



Comparison between F₂ hybrid wheat (*Triticum aestivum* L.) and their parents based on heterosis assessment

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Abstract

As wheat *Triticum aestivum* L. is the most important crop and among the major three cereal crops that provides 20 % of the total energy requirement in human food a comparison between six F₂ bread wheat (*Triticum aestivum* L.) is very encouraged to improve wheat yield and qualities. The main goal was to select hybrids according to their heterosis estimations. The methodology of our study is focused on crosses obtained with their respective parents, layed out and evaluated in a randomized complete block. Morphological, physiological and biochemical parameters have been measured. Heterosis are estimated using mid and high parent measurements. Results show that overall heterosis are noticed for plant height, last internodes length, number of grains per spike, leaf area, number of spikes per m² and grain yield. Five hybrids had values above the mid-parent. So, hybrid vigor displayed a marked trend with regard to the variables studied. These hybrids have acquired and maintained a level of higher hybrid vigor in F₂ than the mid-parent. Thus, we agreed with several authors for using F₂ or F₃ hybrids, given difficulties in obtaining F₁ hybrids. In general our results about comparison between F₂ hybrid wheat (*Triticum aestivum* L.) and their parents based on heterosis assessment may provide more useful information for long term improvement in yield and qualities. At the end we recommend identifying the relationship between heterosis and genetic distance based on SSR markers.

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Introduction

Wheat which is a hexaploid species is widely cultivated in the world. *Triticum aestivum* L. which is the most important crop and among the major three cereal crops that provides 20 percent of the total energy requirement in human food (Shewry, 2009) needed to be studied by a comparison between six F2 bread wheat (*Triticum aestivum* L.) to improve wheat yield and qualities. So, hybrids development is currently the main focus of yield improvement and open-pollinated varieties are being important (Usatov *et al.*, 2014). In Algeria wheat and barley, respectively hold 63% and 33% of the utilized agricultural area (MADR, 2014). So, in this country cereals constitute the bulk of the daily diet of the population and occupy an area of 2.7 million hectares (Benbelkacem, 2013). Among cereals, wheat (*Triticum aestivum* L.), is the species most cultivated after durum wheat (*Triticum durum* Desf. L.). However, national productivity remains relatively low, from 12 to 18q/ha (Salmi *et al.*, 2015). This is due to the difficult weather conditions to master (irregular and insufficient rainfall and extreme temperatures) and the sensitivity of the majority of species used by grain at various parasites including fungal diseases. Thus, the genetic improvement of plants to better adapt to biotic and abiotic constraints remains promising. The yield is a complex trait, which is the resultant characters directly and indirectly involved in the training, such as grain weight, number of grains per spike, number of ears per unit area and biomass. Bouzerzour *et al.* (1998) indicate that in variable environments, the efficiency of selection based solely on grain yield is very low, due to the effect of the environment which varies the level of the character and relationships with others, from one year to another. Thus breeders then turn to other potential and less volatile characters that can be used in parallel or independent of performance in a multi characters approach (Benmahammed *et al.*, 2003; Annicchiarico and Iannucci, 2008). Among the multitude of possible morphological traits include early spiking, overhead biomass, the height of

straw, the number and weight of grain and harvest index (Annicchiarico *et al.*, 2005; Reynolds *et al.*, 2005; Slafer *et al.*, 2005). The yield performance is determined by the yield components while tolerance is identified by the morpho-physiological characteristics (Lopes *et al.*, 2012). Phenological parameters of adjustment, or early setting, define the timing of plants in relation to environmental constraints development cycle ; indeed, by acting on these parameters, it is sometimes possible to avoid the coincidence of the critical stages of development and the maximum occurrence dates of some accidents. Nevertheless seeking greater precocity was mentioned by several authors: reduced productivity (Laing and Fischer, 1977), increased risk of late frosts (Single and Fletcher, 1979), reduction of root development (Derera *et al.*, 1969). Morpho-physiological parameters and biochemical adaptation allow highlighting the tolerance phenomena related to the photosynthetic activity, and accumulation osmo-regulators. For the selection, for such characters, to be effective it is necessary to know their genetic determinism, and this variability in segregating populations. The work of East (1936) on corn showed that the product of a cross between two homozygous lines (inbred) was generally more vigorous than their parents (manifestation of vigor hybrid or heterosis). For wheat, the first studies of heterosis began in 1919 with the work of Freeman (1919), who noted that the intensity of the phenomenon depended on the cross made. The two main explanations of the phenomenon of heterosis are those involving dominance or superdominance. The heterosis for a given character is then all higher than the hybrids of parents are complementary to the loci involved in variation of that character. This complementarity is linked to the genetic distance between the hybrid parents. However, in a plant pollinating like wheat, until time the works of this kind have been very limited, because of the F1 seed production difficulties (Zaidi *et al.*, 2016). This study is conducted to evaluate the phenotypic variability and the genetic determinism of traits related to yield performance,

in populations segregating F2 soft wheat (*Triticum aestivum* L.) and their parents. The selection is based on their superiority to parents, estimated by heterosis.

The main goal is to select hybrids according to their heterosis estimations to provide more useful information for long term improvement in wheat yield and qualities.

Material and methods

Experimental site

The experiment was set up at the farm with demonstration and production of seeds in the Guelma Technical Institute of field crops named (ITGC), during the year 2015 campaign at an altitude of 272 m on a bioclimatic class of a subhumid to mild winter. The rainfall distribution is irregular, and ranges between 500 and 600 mm. The soil of the experiment area is characterized by heavy texture, silty-sandy to slightly alkaline pH (7.1) and low organic matter content 2.2%. The dry climate, the atmospheric dust, and low intensity of precipitation accentuate the drawdown of water resources and may also affect the water irrigation and soil qualities generally by increasing the salt content. The region of study is considered to be a semiarid area with a warm summer and low temperature in winter. Temperature rises up to 40°C in summer (Gueroui *et al.*, 2015).

Plant material and experimental device

The Plant material of study realized with six different hybrid combinations of soft wheat segregating populations (F2) with their corresponding four (04) parent varieties which one advanced line (Var1). Hybrids are produced by crossing targeted during the campaign 2012/2013. The genotypes studied are Morocco, Hidhab 1220 Yr 18/3* Avocet, Ciano 79, Morocco x Ciano 79, Morocco x Yr 18/3* Avocet, Morocco x HD 1220, Hiddab x Ciano 79, HD1220 x Yr 18/3* Avocet and Yr 18/3* Avocet x Ciano 79 coded respectively by Mo, HD, Var1, Var2, Mo/Var2, Mo/Var1, Mo/HD, HD/Var2, HD/Var1 and Var1/Var2.

The experimental device of the studied genotypes was sown on 2015. The elementary plots being constituted of six (06) lines of 1 m, 0.20 m apart, in a device in randomized complete block design with three replications. The seed rate is 15g/m². The applied cultivation techniques were established in two inputs of nitrogen fertilizer, the first fraction in the ammonium form (urea 46% nitrogen) at a rate of 1ql/ha done in the early tillering. The second fraction, as Sulphate-N at a rate of 1.30 ql/ha provided on a joint stage.

The measurements were performed on 30 individuals per experimental plot at the rate of one plant per genotype and repetition. The characterization of the different genotypes was based on a set of parameters related to the phenology, morphology and the physiological plant.

Plants morphological parameters, yield and its components

The ratings have focused on the spike tillering (ST), counting the total number of spikes/plant; the stem height (SH) measured in cm from the soil at the base of the spike; the length of the spike (SL) measured in cm from the base of the ear to the end (not included beards); and the last internode length (LINL) measured from the last node to the base of the spike. At maturity the yield was determined by grain and its components. The theoretical grain yield per plant (TY) is calculated from the number of spikes/plant (SN) (determined by counting the number of tillers ears/plant), number of grains spike-1 (SGN), and an average weight of the 1000-grain weight (TGW) which is determined by him counting and weighing 1000 seeds taken from the harvest of each plot. Using the following formula:

$$TY = \frac{SN \times SGN \times TGW}{1000} \quad (1)$$

Physiological, biochemical parameters and heterosis study

The Physiological parameters which are important to complete analysis of agronomic study are identified as follow:

The vegetative stage duration (VSD) is determined by the number of calendar days counted from emergence to date of completion of 50% of heading. The duration of this vegetative stage is an indicator of earliness. Leaf area (LA) is determined by the method of Paul *et al.* (1979).

The Chlorophyll ($\mu\text{g/gMF}$) is determined by extraction of chlorophyll leaf tissue using the method established traditionally by Mackinney, (1941) and improved by Holden (1965), which consists of a maceration of the plant in acetone. Weighed one gram of cut sheets from the middle third, the latter are cut into small pieces and crushed in a mortar with 20 ml of 80% acetone and a pinch of calcium carbonate (CaCO_3).

The reading of the optical density occurs at two wave lengths (λ_1 and $\lambda_2 = 645 = 663\text{nm}$). Chlorophylls (chl. a, chl. b and chl. a+b) are estimated from the equations described by authors (Arnon, 1949; Zaidi *et al.*, 2016); it is expressed in $\mu\text{g/g FM}$.

Biochemical parameters consisted of a measurement of the quantities of the constituents of biological organs: soluble sugars, total protein and proline. For heterosis we studied the manifestations of heterosis for different characters. The estimation of heterosis was calculated in relation to the mid-parent (2) or High-parent (3) as below:

$$\text{Heterosis \%} = \frac{F_2 - (P_1 + P_2)/2}{(P_1 + P_2)/2} \quad (2)$$

$$\text{Heterosis \%} = \frac{F_2 - P_{\text{max}}}{P_{\text{max}}} \quad (3)$$

F_2 : Hybrid value.

P_1 : First parent value.

P_2 : Second parent value.

P_{max} : High-parent value.

The reference to the mid-parent highlights the effects of dominance thus the deviation from the high-parent additivity that highlights superdominance effects (Lefort-Buson, 1985).

Statistical analysis

Collected data were analyzed using the device in randomized complete block design with three replications. For statistical analysis we used STATISTICA software 8.0 version.

Results and discussion

Yield components results

According to the results (Table 1), we note that the grain yield gives a highly significant positive correlation with a major component of yield, like the number of spike (0.812). Very highly significant positive correlations were also observed between the length of the last internode and stem height (0.919). Positive correlations were observed between the TWG, the number of grain corn and yield. Significant negative correlations were observed between the TWG and precocity genotype (-0.726) indicating that over the growing cycle of the plant is shorter, the TWG is high.

Table 1. Correlation matrices of yield components.

| | SH | SL | LINL | SGN | LA | TGW | SN | TY | VSD |
|------|--------|--------|--------|--------|--------|--------|-------|-------|-----|
| SH | 1 | | | | | | | | |
| SL | 0,277 | 1 | | | | | | | |
| LINL | 0,919 | 0,314 | 1 | | | | | | |
| SGN | 0,346 | 0,521 | 0,486 | 1 | | | | | |
| LA | 0,109 | 0,191 | 0,304 | 0,606 | 1 | | | | |
| TGW | 0,238 | -0,205 | 0,159 | 0,295 | -0,268 | 1 | | | |
| SN | -0,078 | 0,365 | -0,208 | -0,100 | -0,096 | -0,083 | 1 | | |
| TY | 0,211 | 0,601 | 0,147 | 0,486 | 0,276 | 0,101 | 0,812 | 1 | |
| VSD | -0,461 | -0,072 | -0,388 | -0,200 | 0,201 | -0,726 | 0,226 | 0,064 | 1 |

Classification assessment

The grouping of 10 genotypes based on morphological parameters, physiological and biochemical, using a dendrogram with a single link

and the euclidean distances and the level of similarity of 85.37%, allows distinguishing six separate homogeneous groups that are as below (Fig. 1).

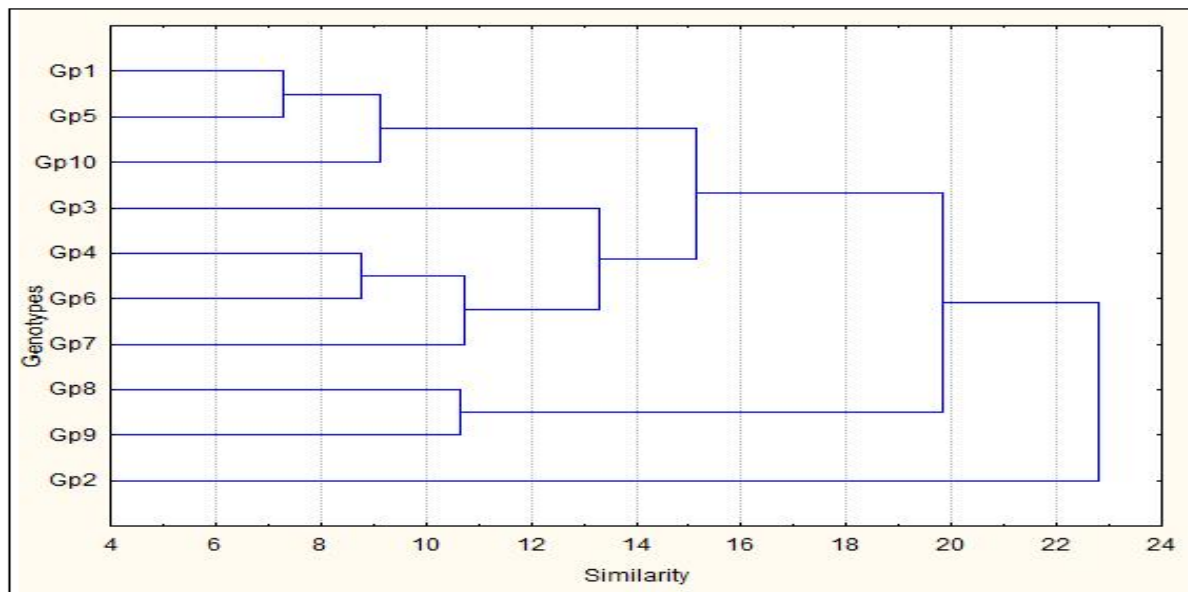


Fig. 1. Dendrogram grouping ten genotypes.

Group 1 includes the HD genotype and Group 2 concerns the genotype MO.

Group 3 is represented by the genotypes Var2 Var1/Var2, MO/Var1, HD/HD Var1et /Var2.

Group 4 holds MO/HD and Group 5 includes Var1 genotype but Group 6 concerns the group genotype MB/Var2.

Moreover, the grouping of variables (parameters) using the single link and the Euclidean distances based on the correlation coefficient gives six homogenous groups (Fig.2), at a rate of similarity of 88.60%, which are as follows:

The first group consists of the variable total protein content.

A second group is composed by two variables: proline content and the TWG with 100% similarity levels.

A third group consisting of precocity.

A fourth group of the soluble sugar.

A fifth group consisting composed by the combination of three characteristics (Fig. 3):

Chl content (a + b) and Chl (b) with a level of similarity of 100% and the content of Chl (a).

Finally, a sixth group including variables: number of grain per spike and leaf area, number of spike and yield, spike's length and plant height and length of the last internode to all a similarity of 100%.

Heterosis assessment

For Heterosis assessment (Fig. 3) we note that dominance case study of plant height is an important selection criterion particularly for arid for estimating the yield major flaw in these regions. It was admitted for a long time by breeders as varieties with high straw are more drought tolerant. The fact is that tall stubble is often associated with a deep root system (Ali-Dib, 1990) and therefore to a better ability to extract water from the soil. The results have shown a heterosis in relation to the mid-parent with the MO/Var1 hybrid HD/VAR1 and HD/Var2, it is respectively 5.38 %, 45.54% and 45.60% (Fig. 3). For the character length of the ear, only the hybrid Var1/Var2 has a value greater than the mid-parent.

However, for superdominance case the heterosis from the high-parent is observed in the case of the stem height, spike length, the length of the last internode, number of spike grain, leaf area, spike length, number tillers spikes, grain yield, vegetative stage duration, protein content, proline, chlorophyll (a) and chlorophyll (a + b).

The study of different parameters highlights interesting hybrid as follow:

The hybrid Var1/Var2, presents values above the mid-parent for variables spike length, length of the last internode, number of grain per spike, leaf area, number of ears, grain yield and earliness.

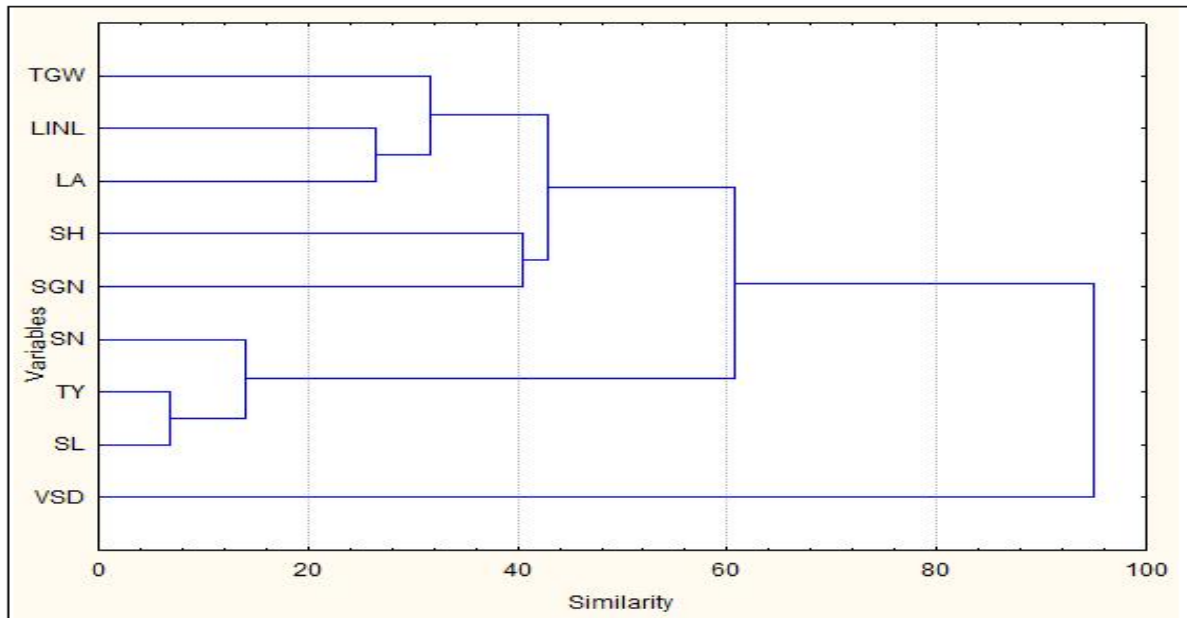


Fig. 2. Dendrogram grouping nine variables.

The hybrid HD/Var1, presents values above the mid-parent for the variables plant height, length of the last inter-node, number of spike grain, leaf area and yield. The hybrid HD/Var2, presents values above the mid-parent for variables plant height, length of the last internode, number of spike grain, leaf area, yield and proline content.

The hybrid MO/Var2, presents values above the mid-parent for variables spike length, leaf area, number of ear, grain yield, earliness and protein content.

The hybrid MO/Var1, presents values above the mid-parent for variables plant height, length of the last internode, leaf area, number of ear and the content of chlorophyll (a, b and a+ b).

Yield components, classification and heterosis results discussion

Positive correlations (Table 1) observed between the TWG, the number of grain corn and yield coincides with results by Benbelkacem and Kellou (2001),

who note that the average grain weight, expressed as weight of 1000 grains does not seem to affect the yield. This component remains virtually uncontrolled response to the combined effects of the compensation with the number of grains per spike and those of the stress cycle end (Zaidi *et al.*, 2016). These results agree with those obtained by Busch and Kofoid (1982), they note that the cycle and fertility decline as the weight of 1000 grains improves, but with no effect on grain yield. Bahlouli (1998) examines the efficiency of selection on the basis of the duration to heading stage. He observed that the effectiveness of this selection is subject to inter-annual climate variation. Other work note that yield per spike may be increased, under irrigated condition, by selecting plants with thicker culm and longer spike and, under non irrigated late season water stress conditions, by choosing taller plants with thicker culm and longer spikes (Okuyama *et al.*, 2005).

So, we note in our work that some negative correlations were also observed between the number of ears per plant products and the number of grains per ear, the same results was found by Bahlouli *et al.* (2005) and Salmi *et al.* (2015),

they show a negative compensation effect between its two components under semi-arid conditions. Improving one of these components reduces the other, with uncontrolled effects on grain yield.

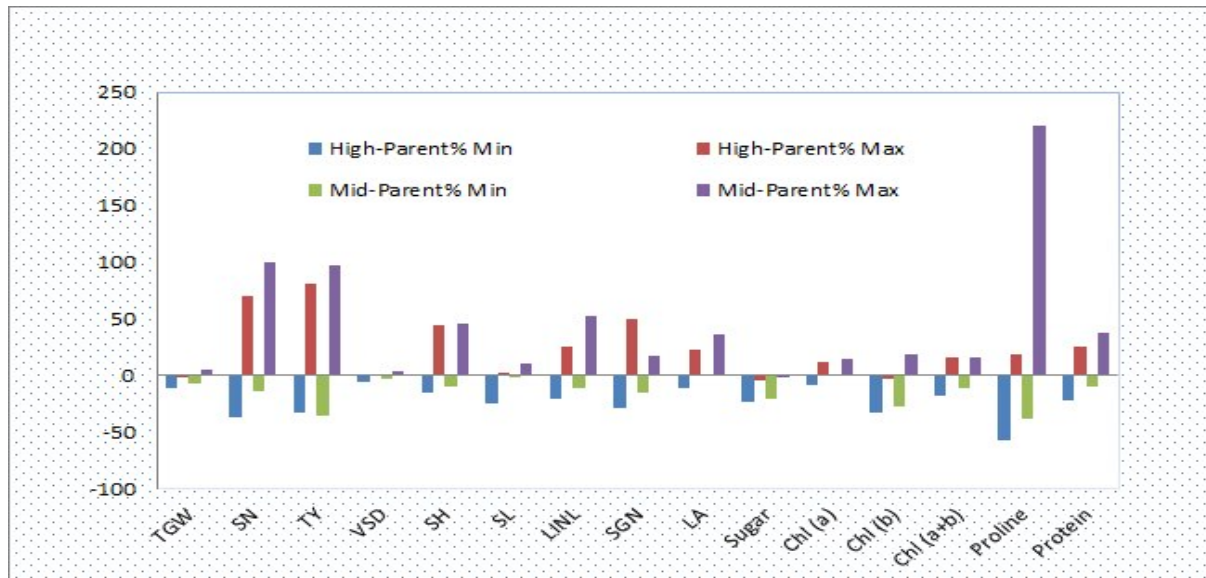


Fig. 3. Heterosis of Mid and High-Parent relations.

According Bammoun (1993), the heterosis being 10.6% is important for adaptation to drought that spike involved much more than flag leaf photosynthesis in case of water deficit. Regarding the character tillering ear, data analysis, shows a spike tillering than the mid-parent for MB/hybrid Var1 Var1/Var2 and MB/Var2, which is 5.26%, respectively, 13.64 % and 100%, revealing by this way effects of dominance. These results confirm those obtained by Jatasra and Paroda (1980), Cox and Murphy (1990), Singh and Behl (1991) and Vitikare and Atali (1991). In terms of the length of the last internode, the Mo/hybrid Var1 Var1/Var2, HD/Var1 and HD/Var2, exhibit superior heterosis through parent, the values are respectively 9.30%, 8.21 %, 37.17% and 52.30%. The analysis of heterosis for leaf surface character indicates that all hybrids are higher than the mid-parent revealing dominance. The study of early showed that only hybrid Var1/Var2 and MB/Var2 have identical precocity at the earliest parent. This heterosis was estimated at 3.03% for the two hybrids. These results are comparable to those obtained by Goujon and Paquet (1968), Deshmukh

and Deshmukh (1989), Ziaddin *et al.* (1988) and Tarkeshiwar and Mishara (1990). Precocity is a phenological parameter adaptation to drought (Benabdallah and Bensallem, 1993). It is investigated by breeders of shortcycle cultivars growing period is within the favorable season or so cultivars whose vegetative stages are controlled by the photoperiod to coincide with the most favorable period (Fox *et al.*, 1990). Hybrid Var1/Var2, HD/Var1, HD/Var2 have a grain yield higher than the mid-parent heterosis being 13.40%, 13.16% and 17.56%. Regarding the TWG, only the hybrid HD/Var2, has a value greater than the mid-parent, 4.46%. The results show that almost all hybrids have a grain yield higher than the mid-parent. However the most important value is that of the hybrid MO/Var2 (96.48%). For the character content of chlorophyll (a), the MO/HD hybrid, MO/Var1 and HD/Var showed superiority over the mid-parent, whose values of heterosis are respectively 10.6 7%, 14.42 % and 14%. Regarding chlorophyll (b), only the hybrid MO/Var1 presented a higher value than the mid-parent (7.52%). The heterosis for chlorophyll content (a + b) was evident

in MO/Var1 hybrids (10.57%) and HD/Var1 (16.1%). Hybrid MO/HD and HD/Var2, have shown values above the mid-parent with values of heterosis of 17.05% and 220.61% respectively. For the character content of soluble sugar, no hybrid showed superiority over the mid-parent. Analysis of the results for the single protein content only MO/HD and MO/Var2 hybrids have presented vigor in relation to the mid-parent.

Reflection and key components

Finally, we reach the conclusions of several authors who want to use large-scale F2 or F3 hybrids, given the difficulty in obtaining F1 hybrids and their high cost. Some researchers, Brim and Cockerham (1961) cited by Tarkeshwar and Mishara (1990) note that more the contribution of additive gene is more great the depression in F2 will be smaller. It is important to note that the HD/Var1 hybrid HD/Var2 are superior to their parents for the characters plant height, length of the last internode, number of spike grain, leaf area, grain yield, which are morphological parameters adjustment to water stress. Gate *et al.* (1992) as some authors have attributed to the plant in case of water deficit; a better ability to tolerance with the amount of assimilates stored at this level. Important aspects of wheat physiology, such as lodging resistance, the use of growth regulators for wheat growth, weed competition, soil mechanical impedance and nutrient toxicities/deficiencies, should be discussed as a priority and given to yield and yield forming processes with the idea that the application of these concepts would have a higher impact on wheat production around the world (Acevedo *et al.*, 2016). Wheat ear morphology parameters are of great concern of the breeding experts and are important for the reflection of wheat growth status. In order to realize the fast nondestructive measurement of wheat ear morphology parameter and other characteristics we recommend exploring remote sensing based on image processing. This method can be used to auto extract quickly the awn number, average awn length; ear length and ear type (Kun *et al.*, 2011). To increase wheat production and progressive sustainable agricultural development,

several studies should be conducted also on climate change, environmental conditions, health risk assessment and water irrigation quality (Labar *et al.*, 2013; Medjani *et al.*, 2016). Knowledge of genetic diversity in a crop species is fundamental to its improvement (Kara *et al.*, 2016). Evaluation of genetic diversity levels among adapted, elite germplasm can provide predictive estimates of genetic variation among segregating progeny for pure-line cultivar development (Manjarrez-Sandoval *et al.*, 1997). The use of molecular markers for the evaluation of genetic diversity to identify the relationship between heterosis and genetic distance is very recommended. Many wheat scientists (Chen *et al.*, 1994; Vollmann *et al.*, 2005; Kara *et al.*, 2016) have studied genetic diversity in common wheat using different molecular markers.

Conclusion

Our results about comparison between F2 hybrid wheat (*Triticum aestivum* L.) and their parents based on heterosis assessment may provide great useful information for long term improvement in yield and qualities. The values we get are comparable to values reported by other authors. Regarding the yield we get heterosis values above the mid-parent for 04 hybrids (Var1/Var2, HD/Var1, HD/Var2 and MO/Var2) and the maximum value we get for heterosis compared at high-parent of 81.26% for the hybrid MO/Var2. For leaf area, all hybrids have a higher value than the mid-parent. These hybrids have acquired and maintain a level of hybrid vigor in F2 higher than the mid-parent. The main strategy used in the past to deal with environmental stress has been to alleviate the stress through irrigation, soil reclamation, fertilizer use and others. Economics, as well as ecological limitations associated with these practices, however, have prompted an interest in searching for plant genetic resistance to environmental stresses. Wheat yields are depressed, among other factors, by drought, heat, low temperatures, low fertility, especially nitrogen, and soil salinity. Yield under stress is generally less understood, but available physiological knowledge should allow better and more rapid progress in the future.

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