistics; and (vi) enhanced success in the marker-assisted-selection (MAS), introgression of gene, and genetic gain as compared to pedigree lines (Sserumaga *et al*., 2016). Odiyo *et al*. (2014) suggested supremacy in overall performance through DH-hybrids. Nowadays, doubled haploid technology is frequent technique in business of hybrid maize breeding around the World (Gupta *et al*., 2022).

Pakistan usually imports hybrid maize seed which proves to be highly expensive. It is mandatory to develop such breeding methods which were cost-effective. That is why current research was conducted with the objective--to produce own hybrids for best and sustained maize production in the country.

Material and Methods

Experiment location

The following research study was conducted in the research fields of Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad.

Plant materials

Eleven doubled haploid single cross maize hybrids for the current experiment were obtained from Tissue Culture Lab Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad which named as DH-2XDH-1, DH-5XDH-1, DH-27XDH-26, DH-14XDH-11, DH-8XDH-6, DH-23XDH-21, DH-17XDH-16, DH-40XDH-36, DH-38XDH-3, DH-3XDH-1, and DH-33XDH-31.

Climate data

Average values of different climate's factors of experimental area are shown in Table A.

Table A:

Months	Temperature (°C)			Relative humidity (%)	Rain Fall (mm)	Pan Evaporation (mm)	Sunshine Radiation (Hours)	ET_o (mm)	wind Speed (Km/h)
	Max.	Min.	Avg.						
August	39	29.5	34.2	67.4	5.4	6	8.5	4.2	4.5
September	37.2	25.6	31.4	65.1	41.7	4.8	8.7	3.4	4.3
October	32.6	18.7	25.6	64.4	0.0	3.2	0.8	2.4	3.6
November	27	12.4	19.7	74.6	0.6	1.9	6.9	1.4	3.4

All data were collected from Meteorological Cell of University of Agriculture Faisalabad.

Experimental design

These hybrids were cultivated using a randomized complete block design (RCBD) with three replications on ridges with maintaining 20 cm and 70 cm distance between plants (P×P) and rows (R×R), respectively. The sowing time was 8th of august during kharif season of 2018. These cultivated hybrids were harvested on 22nd of November 2018.

Cultural practices

The land (moist soil) was prepared for sowing by three applications of tined cultivator followed by planking, 70 cm wide ridges (East-west) were made by the help of the ridger. The seeds were treated by Chlorostrobin™ 56 SC (accordingly, 9g/kg) and sown by hand manually (Choka) followed by light irrigation application. For the fertilizer application, complete recommended dose of DAP applied during land preparation at sowing time and recommended urea dose were split into three doses first dose applied on 5-6 leaves stage, second on 8-10 leaves stage and third on 15 days before flowering. The weeds were control by application of herbicide GENGWIE™ 550 SC (Mesotrione + Atrazine) after 30 days of emergence. Total 10 irrigation were applied, first after 12 days of emergence then remaining applications were applied with 7-8 days difference and last application applied 30 days before harvesting.

Date observation

Various agro-morphological parameters were recorded. Plant height (cm), Leaf area (cm^2), Leaves per plant, Flag leaf angle, Leaf temperature (°C) were recorded before harvest and Cob length(cm), Cob diameter (mm), Number of grain rows per cob, Grains per grain row of cob, 100 grain weight (g), Grain yield per plant (g) and Total biomass were recorded after harvest.

Data for plant height and cob length are recorded with measuring tape, whereas Vernier caliper are used for cob diameter measures. Leaf temperature is recorded with an infrared thermometer. Number of grains per rows, grain rows per cob and number of leaves per plant calculated by manual counting. 1000 grain weigh, grain yield per plant and total biomass was recorded by using electrical weight balance. Leaf area was calculated with the following formula:

$$Leaf\ area\ (cm^2) = (length \times width)\ 0.74$$

Statistical analysis

Data for different growth, yield, and agro-morphological parameters was analyzed utilizing ANOVA and the pairwise comparison HSD test at a 5% probability which was computed by Statistix 8.1 software. Heritability, Genetic Advance, Correlation and path analysis was computed by R 4.1.2 software.

Results and Discussion

Growth parameters

Data for plant height (Table 1) showed significant differences among all doubled haploid maize hybrids under study. The highest plant height (196.67 cm) was recorded from maize hybrids DH-8XDH-6. Minimum plant height (144.5 cm) was found in DH-2XDH-1 (144.5 cm). For plant height, percentages of heritability (broad-sense) and genetic-advance were recorded as 72.47% and 24.39%, respectively.

Significant differences among all doubled haploid maize hybrids were observed from data analysis for No. of leaves. Data recorded is provided in (Table 1). DH maize hybrids DH-8XDH-6 produced maximum leaves (14), while DH-38XDH-3 produced the minimum leaves per plant (10). For no. of leaves per plant, percentages of heritability (broad-sense) and genetic-advance were recorded as 42.72% and 1.188%, respectively.

Table 1: *Growth parameters.*

Hybrids	Plant Height (cm)	Leaves per plant	Leaf temperature (°C)	Flag leaf angle	Leaf area (cm²)	Total biomass (g)
DH-8XDH-6	196.67 a	14 a	13.5 ab	50.00 abc	107.84 cd	440.91 a
DH-17XDH-16	184.33 ab	12 ab	11.7 ab	40.00 cde	117.17 cd	447.01 a
DH-3XDH-1	176.33 abc	11 ab	13.667 ab	48.33 abcd	43.66 d	446.01 a
DH-33XDH-31	169.50 abcd	11 ab	9.567b	41.66 bcde	142.80 bcd	374.26 bc
DH-38XDH-3	167.33 abcd	11 ab	15.633 a	55.00 a	115.88 cd	369.47 bcd
DH-14XDH-11	166.17 bcd	12 ab	10.067 b	36.66 de	168.97abc	383.56 b
DH-23XDH-21	156.00 bcd	10 b	14.4 ab	53.33 ab	95.95 cd	351.11 cde
DH-5XDH-1	150.00 cd	10 b	15.567 a	34.33 e	269.61 a	342.14 def
DH-27XDH-26	145.33 d	11 ab	12.533 ab	43.33 abcde	197.70 abc	327.56 ef
DH-40XDH-36	145.33 d	10 b	11.567 ab	42.67 abcde	87.54 cd	320.90 f
DH-2XDH-1	144.50 d	11 ab	15.333 a	43.33 abcde	252.2 ab	322.39 ef
HSD value	29.726	3.5262	5.0299	13.269	116.62	29.66
Coefficient of variance (CV)	6.16	10.28	13.07	10.13	27.20	2.68

All values are significant at $p<0.05$

Significant differences in leaf temperature were noticed from different maize hybrids (Table 1). Maximum leaf temperature (15.63 °C) was recorded from maize hybrids DH-38XDH-3 followed by DH-5XDH-1 (15.56 °C). Whereas the minimum leaf temperature (9.56 °C) was observed in hybrid DH-33XDH-31 For leaf temperature, percentages of heritability (broad-sense) and genetic-advance were recorded as 55.46% and 2.496%, respectively.

Among the maize hybrids sown, DH-38XDH-3 reflected the highest leaf angle (55.00°) followed by DH-23XDH-21 (53.33°), while hybrid DH-5XDH-1 showed the minimum leaf angle (34.33°). For flag leaf angle, percentages of heritability (broad-sense) and genetic-advance were recorded as 64.29% and 2.49%, respectively.

Maize hybrid DH-5XDH-1 provided the highest (269.61 cm²) whereas, maize hybrids DH-33XDH-31 produced the lowest leaf area (43.66 cm²) among the used hybrids. For leaf area, percentages of heritability (broad-sense) and genetic-advance were recorded as 73.83% and 100.472%, respectively.

Yield Parameters

Data recorded for all yield-related parameters is presented in (Table 2) significant variation. DH-17XDH-16 produced the maximum biomass per plant (447.01g) followed by DH-33XDH-31 (446.01g), while maize hybrids DH-38XDH-3 was lowest in producing biomass (320.9g). For total biomass, percentages of heritability (broad-sense) and genetic-advance were recorded as 95.94% and 84.338%, respectively.

The highest cob length (20.06 cm) was noticed from maize hybrids DH-23XDH-21, whereas the lowest cob length (15.49 cm) was observed from maize hybrid DH-38XDH-3. For cob length, percentages of heritability (broad-sense) and genetic-advance were recorded as 85.49% and 2.541%, respectively.

Cob diameter also showed significant variations among maize hybrids cultivated. Significantly, the maximum cob diameter (63.33 mm) was attained from hybrid DH-33XDH-31, while the minimum cob diameter (43.74 mm) was depicted from hybridDH-14XDH-11. For cob diameter, percentages of heritability (broad-sense) and genetic-advance were recorded as 74.45% and 8.22%, respectively.

In the case of No. of grains per row, maize hybrid DH-33XDH-31 was significant over other hybrids with (20) grain rows. While maize hybrids DH-38XDH-3 retained the lowest grain rows (12). No. of grain rows per cob, percentages of heritability (broad-sense) and genetic-advance were recorded as 81.85% and 3.453%, respectively.

Grains per row also showed significant differences among maize hybrids with DH-23XDH-21 indicating

Table 2: *Yield parameters.*

Hybrids	Cob length (cm)	Cob diameter (cm)	Number of grain rows per cob	Grains per grain row of cob	100 Grain weight (g)	Total grain yield per plant (g)
DH-8XDH-6	18.16 AB	50.52 BCD	16 B	36 AB	40.47 A	274.92 A
DH-17XDH-16	17.10 B	46.287 BCD	14 BC	34 BC	28.66 C	228.85 ABCD
DH-3XDH-1	15.70 B	54.437 AB	16 BC	31 BC	29.49 BC	212.82 BCD
DH-33XDH-31	17.00 B	63.333 A	20 A	34 BC	33.66 ABC	263.55 AB
DH-38XDH-3	16.08 B	53.057 BC	14 BC	32 BC	33.77 ABC	256.58 ABC
DH-14XDH-11	16.34 B	43.743 CD	14 CD	32 BC	33.77 ABC	256.64 ABC
DH-23XDH-21	20.06 A	48.827 BCD	16 B	40 A	30.46 BC	254.31 ABC
DH-5XDH-1	18.11 AB	50.8 BCD	16 B	36 AB	34.98 ABC	201.8 CD
DH-27XDH-26	17.69 AB	46.287 BCD	14 BC	35 ABC	37.50 AB	194.08 D
DH-40XDH-36	15.49 B	42.9	12 D	30 C	27.88 C	172.36 D
DH-2XDH-1	17.35 B	49.39 BCD	14 BC	35 ABC	30.08 BC	215.71 BCD
HSD value	2.7034	9.3510	3.0103	5.5088	8.0197	56.977
Coefficient of variance (CV)	3.683	6.35	6.48	3.30	8.29	8.40

All values are significant at p<0.05

Table 3: *Genetic advance of various physio-genetic parameters.*

Physio-genetic parameter	Plant height	Number of leaves	Leaf temperature	Flag leaf angle	Leaf area	Total biomass	Cob length	Cob diameter	Number of grain rows per cob	Grains per grain row of cob	100 grains weight	Total grain yield per plant
Genotypic variation	267.64	1.066	3.624	36.472	4413.83	2393.42	2.438	29.310	4.703	12.096	12.865	981.423
Phenotypic variance	369.29	2.497	6.535	56.727	5978.23	2494.67	2.852	39.368	5.745	13.400	20.264	1354.87
Environmental variance	101.64	1.430	2.910	20.254	1564.39	101.249	0.413	10.058	1.042	1.309	7.398	373.446
Broad sense Heritability	0.724	0.4272	0.554	0.642	0.738	0.959	0.854	0.744	0.818	0.902	0.639	0.724
Genetic advance %	24.512	1.188	2.496	8.523	100.472	84.338	2.541	8.222	3.453	5.815	5.030	46.927

the highest grains per row (40). While maize hybrid DH-38XDH-3 confined the least grains per row (30). For grains per row, percentages of heritability (broad-sense) and genetic-advance were recorded as 90.23% and 5.81%, respectively.

Significantly, the maximum 100 grains weight (40.46 g) was recorded from maize hybrids DH-8XDH-6, whereas DH-38XDH-3 was lowest in grain weight with (27.88 g). For 100 grains weight, percentages of heritability (broad-sense) and genetic-advance were recorded as 63.49% and 5.030%, respectively.

Results for grain yield per plant were also significant among maize hybrids. The maximum grain yield (274.92 g plant^{-1}) was received from maize hybrid DH-8XDH-6. In contrast, maize hybrids DH-38XDH-3 was the lowest in producing grain yield (172.36 g plant^{-1}). For Grain yield per plant, percentages of heritability (broad-sense) and genetic-advance were recorded as 72.43% and 46.927%, respectively.

Genetic advance

Agro-morphological parameters having higher genetic advance with higher broad sense heritability, are most favorite for selection and also for future study and improvements. In this research we have selected five agro-morphological parameters which are having positive and higher genetic advance with higher broad sense heritability as compared to other agro-morphological parameters (Table 3). These selected parameters are Leaf Area (GA= 100.472%, H^2=73%), Total biomass (GA= 84.338%, H^2=95.9%), Plant height (GA= 24.512%, H^2=72.4%), total grain yield per plant (GA= 46.927%, H^2=72.4%) and 100 grains weight (GA= 5.030%, H^2=63%). Selection on the bases of leaf area, total biomass, pant height, total grain yield per plant and 100 grains weight will be beneficial because they have high heritability and high genetic advances. It will help in selection of improved and best performed under study doubled haploid single crossed maize hybrids. The reported findings of this research have similarity with the findings of Yahaya *et al*. (2021) and Mohammedali *et al*., (2021).

Further we will discuss correlation analysis and path analysis for theses parameters for selection of the best single cross doubled haploid hybrids.

Correlation analysis

Correlation among selected agro-morphological parameters: Plant height was positively revealed positive phenotypic correlation with no. of leaves per plant, biomass, and grain yield, but the non-significant phenotypic correlation with leaf angle, grain rows per cob, cob diameter and 100 grains weight were non-significant (Table 4). For genotypic parameters, correlation of plant height with no. of leaves per plant, biomass, and grain weight. Positive correlation of these parameters with plant height is an indication of plant selection for higher yield on height basis. Genotypic correlation of plant height observed was negative with leaf area but non-significant positive with flag leaf angle, grain rows per cob, cob diameter, and 100 grains weight. Genotypic correlation of plant height was significantly negative with leaf temperature, grains per row and cob length (Table 5). comparable outcomes were stated by İlker (2016), Munawar *et al*. (2013) and Reddy *et al*. (2013).

Phenotypic correlation of leaf area was non-significant positive with leaf temperature, grain rows per cob, cob length, grains per row, and grains weight. Leaf area showed significantly negative phenotypic correlation with leaf angle, plant height, and total biomass. Leaf area exposed negative but non-significant phenotypic correlation with cob diameter, no. of leaves per plant and grain yield (Table 4). Positive and significant correlation (genotypic)of leaf area was reflected with 100 grains weight. So, a significantly positive association of this parameter with leaf area visualized the benefits of doubled haploid selection for higher yield on leaf area basis. Leaf area disclosed non-significant but a positive genotypic correlation with cob length, leaf temperature, grain rows per cob, and gains per row of cob. Highly significant but negative genotypic correlation of leaf area with flag leaf angle, plant height, and total biomass was noticed. Leaf area showed non-significant and a negative genotypic correlation with cob diameter and grain yield (Table 5). Similar results have been presented by Sharifai *et al*. (2006), Nataraj *et al*. (2014) and Adesoji *et al*. (2015).

Total biomass showed positively significant phenotypic correlation with plant height, leaves per plant, and grain yield but the positive and non-significant correlation (phenotypic) was recorded with cob diameter, flag leaf angle, grain rows per cob, and 100 grains weight. Total biomass exhibited negative and significant correlation (phenotypic) with leaf area and non-significantly negative phenotypic correlation had been found with leaf temperature, cob length, and grains per rows of cob (Table 4). Total biomass disclosed significantly positive genotypic correlation with plant height, leaves per plant, and grain yield. Positive and significant association of these parameters with total biomass indicated biomass-based selection would be productive for developing maize doubled haploid hybrids with better yield. But flag leaf angle, cob diameter, and 100 grains weight were non-significantly but positively genotypically correlated with total biomass. Total biomass possessed negative but highly significant genotypic correlation with the leaf area but negatively non-significant genotypic correlation with leaf temperature, cob length, and gains per rows of cob (Table 5). Results are similar to that reported by Sharifai *et al*. (2006), Raghu *et al*. (2011) and Adesoji *et al*. (2015).

100 grains weight disclosed positive and highly significant phenotypic correlation with no. of leaves per plant and positively significant with cob length and grain yield. 100 grains weight showed a non-significant but correlation with leaf temperature, leaf area, plant height, total biomass, cob diameter, grain rows per cob, grains per rows of cob. 100 grains weight exposed negative phenotypic correlation with flag leaf angle (Table 4). 100 grains weight showed positive

Table 4: *Phenotypic correlation among physio-genetic parameters.*

Physio-genetic parameters	Plant height	Number of leaves	Leaf Temperature	Flag leaf angle	Leaf area	Total biomass	Cob length	Cob diameter	Number of grain rows per cob	Grains per grain row of cob	100 grains weight	Total grain yield per plant
Plant height	1.000	0.750**	-0.159 n.s.	0.227 n.s.	-0.481**	0.856**	-0.012n.s.	0.230 n.s.	0.195 n.s.	-0.157 n.s.	0.202 n.s.	0.469**
Number of leaves		1.000	0.04336 n.s.	0.141 n.s.	-0.195 n.s.	0.620**	0.071 n.s.	0.118 n.s.	0.140 n.s.	-0.014 n.s.	0.449**	0.353*
Leaf temperature			1.000	0.306 n.s.	0.163 n.s.	-0.133n.s.	0.204 n.s.	0.119 n.s.	0.080 n.s.	0.203 n.s.	0.071 n.s.	-0.055 n.s.
Flag leaf angle				1.000	-0.480**	0.151 n.s.	0.160 n.s.	0.149 n.s.	0.060 n.s.	0.087 n.s.	-0.055n.s.	0.295 n.s.
Leaf area					1.000	-0.523**	0.193 n.s.	-0.075n.s.	0.129 n.s.	0.240 n.s.	0.234 n.s.	-0.162 n.s.
Total biomass						1.000	-0.132n.s.	0.190 n.s.	0.144 n.s.	-0.201 n.s.	0.070 n.s.	0.431*
Cob length							1.000	0.035 n.s.	0.479**	0.956**	0.340*	0.279 n.s.
Cob diameter								1.000	0.822**	0.015 n.s.	0.146 n.s.	0.327 n.s.
Number of grain rows per cob									1.000	0.470**	0.283 n.s.	0.419*
Grains per grain row of cob										1.000	0.286 n.s.	0.239 n.s.
100 grains weight											1.000	0.375*
Total grain yield per plant												1.000

'' = significant at 5% probability level, '**' = significant at 1% probability level, 'n.s.' = non-significant.*

Table 5: *Genotypic correlation among physio-genetic parameters.*

Physio-genetic parameter	Plant height	Number of leaves	Leaf Temperature	Flag leaf angle	Leaf area	Total biomass	Cob length	Cob diameter	Grain rows per cob	Grains per grain row	100 grains weight	Grain yield per plant
Plant height	1.000	0.929**	-0.237n.s.	0.270 n.s.	-0.559**	0.997**	-0.033 n.s.	0.313 n.s.	0.280 n.s.	-0.134 n.s.	0.329 n.s.	0.784**
Number of leaves		1.000	-0.133 n.s.	0.317 n.s.	-0.342*	0.934**	0.056 n.s.	0.186 n.s.	0.146 n.s.	-0.043 n.s.	0.587**	0.715**
Leaf temperature			1.000	0.509**	0.309 n.s.	-0.216 n.s.	0.254 n.s.	-0.091 n.s.	-0.020 n.s.	0.276 n.s.	0.004 n.s.	-0.165 n.s.
Flag leaf angle				1.000	-0.673**	0.171 n.s.	0.247 n.s.	0.256 n.s.	0.122 n.s.	0.173 n.s.	0.079 n.s.	0.439*
Leaf area					1.000	-0.576**	0.186 n.s.	-0.086 n.s.	0.086 n.s.	0.263 n.s.	0.359*	-0.244 n.s.
Total biomass						1.000	-0.118 n.s.	0.224 n.s.	0.173 n.s.	-0.214 n.s.	0.079 n.s.	0.488**
Cob length							1.000	-0.017 n.s.	0.448**	1.001**	0.411*	0.381*
Cob diameter								1.000	0.884**	0.024 n.s.	0.125 n.s.	0.497**
Number of grain rows per cob									1.000	0.477**	0.309 n.s.	0.517**
Grains per grain row of cob										1.000	0.299 n.s.	0.232 n.s.
100 grains weight											1.000	0.437*
Total grain yield per plant												1.000

'' = significant at 5% probability level, '**' = significant at 1% probability level, 'n.s.' = non-significant*

and highly significant genotypic correlation with no. of leaves per plant and significantly positive correlation with leaf area, cob length, and grain yield per plant. A positive correlation of 100 grain weight with other parameters widest the way to select the maize doubled hybrid on 100 grain weight bases for escalated yield outcomes. 100 grains weight exposed non-significant but positive genotypic correlation with leaf temperature, flag leaf angle, total biomass, cob diameter, plant height, grain rows per cob, and grains per grain row of cob. 100 grains weight revealed a negative but non-significant genotypic correlation with none of any parameters under study (Table 5). Current results are supported with those reported by Sharifai *et al*. (2006), Rafiq (2010), and Adesoji *et al*. (2015).

Grain yield also showed a positive and highly significant phenotypic correlation with leaves per plant, plant height, total biomass, grain rows per cob, and 100 grains weight. It results a non-significant but positive phenotypic correlation with cob length, flag leaf angle, cob diameter, and grains per rows of cob. While it showed a negative non-significant phenotypic correlation for leaf temperature and area (Table 4). Grain yield per plant revealed a positively significant genotypic correlation with leaves per plant, total biomass, cob diameter, plant height, grain rows per cob, and significantly positive correlation with flag leaf angle, cob length, and 100 grains weight. So, a positive and significant correlation with these parameters with grain yield triggers the grain yield-based selection of doubled haploid hybrids to achieve the productivity target in maize. Grain yield showed a non-significant but positive and non-significant negative genotypic correlation with grains per row and leaf temperature, respectively (Table 5). Similar results were published by Sharifai *et al*. (2006), Rafiq (2010) and Adesoji *et al*. (2015).

Direct and indirect effects of selected agro-morphological parameters on grain yield: Plant height had negative direct effect on grain yield. It indicted that direct selection based on plant height will not best for improving yield in the doubled haploid maize hybrids. But plant height had positive genotypic (Table 5) and phenotypic correlation (Table 4) with total grain yield per plant, that why we will consider indirect selection. Leaves per plant, cob length, leaf area, total biomass, grains rows per plant, grains per grain row of cob and 100 grains weight revealed positive indirect effect on total grain yield. These results showed that indirect selection on based on these parameters will be fruitful grain yield of doubled haploid maize hybrids. Negative indirect effect through plant height on total grain yield per plant was showed by leaf temperature, flag leaf angle and cob diameter. So, selection based on these parameters will not be useful for improving total grain yield of doubled haploid maize hybrids (Table 6). Current findings are similar to those reported by İlker (2016) and Munawar *et al*. (2013).

Direct effect of leaf area was negative on total grain yield. It revealed that direct selection based on leaf area will not be fruitful for the greater total grain yield of doubled haploid maize hybrids. But positive indirect effects were depicted for plant height, leaf temperature, flag leaf angle, cob length, grain rows per cob and 100 grains weight through leaf area on total grain yield. So, indirect selection on the basis of these parameters should be useful for developing doubled haploid maize hybrids with greater total grain yield. Leaf area had also negative phenotypic correlation (Table 4) and genotypic correlation (Table 5) with total grain yield. Negative indirect effects were showed by number of leaves per plant, total biomass, cob length and grains per rows of cob through leaf area on total grain yield per plant. It indicated that selection on these parameters will not be beneficiary for developing doubled haploid maize hybrid with better total grain yield (Table 6). These results are in accordance to Sharifai *et al*. (2006), Nataraj *et al*. (2014) and Adesoji *et al*. (2015).

Total biomass exhibited positive direct effect on total grain yield. It indicated that direct selection based on total biomass will be a smart decision for developing doubled haploid maize hybrids with greater total grain yield. Total biomass also had positive genotypic correlation (Table 5) and phenotypic correlation (Table 4) with total grain yield per plant. Positive indirect effects were found by leaves per plant, cob length, grain rows per cob, leaf area, grains per grain row of cob and 100 grains weight on total grain yield. So, indirect selection based on these parameters will be fruitful for i improving the doubled haploid maize hybrids for better total grain yield. But negative indirect effect had been exhibited by plant height, leaf temperature, flag leaf angle and cob diameter on total grain yield per plant through total biomass. That's why indirect selection based on these parameters will

Table 6: *Direct (diagonal) and indirect effects of various physio-genetic parameters on grain yield per plant.*

Physio-genetic parameters	Plant height	Number of leaves	Leaf temperature	Flag leaf angle	Leaf area	Total biomass	Cob length	Cob diameter	Number of grain rows per cob	Grains per grain row of cob	100 grains weight
Plant height	-3.337	0.687	-0.059	-0.038	0.806	1.988	0.013	-0.502	0.747	0.085	0.394
Number of leaves	-3.101	0.739	-0.033	-0.044	0.494	1.862	-0.021	-0.298	0.389	0.028	0.701
Leaf temperature	0.791	-0.098	0.250	-0.070	-0.447	-0.431	-0.097	0.159	-0.053	-0.174	0.005
Flag leaf angle	-0.904	0.235	0.126	-0.139	0.971	0.341	-0.094	-0.410	0.327	-0.110	0.095
Leaf area	1.867	-0.253	0.078	0.094	-1.441	-1.149	-0.071	0.138	0.232	-0.166	0.430
Total biomass	-3.328	0.690	-0.054	-0.024	0.831	1.993	0.045	-0.360	0.463	0.136	0.095
Cob length	0.113	0.041	0.064	-0.035	-0.269	-0.237	-0.380	0.028	1.194	-0.631	0.491
Cob diameter	-1.047	0.138	-0.025	-0.036	0.124	0.448	0.007	-1.600	2.354	-0.016	0.149
Number of grain rows per cob	-0.936	0.108	-0.005	-0.017	-0.125	0.347	-0.170	-1.414	2.662	-0.301	0.370
Grains per grain row of cob	0.450	-0.032	0.069	-0.024	-0.380	-0.428	-0.380	-0.039	1.271	-0.631	0.357
100 grains weight	-1.101	0.434	0.001	-0.011	-0.519	0.159	-0.156	-0.200	0.824	-0.189	1.194

not be useful for developing doubled haploid maize hybrids having better total grain yield (Table 6). Currents results are advocated by Raghu *et al.* (2011), Sharifai *et al.* (2006) and Adesoji *et al.* (2015).

Positive direct effect had been showed by 100 grains weight on total grain yield. It indicated that direct selection based on 100 grains weight will be beneficiary for the improvement of doubled haploid maize hybrids with greater total grain yield. Also, 100 grains weight had positive phenotypic correlation (Table 4) and genotypic correlation (Table 5) with total grain yield. Positive indirect effects had been showed by number of leaves, leaf temperature, total biomass and grain rows per cob through 100 grains weight on total grain yield. So, indirect selection based on these parameters will be beneficiary for developing doubled haploid maize hybrids with improvement of total grain yield. Negative indirect effects had been showed by flag leaf angle, leaf area, plant height, cob diameter, cob length and grains per grain row of cob. So, indirect selection based on these parameters will not be beneficiary for the developing doubled haploid maize hybrids with improved total grain yield (Table 6). Earlier, Sharifai *et al.* (2006), Adesoji *et al.* (2015) and Rafiq *et al.* (2010) have reported similar results.

Conclusions and Recommendations

Among doubled haploid maize haploids understudy, DH-8XDH-6 showed the best performance with the highest values for total grain yield per plant (274g), 100 grains weight (40.47g), plant height (196.67cm), and the number of leaves per plant (14), second-highest values for cob length (18.169cm), optimum value for flag leaf angle (50°), grains per grain row of cob (36) and total biomass (440.91g) and average values of leaf area (107.84 cm^2) and cob diameter (50.52cm). DH-8XDH-6 can be used as a good doubled haploid maize hybrid.

Novelty Statement

Different doubled haploid maize hybrids were screened out to take a step to develop our hybrids rather than to import and become dependents of other countries.

Author's Contribution

Madad Ali: Conducted experiment, collected data and prepare initial draft.
Muhammad Ahsan: Supervise the experiment and did scrutiny of final manuscript.
Muhammad Zubair Akram: Did statistical analysis and prepare manuscript in collaboration with others.
Samreen Nazeer: Helped in collection of data and preparation of manuscript.

Conflict of interest
The authors declare that there is no conflict of interest among the authors of the manuscript.

References

Adesoji, A.G., I.U. Abubakar and D.A. Labe. 2015. Character association and path coefficient analysis of maize (*Zea mays* L.) grown under incorporated legumes and Nitrogen. J. Agron., 14: 158-163. https://doi.org/10.3923/ja.2015.158.163

Ali, A., A. Beshir Issa and D.B. Rahut. 2020. Adoption and impact of the maize hybrid on the livelihood of the maize growers: Some policy insights from Pakistan. Scientifica, 2020: 1-8. https://doi.org/10.1155/2020/5959868

Aslam, M., B. Farid, K. Khakwani, M.A. Maqbool and H. Zou. 2017. In vivo Maternal Haploid Seed Production and Chromosome Doubling with different Anti-microtubular Agents in Maize. Int. J. Agric. Biol., 19(1): 115-120. https://doi.org/10.17957/IJAB/15.0251

Beyene, Y., S. Mugo, K. Pillay, T.S.A. Tefera, S. Njoka and H. Karaya, J. Gakunga. 2011. Testcross performance of doubled haploid maize lines derived from tropical adapted backcross populations. Maydica, 56: 351-358.

Chaikam, V., W. Molenaar, A.E. Melchinger and P.M. Boddupalli. 2019. Doubled haploid technology for line development in maize: technical advances and prospects. Theor. App. Genet., 132(12): 3227-3243. https://doi.org/10.1007/s00122-019-03433-x

Durand, E., S. Bouchet, P. Bertin, A. Ressayre, P. Jamin, A. Charcosset, C. Dillman, and M. I. Tenaillon. 2012. Flowering time in maize: Linkage and epistasis at a major effect locus. Genetics, 190: 1547-1562. https://doi.org/10.1534/genetics.111.136903

Gupta, M., M. Choudhary, H. Kumar, V. Kaswan, Y. Kaur, J.R. Choudhary, S. Yadav, M. Gupta, A. Kumar, S. Sharma and K. Rana. 2022. Doubled Haploid Technology in Maize (Zea mays): Status and Applications. Indian J. Agric. Sci., 92(3): 283-291.

İlker, E., 2011.correlation and path coefficient analyses in sweet corn. Turk. J. Field Crops, 16(2): 105-107.

Khakwani, K., M. Ahsan, H.A. Sadaqat and R. Ahmad. 2019. Development and genetics of maize doubled haploid lines. Maydica, 63(3): 1-15.

Khakwani, K., M.R. Dogar, M. Ahsan, A. Hussain, M. Asif, A.R. Malhi and M. Altaf. 2015. Development of maize haploid inducer lines and doubled haploid lines in Pakistan. Br. Biotechnol. J., 8: 1-7. https://doi.org/10.9734/BBJ/2015/18394

Lourençoni, L.J., R.D.S. Trindade, L.C.A. Ribeiro, N.N.L.D. Parrella, P.D.O. Guimaraes, L.J.M. Guimaraes, B.M.C. Trindade and M.R. Ribeiro. 2021. Agronomic performance of maize hybrids derived from doubled haploid lines compared to conventional hybrids. Rev. Bras. Milho Sorgo, 20: 1-16. https://doi.org/10.18512/rbms2021v20e1218

Maqbool, M.A., A. Beshir and E.S. Khokhar. 2020. Doubled haploids in maize: Development, deployment, and challenges. Crop Sci., 60(6): 2815-2840. https://doi.org/10.1002/csc2.20261

Meng, D., C. Liu, S. Chen and W. Jin. 2021. Haploid induction and its application in maize breeding. Mol. Breed., 41(3): 1-9. https://doi.org/10.1007/s11032-021-01204-5

Mohammedali, M.A., O.A. Mohamed, M.G. Abdalla, K. AMA and E.M.M. Taha. 2021. Genetic variability, heritability and genetic advance estimates in some maize (Zea mays L.) Varieties in Sudan. Int. J. Food Sci. Agric., 5(1): 85-90. https://doi.org/10.26855/ijfsa.2021.03.012

Munawar, M., M. Shahbaz, G. Hammad and M. Yasir. 2013. Correlation and path analysis of grain yield components in exotic maize (*Zea mays* L.) hybrids. IJSBAR, 12(1): 22-27.

Nataraj, V., J.P. Shahi and V. Garwal. 2014. Correlation and path analysis in certain inbred genotypes of maize (*Zea mays* L.) at Varanasi. Int. J. Innov. Res. Sci. Eng. Technol., 3(1): 14-17.

Odiyo O., K. Njoroge, G. Cheminingwa and Y. Beyene. 2014. Performance and adaptability of doubled haploid maize testcross hybrids under drought stress and non-stress conditions. Int. Res. J. Agric. Sci. Soil Sci., 4: 150-158.

Rafiq, C.M., M. Rafique, A. Hussain and M. Altaf. 2010. studies on heritability, correlation and path analysis in maize (*Zea mays* L.). J. Agric. Res., 48(1): 35-38.

Raghu, B., J. Suresh, S.S. Kumar and P. Saidaidh. 2011. Character association and path analysis in maize (*Zea mays* L.). Mad. Agric. J., 98 (1/3): 7-9.

Reddy, R.V., F. Jabeen, M.R. Sudarshan and R.A. Seshagiri. 2013. Studies on genetic variability, heritability, correlation and path analysis in maize (*Zea mays* L.) Over locations. Int. J.

Appl. Biol. Pharma. Tech., 1: 195-199.

Sharifai, A.I., M. Mahmud, A.B. Lawal, I.U. Abubakar and S.G. Mohammed. 2006. Correlation and path coefficient analysis for growth, yield and yield components of early maturing maize (*Zea mays* L.) varieties. Savannah J. Agric., 1: 103-109.

Sserumaga, J.P., O. Sylvester, Oikeh, S. Mugo, G. Asea, M. Otim, Y. Beyene, G. Abalo and J. Kikafund. 2016. Genotype by environment interactions and agronomic performance of doubled haploids testcross maize (*Zea mays* L.) hybrids. Euphytica, 205(3): 253-365. https://doi.org/10.1007/s10681-015-1549-2

Tang, F., Y. Tao, T. Zhao and G. Wang. 2006. In vitro production of haploid and doubled haploid plants from pollinated ovaries of maize (*Zea mays*). Plant Cell, Tissue and Organ Culture, 84: 233-237. https://doi.org/10.1007/s11240-005-9017-7

Yahaya, M.S., I. Bello and A.Y. Unguwanrimi. 2021. Correlation and path-coefficient analysis for grain yield and agronomic traits of maize (*Zea mays* L.). Sci. World J., 16(1): 10-13.