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RELATIONSHIP OF EXPRESSION LEVEL OF TWO YIELD RELATED GENES WITH MORPHO-AGRONOMIC VARIABILITY IN COMPOSITE CORN LINES UNDER DRY CLIMATE

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ABSTRACT

The grain harvest in corn is measurable and controlled polygenically. Therefore, effective yield enhancement and concurrent modification of yield-related genes are imperative. Six newly composite corn lines were characterized and their morpho-agronomic variability was evaluated. The expression level of two yield-related genes *(Sh2* and *Bt2b)* and their relationship with morpho-agronomic characters were considered using quantitative real-time PCR (qRTPCR). The outcomes of the principal component analysis showed that 2 components with Eigen values larger than one explained 86.62 percent of the total variability. The highest phenotypic and genotypic coefficients of variance (PCV and GCV, respectively) were recorded for grain yield per plant. The greatest phenotypic and genotypic correlation of grain yield per plant was noted with 1000- kernel weight (0.84 and 0.98, respectively). Kernel number showed the highest genetic advance (1990.12%) and heritability (75.27%). Kernel number and its 1000 weights were positively correlated with grain yield per plant. The *Sh2* and *Bt2b* genes presented upregulated expressions in leaf and endosperm tissues which was significantly and positively related to the grain yield and its primary components. It was observed that by altering the expression level of *Sh2* and *Bt2b* genes via hybridization in the composite corn lines, yield enhancement and improvement in genetic parameters of yield components occurred.

Keywords: cross transformation, morpho-agronomic variability, yield-related genes.

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INTRODUCTION

Corn (*Zea mays* L.) is the 3rd largest cereal crop and contributes significantly to global agricultural production (Zhu*et al.,* 2011). Corn is utilized like human food, food for livestock, building material, like fuel, and ornamental and medical plants (Bekrić and Radosavljević, 2008). To achieve favorable corn production with the highest yield, it is crucial to consider the physiological and morphological aspects of the plant and the functions of the different yield components. Therefore, the consideration of these significant factors can potentially increase the grain yield (Azizi *et al.,* 2012). The study of associations among the traits is also important for effective selections during breeding programs and is extensively used among several methods that can be utilized (Yagdi and Sozen 2009). Information on character correlation in crops is significant for rapid and efficient selection in crop development (Binodh *et al.,* 2008). Hadji (2004) noted significant and positive associations between grain yield and ear height, ears per plant, plant height, ear diameter, kernel weight, and kernels per row. Similarly, Monneveux *et al.,* (2005) reported a considerable association between grain yield and grain weight under optimum N, along with grains per ear and anthesis-silking interval under low N scenarios.

Most traits of economic importance are quantitative and controlled by several to many major genes (Ali *et al.,* 2012). Corn starch is a key industrial raw material in the production of various films and coatings, paper, textiles, medical instruments, biodegradable electronics and packaging, and first-generation biofuel products (Guan *et al.,* 2011). AGPase is a heterotetramer that produces nucleotide sugars that are used by starch synthases to connect glucosyl units with starches. This heterotetramer is consists of dual larger subunits (AGP-L) programmed via Shrunken2 (*Sh2*) plus 2 smaller subunits (AGP-S) programmed via Brittle2 (*Bt2*) (Hannah *et al.,* 2001). In plants increasing the kernel weight has a direct relationship with overexpression of wild kinds of *Sh2* and *Bt2* genes, which represents the total increased starch synthase (Li *et al.,* 2011). Jiang *et al*., (2013) reported overexpression of some genes including *Sh2* and *Bt2* can enhance the activity of sucrose synthase, AGPase, and granule bound starch synthase. It causes an increase in the endosperm starch content the proportion of amylose. Therefore, the composition of the kernel starch content can be altered for both the quality (structure and chemical diversity) and

quantity (Jiang *et al*., 2013). Expression of allosterically-customized AGPase also intensified yield in transitory starches, causing improved growth in the leaf tissues of mouse ear cress (*Arabidopsis thaliana* L. Heynh.) (Obana *et al.,* 2006) meanwhile increase in the fresh weight of lettuce leaves (*Lactuca sativa* L.) (Lee*et al.,* 2009).

The present study is an endeavor to evaluate the genetic behavior and relationship between the yield and yield components in some composite corn lines. Furthermore, the expression level of *Sh2* and *Bt2b* genes in leaf and endosperm tissues were measured via quantitative real-time PCR (qRTPCR). After that, the influence of the altering in the expression level of these two genes on increasing grain yield per plant and improving in genetic parameters of yield components was measured via analyzing morpho-agronomic characterization.

MATERIALS AND METHODS

Materials and experimental design: A dozen composite corn lines were collected from the repository of the Institute of Biological Sciences (ISB), University of Malaya, Malaysia. These cross hybrids were derived from yellow sweet corn from the International Institute of Tropical Agriculture (IITA, Nigeria) and white field corn was collected from the International Maize and Wheat Improvement Centre (CIMMYT), Mexico. Finally, six superior lines (UM1, UM2, UM3 UM4, UM6, UM1) were selected to plant under the dryland environment (Iran). The experiment was conducted as Randomised Complete Block Design (RCBD) with 3 replications. The plot size was 7m x 14 m. Corn seeds were sown in rows of 4 m in length each. Similar corn lines were planted in two rows with a 50 cm distance. Distances between rows of various corn lines, plants and plots were kept as 75 cm, 50 cm, and 1 m, respectively. Quantitative data such as silking date, tasseling date, plant height, number of ears per plant, ear length, ear weight, 1000- kernel weight, number of kernels per ear and grain yield per plant were recorded in 10 guarded plants selected randomly.

Statistical analysis: The recorded data were subjected to ANOVA (SAS version 9.1) and assessed for simple statistics (standard deviation, mean, and variance), distribution of frequency, and phenotypic correlation coefficients using the techniques of Steel and Torrie (1980). . Means were compared using the Duncan Multiple Range Test (Duncan, 1955). The multivariate assessment, especially the chief component and cluster assessment, was carried out following different methods (Cruz and Regazzi, 2012; Mardia *et al.,* 1979). Cluster assessment using UPGMA (among group linkages) was utilized to examine distance, relatedness, and similarity of populations or genotypes so that similar genotypes can be categorized into the same group and dissimilar ones into other groups (Michener and Sokal, 1957). Principal component analysis (PCA) was conducted to comprehend a balanced weighing of traits and variable independence, which causes significant contributions of various characters based on the respective variation (Pearson, 1901). The data assessed based on RCBD was utilized to divide the gross (phenotypic) variability into the constituents due to genetic and non-genetic parameters and to determine their magnitude. Variance components (phenotypic, genotypic, and error variance) were determined using the technique of Prasad *et al.* (1981) and Wricke and Weber (1986).

Extraction of DNA and RNA from leaves and endosperm: The genomic DNA of all corn plants was extracted from 100 mg of new leaf tissues collected from each type, using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The leaf RNA isolate was obtained by using an RNA extraction kit, the SV Total RNA Isolation System (Promega, Madison, WI, USA). Entire endosperm RNA isolate was obtained from the dismembered mid-ear endosperm 20 DAP (Shang-Jing *et al.,* 2006), by using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) following manufacturer protocol. The RNA taken from three distinct kernels from each ear was pooled.

Polymerase Chain Reaction (PCR): The polymerase chain reaction (PCR) amplification for all primer sets was carried out using the C1000 Thermal Cycler (Bio-Rad, Hercules, CA, USA) within a reaction solution of 10µl total volume, consisting of 1.5µl of DNA extracted from leaf tissues, 5µl of Gotaq Green master blend (Promega, Madison, WI, USA), and also 1µl from primer (10mM). PCR reactions were conducted in the following manner: Initial denaturation was conducted at 95°C for 5 minutes, and then 35 cycles of denaturation were carried out at a temperature of 94°C for 30 seconds, with annealing temperatures for 30 seconds, including a 1-minute extension step at a temperature of 72°C. The process was concluded with a final step conducted at a temperature of 72°C for 10 minutes. PCR product incidence was verified by using electrophoresis. A 1.0% agarose gel was used for conducting the PCR product electrophoresis. Gel electrophoresis was conducted at levels of 80 volts, 175 mA, utilizing a 1 x TBE running buffer for 30 minutes. All gels were visualized under ultraviolet lighting (Alpha Imager Gel Documentation System, Siber Hegner, Germany). Similarly, the sequencing product was analyzed using the ABI 3730xl DNA analyzer (Applied Biosytems, Foster City, CA, USA).

Real-Time PCR and data analysis: The real-time quantitative PCR amplification was performed for extracted samples of RNA from endosperm and leaf tissues of 6 composite corn lines. Real-time quantitative PCR was carried out by using CYBR green fluorescence

through RTqPCR (CFX96 Touch™ Real-Time PCR Detection System, Bio-Rad, Hercules, CA, USA) on a reaction amount of 20 μl in total, which comprised primer (forward and reverse), SYBR Green blend (GoTaq 1-step RTqPCR reaction mix, Promega, Madison, WI, USA), nuclease-free water, and also template of RNA (100 ng), with negative control (missing reverse transcriptase). Neither positive controls (no primer) nor template (no RNA) were included in the reaction groups, which were each examined in triplicate for three biotic replicates. Thermal cycling specifications were set at a 48°C for 15 minutes (inverse transcription), for 10 minutes at 95°C (reverse transcription inactivation), before 40 cycles for 10 seconds at 95°C (denaturation), further at 58°C, 52°C, and 55°C related to *18s*, *Bt2b* and *Sh2* for 30 seconds (annealing), and for 30 seconds at 72°C (extension). Following amplification, all samples were kept for 10 seconds at 95°C and for 5 seconds at 65°C, which was steadily increased by 0.5°C at every 5 seconds to obtain the melt curves (Cholet, Ijaz *et al.* 2019).

Statistical analysis: The amplification results from CFX Manager Software (Included with CFX96 Touch™ Real-Time PCR Detection System, Bio-Rad, USA) were exported to an Excel file and the quantification of gene expression was conducted according to the relative quantification methods of Livak and Schmittgen (Livak and Schmittgen, 2001). The C_T values of the *18s* gene were used as the internal control (Endogenous reference) and C_T values of the parent line for each corn advance line were used as calibrator (Control). The effects of hybridization on internal control, *Sh2* and *Bt2b* genes were estimated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) where the same amount of RNA was used for external normalization. Before data analysis, all variables were subjected to a normality test using Kolomogorove –Smirnove test, and results indicated that all variables were distributed normally. To equate the relative transcript level of *Sh2* and *Bt2b* a one-way ANOVA was applied to compare among genotypes. Pearson correlation coefficients were employed to examine the correlation between molecular data and morphological traits (Pearson, 1985).

RESULTS

Morpho-agronomic and yield variability amongst composite corn lines: Descriptive statistics including mean and standard deviation were used to measure the quantitative traits (Table 1). To compare each of the traits among 6 corn lines, variance assessment based on RCBD was done and the outcome showed a significant difference between all the 6 corn lines for all characteristics except for ear number per plant and silking date which showed no considerable variation (Table 2).

Table 1: Basic statistics for the quantitative traits of six composite corn lines.

Trait	Mean ± SD	Minimum	Maximum
Tasseling (days)	69.56 ± 1.54	67.00	72.00
Silking (days)	74.89 ± 1.28	73.00	77.00
Plant height (cm)	181.26 ± 15.97	138.80	203.50
Ear no. /plant	2.31 ± 0.32	1.6	3.7
Ear wt. (g)	178.82 ± 3.25	172.80	181.70
Ear length (cm)	14.40 ± 1.07	12.50	15.40
No. kernel/ ear	254.11 ± 33.84	191.60	298.80
1000-kernel wt. (g)	126.28 ± 8.71	110.00	135.00
Grain yield/plant	75.13 ± 20.13	41.41	102.02

Table 2. Mean square (MS) values from ANOVA for the yield and yield components of six composite corn lines.

Source of variation	Tasseling (days)	Silking (days)	Plant height (cm)	Ear no. /plant	Ear wt. (g)	Ear length (cm)	No. kernels/ ear	1000 kernel wt.(g)	Grain yield/ plant
Genotypes	3.96**	2.09 ns	589.07**	0.16 ns	26.24*	3.66**	3215.73**	234.59**	916.85*
Block	7.72**	2.06 ns	259.74 ns	0.12 ns	0.63ns	0.031ns	106.40 ns	7.39 ns	148.25 ns
Error	0.52	2.32	86.91	0.25	4.73	0.09	317.36	10.19	200.54
F-value for Gen	7.57**	0.90 ns	6.78**	0.62 ns	5.55*	38.22**	10.13**	23.02**	4.57*
CV (%)	1.038	2.034	5.14	21.69	1.22	2.15	7.01	2.53	18.85
R^2	0.87	0.39	0.80	0.29	0.74	0.95	0.84	0.92	0.71

Principal component analysis: The statistical data were subjected to PCA where 2 components with Eigen values larger than one were extracted and these 2 components were responsible for 86.62 percent of the total variability. This proves great variation amongst the genotypic traits under examination. The PC1 (first principal component)

involved nearly all the traits excluding ear number per plant which explained 70.60 percent of the total variability. The characters with the highest positive weight on PC2 were just the ear number per plant and this component was responsible for 16.02 percent of the total variance among corn lines (Table 3). The Scatter diagram of six composite corn lines for the first two PCs score is shown in Figure 1.

Table 3. Principal components (PCs) of the morphological traits of six composite corn lines.

Trait	1st component	2nd component
Ear length	**1.00**	0.02
Ear wt.	**0.98**	0.19
No. kernels/ ear	**0.98**	0.19
1000 kernel wt.	**0.93**	0.35
Silking	**-0.92**	-0.15
Tasseling	**-0.84**	0.10
Plant height	**-0.58**	0.34
Ear no./plant	0.14	**0.97**
Eigenvalue	5.65	1.28
Proportion σ2%	70.60	16.02
Cumulative σ2%	70.60	86.63

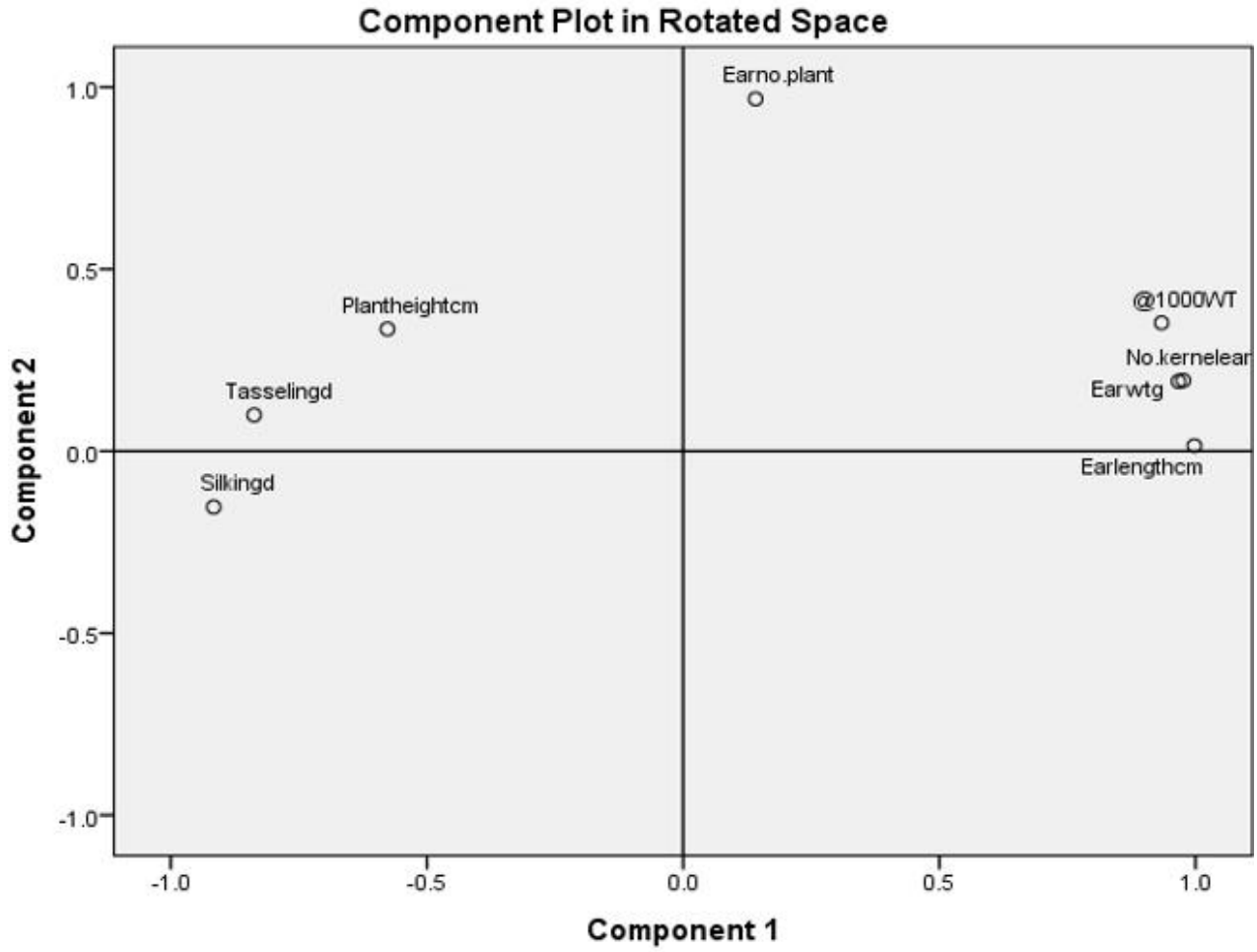

Figure 1. Scatter diagram of six composite corn lines for the first two PCs score

Using cluster assessment by unweighted pair group technique with arithmetic mean (UPGMA) method, 6 composite corn lines were categorized into 2 main groups (Figure 2).

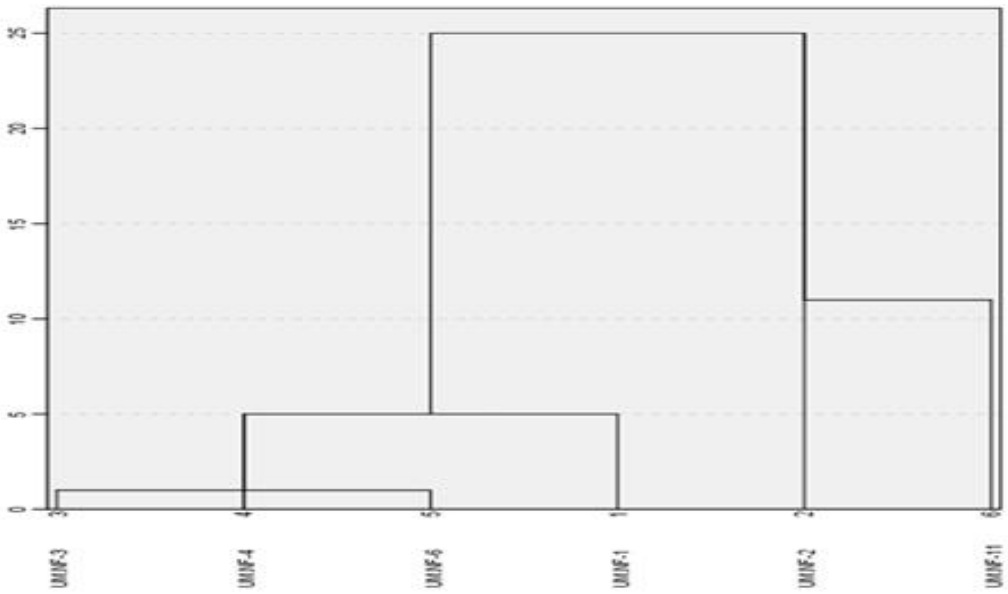

Figure 2. Dendrogram using average linkage (between groups) rescaled distance cluster combine

Genetic parameters of yield and yield components of composite corn lines: Greater variation was observed among the phenotypic and genotypic variances for the silking date, plant height, ear weight, kernel number per ear, 1000- kernel weight, and grain yield per plant suggesting an environmental influence on these traits also, indicating that these characteristics were highly inherited (Table 4). The phenotypic and genotypic coefficient of variability was greatest for yield per plant with PCV= 27.90% and GCV=20.57% respectively. Most of the traits showed high heritability, except silking (14.36%) and ear number per plant (19.23%). Maximum genetic advance (1990.12) with higher heritability (92.58) was observed in kernel number per ear. A higher heritability estimate, correlated with good estimates of genetic advance expected in the next generation for kernel number of per ear, indicated that this character is supported by additive gene effects.

Genotypic and phenotypic correlation coefficients: As per the outcome of the phenotypic correlation, the greatest positive and significant relationship of grain yield per plant was noted with 1000- kernel weight (0.84) (Table 5). Also, the yield was associated positively and significantly with ear weight (0.66^{**}), ear number per plant (0.72^{**}), kernel number per ear (0.73^{**}), and ear length (0.67^{**}).

Table 4. Genetic components of variation for morphological traits in six composite corn lines.

	Tasseling (days)	Silking (days)	Plant height (cm)	Ear no. /plant	Ear wt. (g)	Ear length (cm)	No. kernel/ ear	1000 kernel wt. (g)	Grain yield/ plant
Genotypic Variance	1.14	0.23	167.38	0.02	7.17	1.19	966.12	74.80	238.77
Phenotypic variance	1.67	1.62	254.30	0.11	11.90	1.28	1283.48	84.99	439.31
Genotypic coefficient of variation %	1.54	0.64	7.14	6.37	1.50	7.57	12.23	6.85	20.57
Phenotypic coefficient of variation %	1.86	1.70	8.80	14.52	1.93	7.86	14.10	7.30	27.90
Heritability in broad sense %	68.67	14.36	65.82	19.23	60.26	92.58	75.27	88.01	54.35
Genetic advance	2.36	0.48	344.80	0.04	14.77	2.44	1990.12	154.08	491.85
Genetic advance Expressed as percent of mean	3.39	0.64	190.29	1.92	8.26	16.98	783.20	122.09	654.76

Table 5. Estimates of phenotypic correlation coefficients (rp) among different characters of composite corn lines.

Characteristics	Tasseling (days)	Silking (days)	Plant height (cm)	Ear no. /plant	Ear wt. (g)	Ear length (cm)	No. kernel/ ear	1000-kernel wt. (g)	Grain yield/ plant
Tasseling (days)	1								
Silking (days)	0.62**	1							
Plant height (cm)	-0.21	-0.03	1						
Ear no. /plant	0.18	0.06	-0.18	1					
Ear wt. (g)	-0.46	-0.53*	-0.40	0.01	1				
Ear length (cm)	-0.51*	-0.39	-0.49*	0.05	0.83**	1			
No. kernel/ ear	-0.48*	-0.58*	-0.44	0.08	0.96**	0.85**	1		
1000-kernel wt. (g)	-0.52*	-0.42	-0.33	0.35	0.77**	0.90**	0.81**	1	
Grain yield/plant	-0.23	-0.35	-0.42	0.72**	0.66**	0.67**	0.73**	0.84**	1

* Correlation is significant at p = 0.05 level (2-tailed).
** Correlation is significant at p = 0.01 level (2-tailed).

The Grain yield was correlated significantly and positively with ear weight (0.93), ear length (0.86), the number of kernels per ear (0.94) and 1000- kernel weight (0.98) (Table 6). Grain yield also demonstrated a significant and negative correlation with the silking date (-0.81) at level P < 0.05.

Table 6. Estimates of genotypic correlation coefficients (rg) among different characters of composite corn lines.

Characteristics	Tasseling (days)	Silking (days)	Plant height (cm)	Ear no. /plant	Ear wt. (g)	Ear length (cm)	No. kernel/ ear	1000-kernel wt. (g)	Grain yield/plant
Tasseling (days)	1								
Silking (days)	0.82*	1							
Plant height (cm)	0.13	0.34	1						
Ear no. /plant	0.08	-0.24	0.09	1					
Ear wt. (g)	-.83*	-0.91*	-0.46	0.32	1				
Ear length (cm)	-0.80	-0.91*	-0.62	0.17	0.97**	1			
No. kernel/ ear	-0.75	-0.86*	-0.55	0.34	0.99**	0.97**	1		
1000-kernel wt. (g)	-0.72	-0.90*	-0.47	0.49	0.98**	0.94**	0.98**	1	
Grain yield/plant	-0.60	-0.81*	-0.41	0.64	0.93**	0.86*	0.94**	0.98**	1

* Correlation is significant at the P = 0.05 level (2-tailed).
** Correlation is significant at the P = 0.01 level (2-tailed).

The expression level of the *Sh2* and *Bt2b* genes in the different tissues of corn lines: The *Bt2b* and *Sh2* genes exhibited upregulation in the leaf and endosperm tissues of all six composite corn lines. To compare the expression levels of the *Bt2b* and *Sh2* genes in the leaf and endosperm tissues of six corn lines, an analysis of variance was done. The results show the variability of these two genes in different lines (Table 7). The expression levels of the *Bt2b* and *Sh2* genes in leaf tissue displayed significant differences among the six composite corn lines.

Table 7. Mean square values (MS) from ANOVA for *Bt2b* and *Sh2* expression level in leaf and endosperm tissues among six composite corn lines.

Source of variation	Degrees of freedom	*Sh2* (endosperm)	*Bt2b* (endosperm)	*Sh2* (leaf)	*Bt2b* (leaf)
Genotype	5	0.97ns	2.07**	2.288**	2.365 **
Error	12	0.57	0.24	0.650	0.325
CV (%)		31.50	19.348	14.981	19.667
Coefficient of determinations (R^2)	0.42	0.783	0.594	0.751	

The highest and the lowest expression level of the *Bt2b* gene in the leaf tissue was observed in UM 11 (4.08 fold) and UM 2 (1.66 fold), respectively (Figure 3).

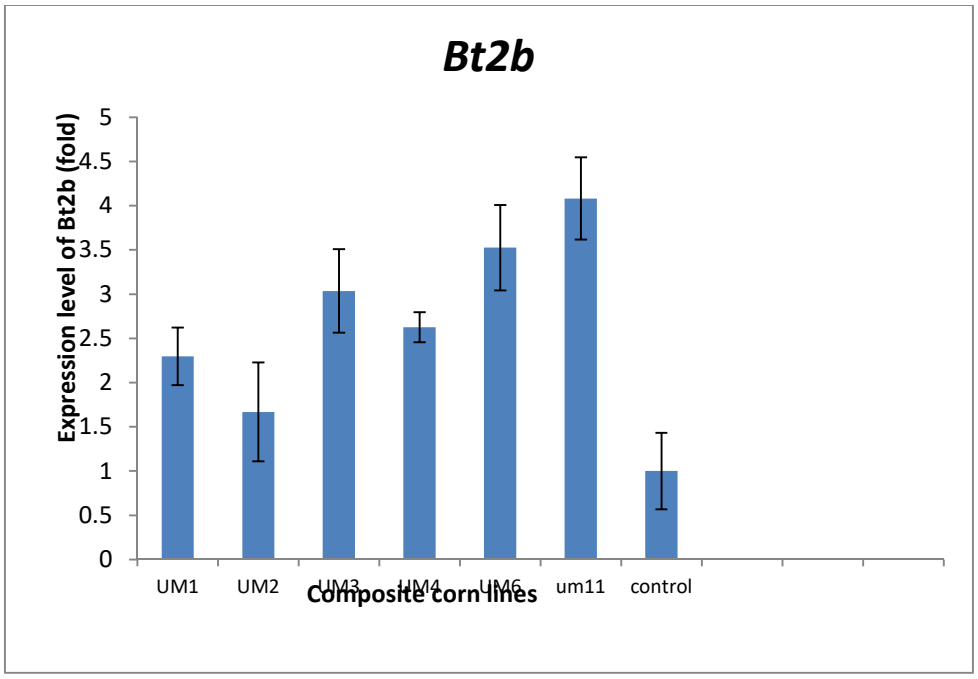

Figure 3. The relative transcript level of *Bt2b* gene in leaf tissue of six composite corn lines

The highest and the lowest expression level of the *Sh2* gene was observed in UM 1 (6.4 fold) and UM 3 (4.03 fold), respectively (Figure 4). A comparison of the *Bt2b* and *Sh2* gene expression levels indicated that the relative transcription level of the *Sh2* gene in the leaf tissue of all six composite corn lines was in a higher range than the *Bt2b* gene.

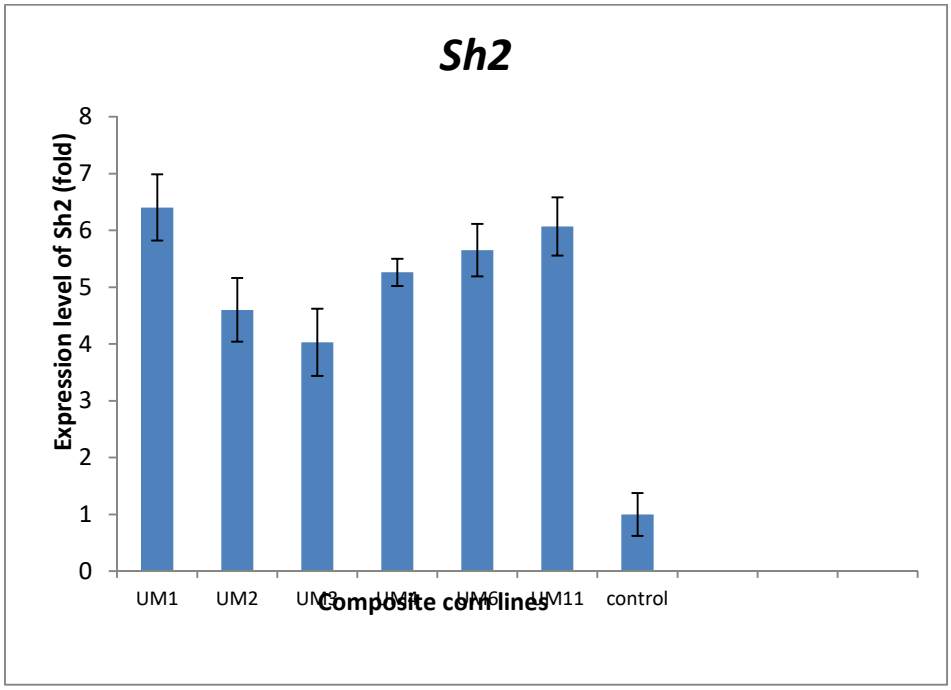

Figure 4. The relative transcript level of *Sh2* gene in leaf tissue of six composite corn lines

Similarly, the highest expression level of *Bt2b* in the endosperm tissue was observed in UM 1 (3.96 fold) (Figure 5). The results indicated significant differences in the expression level of the *Bt2b* gene in the leaf and endosperm tissues among six composite corn lines (Table 7).

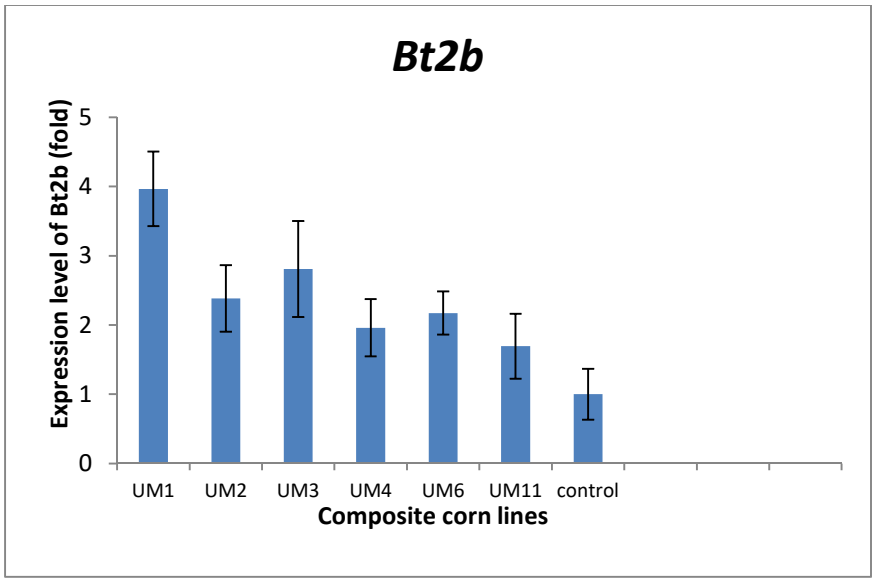

Figure 5. The relative transcript level of *Bt2b* gene in endosperm tissues of six composite corn lines

The highest expression levels of the *Sh2* gene in endosperm tissues were observed in UM 3 (3.05 fold) (Figure 6). The outcomes did not display the considerable differences for the expression level of the *Sh2* gene in the endosperm tissue meanwhile the expression level of this gene was significant in the leaf tissues between six composite corn lines.

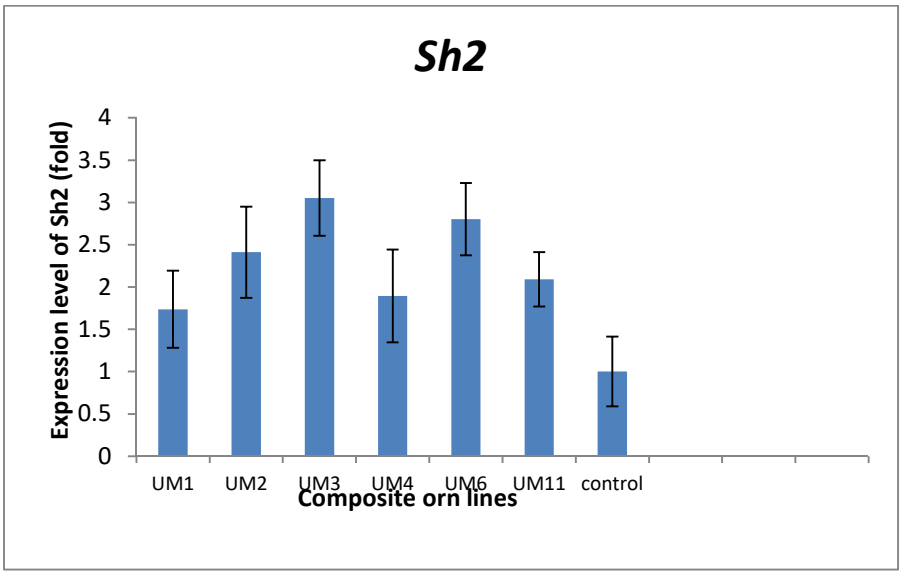

Figure 6. The relative transcript level of *Sh2* gene in endosperm tissues of six composite corn lines

The relationship between yield and yield components with expression Level of *Sh2* and *Bt2b* genes were evaluated under tropical (Malaysia) and dry land (Iran) environments. As per the outcomes of the correlation coefficients, the level of expression of the *Bt2b* gene for leaf tissue demonstrated non-significant with all traits while, for endosperm tissue, it was positively and significantly associated with ear number per plant (0.69), ear weight (0.87), ear length (0.87), kernel number per ear (0.75), 1000- kernel weight (0.93) and grain yield/plant (0.90) (Table 8). The level of expression of the *Sh2* gene for leaf tissue demonstrated a positive but non-significant association with the ear length, ear weight, ear number per plant, 1000- kernel weight, kernel number per ear, and yield. Also, for endosperm tissue, it was positively and significantly associated with ear number per plant (0.63), ear weight (0.74), ear length (0.75), 1000- kernel weight (0.71), and grain yield (0.66).

Table 8. Relationship between morphological characterizations and Expression level of yield-related genes among six composite corn lines at two test locations.

	Sh2 (Leaf)	***Bt2b*** (Leaf)	***Sh2*** (Endosperm)	***Bt2b*** (Endosperm)
Tasselling (days)	-0.08	-0.057	-0.89**	-0.69*
Silking (days)	-0.27	-0.14	-0.79**	-0.72**
Plant height (cm)	-0.28	0.08	-0.70*	-0.92**
Ear number /plant	0.243	-0.18	0.63*	0.69*
Ear weight (g)	0.44	-0.04	0.74**	0.87**
Ear length (cm)	0.45	-0.06	0.75**	0.87**
Number kernel/ ear	0.38	-0.08	0.55	0.75**
1000-kernel weight (g)	0.31	0.02	0.71**	0.93**
Grain yield/plant	0.33	-0.17	0.66*	0.90**

* Significant at $P \leq 0.05$ level
** Significant at $P \leq 0.01$ level

DISCUSSION

Grain yield is a quantitatively inherited trait, therefore, selection using yield component characters as the basis may be more efficient and reliable (Alvi *et al.,* 2003). The important characteristics include kernels per ear, grain yield per plant, and 1000- kernel weight. These traits showed high variation. Overall, this revealed that selection for these traits may be helpful in effectively formulating high yielding varieties of corn. First-order yield characteristics of corn include kernels per ear, number of ears (or ears per plant), and kernel weight. According to Fageria *et al.,* (2006) one can often refer to first-order yield components as primary components. They directly affect the final yield and also have indirect effects via yield components that develop later (Fageria *et al.,* 2006).

This study clarifies the morpho-agronomic variability of selected composite corn lines under dry climate. Meanwhile, for more stability, the relationship between the expression level of two yield-related genes was evaluated under tropical and dryland environments. The primary differences seen among the composite corn lines for the majority of the assessed traits revealed a high degree of genetic variability among the inbred lines. The wide gap between the minimum and maximum values for each trait indicated substantial variation among the composite corn lines under examination. Such variations present corn breeders with the opportunity to enhance traits of interest through hybridization, selection, and recombination of favorable genotypes. Based on Duncan's multiple range test 1000- kernel weight and grain yield per plant as two main traits, showed the highest and the lowest mean comparison were observed in UM 4 and UM 2, respectively.

When the correlation coefficients were considered, it was determined that the coefficients of genotypic correlation were higher compared to the corresponding coefficients of phenotypic correlation. However, both the phenotypic and genotypic correlation coefficients demonstrated almost similar directions. The genotypic correlation displayed that an increase in primary yield traits like ear weight, ear length, number of kernel per ear and 1000- kernel weight, grain yield per plant also showed an increase but increase in tasseling and silking dates, and plant height decreased grain yield per plant. So based on the phenotypic and genotypic correlation results, it seems logical to select for the plant with a short period for tasseling and silking, shorter corn lines for lodging resistance associated with high grain yield.

Genetic traits like coefficients of variability, variance components, genetic advance, and heritability offer genetic variation estimates for quantitative traits. Ear length, kernel number per ear, and grain yield per plant demonstrated high values of genotypic coefficient of variation and phenotypic coefficient of variation. For the majority of the cases, traits that possessed higher phenotypic coefficients of variation also demonstrated a higher genetic coefficient of variation, which is an indicator of the environment's minimum effect on the trait's phenotypic expression.

Most of the traits assessed demonstrated high broad-sense heritability (H_2) estimates. It is fairly easy to select for such traits. Consequently, selection for a character with low heritability may be challenging or impractical since the environment has a masking effect on genotypic effects (Singh, 2015). For instance, the simple selection of plants that possess higher 1000-kernel weight can be successful in enhancing the trait. Moreover, if the character has a very high heritability, 0.8 or more, character selection should be fairly simple (Singh, 2015).

The presence of broad morphological variations among lines was proven further using principal component analysis. Two components possessing Eigen values higher than one were extracted. It was then determined that these two components had a total variability value of 86.62%. It was reported by Beyene *et al.,* (2006) that in 62 traditional Ethiopian highland corn

accessions, 71.8% of the total variation can be attributed to the first four PCs. According to these authors, ear height, thousand kernel weight, number of kernels per row, had a major contribution to the total variation (Beyene *et al.,* 2006). Cluster analysis can be used to determine genotype differences for the breeder through the classification of genotypes (Sabaghnia *et al.,* 2012). The six composite corn lines were classified into 2 major groups. UM 1, UM 3, UM 4 and UM 6 belonged to the same group while, UM 2 and UM 11 were in the same group. One may select representative lines for the hybrid program from specific groups with other approved varieties. For example, Lucchin *et al.,* (2003) clustered 20 Italian flint corn landraces and categorized them into smaller groups using agronomic and morphological traits. Beyene *et al.,* (2006) categorized 62 traditional highland corn accessions and divided them into three groups based on 15 morphological traits.

The expression levels of *Sh2* and *Bt2b* genes (encoding the enzyme AGPase) in newly developed UM composite corn lines were evaluated to consider the possibility of improving the starch content and increasing overall yield by multigene engineering. Their overexpression impact was investigated on the UM composite corn lines. It was reported by Hannah *et al.,* (2012) that most efforts at improving starch accumulation in plants were mainly focused on engineering AGPase activity. To estimate the effect of hybridization and experimental treatment on the expression of an endogenous reference or internal control, the method proposed by Livak and Schmittgen (2001) was used. This method was also used to estimate the effect of hybridization on the target genes (*Sh2* and *Bt2b*). The highest expression level of *Sh2* in leaf tissues was observed in UM 1 among the six composite corn lines and the expression was 6.4 fold. The upregulation of the *Sh2* gene in 1000- kernel weight and grain yield per plant in UM 1 can be due to the positive correlation between the expression level of *Sh2* and 1000- kernel weight and grain yield per plant. The highest expression level of *Bt2b* in leaf tissue was observed in UM 11 (4.08 fold).

As expected, *Bt2b* was weakly expressed in leaf tissues, but the strong expression was found in endosperm tissue, this result was agreed with Cossegal *et al.,* (2008). The results of the comparison of the *Bt2b* expression level of endosperm tissue are an indicator that within the six composite corn lines, UM 1 had the highest expression level with 3.96 fold. The *Bt2b* gene upregulation for grain yield per plant and 1000- kernel weight in UM 1 can be a result of the positive and significant correlation between the *Bt2b* expression level and yield per plant and 1000- kernel weight.

The highest expression level of the *Sh2* gene in the endosperm tissue of composite corn lines was observed in UM 3. The results indicated non-significant difference in the expression levels of *Sh2* in the endosperm tissue of six composite corn lines. A comparison of the relative expression levels for *Bt2b* and *Sh2* in endosperm tissue indicated that the expression level of *Bt2b* was in a higher range than that of *Sh2*. According to the results, the *Sh2* gene was strongly expressed in leaf tissue and its expression was in a higher range than the transcript level of *Bt2b* but, *Sh2* was moderately expressed in endosperm tissue. As expected, *Bt2b* was weakly expressed in leaf tissues, but the strong expression was found in endosperm tissues, this result was agreed with Cossegal *et al.,* (2008).

Relationship studies indicated that increasing the tasseling and silking periods and plant height significantly decreased the expression levels of the *Bt2b* and *Sh2* genes in endosperm tissue. Hence, it seems logical to select corn lines with short periods of anthesis and silking and short plant stature to express high levels of *Bt2b*and *Sh2* in endosperm tissue to increase the overall yield.

In conclusion, the direct effect of yield components on available grain yield per plant and improvement in their genetic parameters can presumably enhance kernel starch content of the composite corn lines. Jiang *et al*., (2013) reported multigene transformation was carried out via assembling plasmid constructs as vector to produce the transgenic plants from a maize elite inbred line. They observed that overexpression of four genes, including *Sh2* and *Bt2* in their transgenic maize lines, caused improvement in the agronomic traits, such as a 20.1–34.7 % increase in 100-grain weight, a 13.9–19.0 % increase in ear weight, and larger kernels also 2.8–7.7 % enhancement in the endosperm starch content (Jiang *et al*., 2013). The genetic advance expected for 1000- kernel weight, kernel number per ear, and grain yield per plant indicated that additive gene effects supported these characters, making them the best for phenotypic selection. The composite corn lines that were obtained can be utilized for effective breeding programs to develop desirable varieties with high productivity.

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