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Development of new early maturing and high yielding bread wheat lines for growing in newly reclaimed desert areas of Egypt

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Conventional wheat breeding programs aim at developing high yielding cultivars suitable for sowing under stress conditions and poor soils especially in newly reclaimed deserts. The objective of the current study was to develop high yielding and early maturing bread wheat (*TRITICUM AESTIVUM* L.) lines adapted to grow in old and new reclaimed desert soils of Egypt. Expressed Sequence Tags (EST) markers were used to detect the genetic variability at molecular level among the new lines as compared to their parents. The promising five lines were evaluated for two years at three locations that is Assiut, Qena and Kafer EL-Shekh districts of Egypt. Grain yield differed significantly among the evaluated wheat lines. Inbred lines Assiut-1, Assiut-2 and Assiut-3 gave significantly higher grain yield as compared with check variety (Giza-168). EST markers showed that the parents and the studied lines had a wide range of genetic variation. Consequently, the EST analysis could be used to detect the genetic variability among parents and the studied lines.

Key words: Bread wheat, expressed sequence tags (EST), grain yield.

INTRODUCTION

Population increase is taking place at an alarming rate of 2.52 to 8% per annum in Egypt. This tremendous increase in population is one of the most serious challenges, the country is facing these days. It hinders efforts of all developmental programs. In addition, majority of Egypt lands are deserts. Newly reclaimed soils in these deserts suffer from various stresses such as drought, salinity and nutrients deficiency etc. (El-Fouly et al., 1984). Therefore, it is imperative to increase yield per unit area of various crops by developing high yielding cultivars suitable for sowing on poor soils and under stress conditions as well as the development and application of improved cultural practices.

Development of tolerant cultivars suitable for sowing under stress conditions is an important objective of many wheat breeding programs. However, limited success has been achieved due to inadequate screening techniques

and lack of genotypes that show clear differences in response to various environmental stresses (Bruckner and Froberg, 1987). Stress tolerant genotypes of major food crops could be developed through breeding for wide (Fischer et al., 1982) or specific adaptation (Hurd, 1975) as well as through the incorporation of certain morphological and/or physiological traits that confer tolerance under stress situations (Blum, 1979; Richards, 1982).

The impact of genotype x environment (G x E) interaction on the relative performance and stability of genotypes across environments is so huge that it is always a challenge for the breeder in developing superior cultivars having broad adapted (Eberhart and Russell, 1966; Asif et al., 2003; Rasul et al., 2006). The mechanisms through which environmental stresses affect plant metabolism, thereby reducing plant growth and development, are still not completely understood (Pessaraki, 1994). Stability of a variety is its ability to hold certain morphological and physiological attributes steady and allowing others to vary. These attributes are

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inherited and are greatly influenced by the environment (Blum, 1979; Polignano and Ugenti, 1984; Polignano et al., 1989). Several molecular markers were used to study the genetic variability and relationship among wheat germplasm (Zhang et al., 2000; Cao et al., 2000; Taghian and Abo-Elwafa, 2003). ESTs "Expressed Sequence Tags" are some of them. ESTs are derived from coding DNA, which generally have a high degree of sequence conservation. They are more likely to be transportable across pedigree and species boundaries than the markers derived from non-expressed sequences (Lazo et al., 2004). Therefore, ESTs can be used in studying genetic variability, QTL analysis, map-based cloning and synteny analysis among related species. The objective of the current study was to develop high yielding and early maturing bread wheat lines adapted to grow in the old and new reclaimed soils in Egypt using EST markers. Furthermore, EST markers were used to detect the genetic variability at molecular level among some F5 families compared to their parents.

MATERIALS AND METHODS

Two lines derived from bi-parental cross and three lines derived from multi-parental cross of wheat (*Triticum aestivum* L. end Thell) were selected according to earliness and higher yield performance at Assiut University. Experimental Farm using a bulk-pedigree method. The original parents which involved in these crosses included two local varieties namely; Sakha-69 and Giza-164, and three exotic varieties Pitic-62, Sonora-64 and Tokowe. Steps involved in development and evaluation of these lines are given in Figure 1.

Development of lines derived from bi-parental cross

During the 1991/92 growing season, one cross (Sakha 69 x Giza 164) was made in clay-loam soil of Assiut University experimental farm to obtain the F1 seeds. Then, the F2 grains population was sown during 1993/94 season to obtain the F2 plants. In the coming seasons from the third to fifth generation (1994/95 and 1998/99), bulked seeds were planted in a non-replicated experiment. In the F6 generation during 1999/2000, single plants were planted in rows (3 m long, 25 cm apart, 15 cm between seeds within row). All cultural practices were carried out as recommended for successful wheat production.

At harvest, the best 10% plants in grain yield were selected. During 2000/01 growing season, the selected families as well as original parents were sown in a randomized complete block design with three replications. Each plot was a single row 3 m long, 25 cm apart and 15 cm between grains within row. At harvest, the best plants from each of best 10% families in yield were selected. During 2001/02 growing season, the highest 20 selected F8 families for grain yield, as well as original parents were sown in a randomized complete block design with three replications. All cultural practices were carried out as recommended for successful wheat production. At harvest, the 3 best lines in yield and earliness were selected.

Development of lines derived from multi-parental crosses

In this experiment, multiple parental cross was made between the

five varieties, Sakha 69, Giza 164, Sonora 64, Pitic 62 and Tokowe (A: Sonora 64 x Pitic 62) and (B: Tokowe x Giza 164) were achieved, then the F1's of these crosses were crossed together in the 1990/91 season to obtain (A x B) hybrid seeds. Finally, the F1 of the cross (Ax B) was cross pollinated with Sakha 69 at 1991/92 season to obtain the F1 seeds of multi-parental cross. The F1 seeds of the multi-parental cross were planted in the next season (1993/94) to produce the F2 seeds. During the coming seasons (1999/95 and 1998/99) from F2 to F5 generation, the progeny of the crosses were grown in a non-replicated experiment. During the (1999/2000) season, 2000 plants in F6 generation were space planted in rows (3 m long, 25 cm apart and 15 cm between seeds within row). All cultural practices were carried out as recommended for wheat production. At harvest, the best 10% plants in grain yield were selected. During 2000/01 growing season, the best 2000 families as well as their parents were grown in a randomized complete block design of three replications. Each plot was a single row 3 m long, 15 cm apart and 15 cm between seeds within row. At harvest, the superior plants from each of the best 20 families were selected. During 2001/02 growing season, the highest 20 selected lines for grain yield were planted in a randomized complete block design and at harvest, the 10 superior lines in grain yield and earliness were selected.

Evaluation of the lines

Three lines derived from bi-parental cross and five lines derived from multi-parental cross of wheat as well as check cultivar were grown in a preliminary yield trail at Sohag experimental farm, faculty of agriculture, south valley university during the succeeding growing seasons of 2002/03 and 2003/04. Three levels of sprinkler irrigations were applied under calcareous soil. During 2004/05, 2005/06 and 2006/07 seasons, the three and five superior lines derived from bi-parental and multi-parental crosses, respectively as well as Sakha-8 and Giza-164 were grown under sprinkler irrigation at Sohag experimental farm. Yield trails were continued at three locations for further two successive seasons of 2007/08 and 2008/09. The superior five lines; three lines derived from multi-parental and two lines derived from bi-parental crosses, were grown in yield trails at three locations of Assiut, Qena, and Kafer El-shekh. The yield trails were laid down in a randomized complete block design with three replications. The experimental unit was 10.5 m². All cultural practices were carried out as recommended for wheat plant production.

Fingerprinting using EST marker

The genetic variations under DNA level of the best five lines in yield that is Assiut-1 (103), Assiut-2 (1006), Assiut-3 (B-52), Line 95 and Lines 92 and their four parents (Sonora-69, Giza-164, Sakha-69 and Tokowe) were analyzed. Genomic DNA was extracted and purified from fresh leaf samples using Dneasy Plant Kit (QIAGEN).

Purified DNA was quantified using agarose mini gel electrophoresis. On the basis of sequences of four wheat EST markers, PCR primer pairs were designed and used to screen the polymorphism among the studied lines. The primer sequences are shown in Table 1. PCR was carried out under the following conditions; one initial strand separation cycle at 94°C for 4 min followed by 32 cycles including a denaturation step at 94°C for 30 s, an annealing step at (50 to 60°C, depending on the primer sequence) for 30 s and polymerization step at 72°C for 1 min, with final extension step at 72°C for 5 min. Reactions were performed in total volume of 25 µl. DNAs of PCR products were digested with two restriction enzymes *Rsa*I and *Ap*aI. Digested DNAs were loaded on 2% agarose gel.

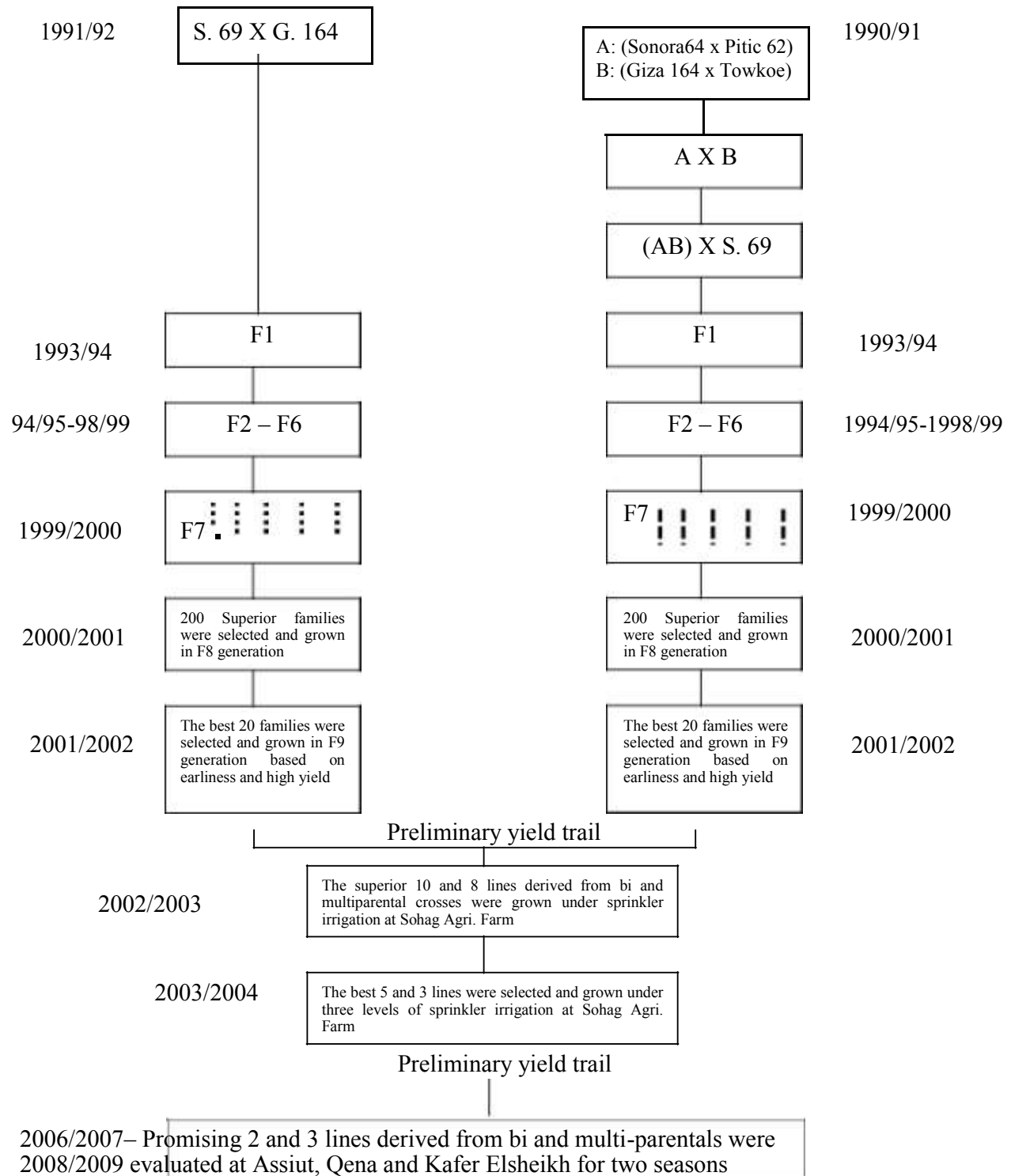


Figure 1. Breeding steps involved in developing the new bread wheat lines.

RESULTS AND DISCUSSION

The combined statistical analysis of variance (Table 2) over the six environments, showed highly significant

differences among the tested genotypes for all the studied traits, indicating a wide range of genetic variability for such traits. Such high significant differences among environments usually indicate the wide range in climatic

Table 1. EST forward and reverse primer pairs sequences.

	EST marker	Primer sequence
1	P04G09860	For.: 5' ACCTTCTCAACGGGTTGATTC 3' Rev.: 5' GCTTCTTTATCCTCGGCTTCT 3'
2	P04G05030	For.: 5' GACATATTTGGAGAACTGTGATCG 3' Rev.: 5' TTCATCCGTTGCAGCTCTTTA 3'
3	P04G14790	For.: 5' AATTCTGAGCAAGCACGGTAA 3' Rev.: 5' TCAGCAAAGAACACGGAAAGT 3'
4	P04G11880	For.: 5' TGGTCGTATGGGAAATGCTAA 3' Rev.: 5' GAGGCAAGACTGATGATACGC 3'

Table 2. Mean squares of environment, genotypes, genotypes x environment and error for the studied traits.

S.O.V	D.F.	Heading days	Plant height (cm)	Spike length (cm)	Spikes/m ²	Grains/ Spike	1000-Kernel Weight (g)	Grain yield kg/ha
Enviro.,	5	649.6**	2014.9**	45.6**	48611.9**	657.7**	4533.2**	84.7**
Rep/Env.	12	123.6	135.2	57.5	1195.5	112.3	97.7	62.7
Genotypes	5	128.8**	363.7**	7.0**	8729.5**	551.6**	139.6**	42.1**
Gen x Env	25	14.4**	41.6**	0.7**	485.5**	46.0**	134.7**	1.3**
Error	60	0.01	1.9	0.001	0.71	0.07	0.03	0.004

and edaphic factors prevailing at different environments.

Moreover, genotypes responded differently to changes in environmental conditions, as genotype x environment interaction mean squares were highly significant for all the studied traits. Rasul et al. (2006), Asif et al. (2003) and Al-Otayk (2010), suggested the importance of the assessment of genotypes under different environments in order to identify the best genetic make up for a particular environment.

Performance of genotypes under different locations over two seasons

Results in Table 3, showed that all the five inbred lines (Assiut-1, Assiut-2, Assiut-3, Line 92 and Line 95) produced heads earlier as compared with the check variety Giza-168 at all locations. Among the five inbred lines, Assiut-3 was the earliest line in producing heads under Assiut and Qena agro-climatic conditions, while under Kafer El-shekh agro-climate, the inbred line Assiut-1 was the earliest one (Table 3). Interestingly, days to heading at Qena for the all genotypes were shorter as compared with Assiut and Kafer El-shekh by about 11 and 9 days, respectively. Generally, weather at Qena is warmer than that in Assiut and Kafer EL-shekh, thus the warm weather assisted the plants to grow faster and head earlier. These results are in agreement with those

found by Porceddu (1976) and Tripathi et al. (1973).

Number of days from planting to heading were converted as percentage and presented as pars. As shown in Figure 2, percentage of earliness in comparisons with the check variety Giza-168, over the six environments was negative and differed significantly. The highest negative percentage (-11.38%) was obtained from line Assiut-3 while the lowest percentage (-5.79%) was obtained from Line 95. Regarding plant height, all the selected inbred lines were taller significantly than the check variety Giza-168 at all locations. Line Assiut-3 and Line 95 gave the tallest plants (106.7 cm) among genotypes as a mean over three locations (Table 3). These two lines increased plant height by 17.9% as compared to the check variety Giza-168 (Figure 3). All genotypes gave shorter plants at Qena as compared with the other two locations (Table 4). This could be explained by the shorter growing conditions at Qena, which led to the development of short plants. These results were in line with what were obtained by Dencic et al. (2000). No inbred line surpassed the check variety Giza-168 significantly for spike length, as it gave the highest spike length (14.7 cm) (Table 3). However, among all the selected inbred lines, line 92 gave the highest spike length (13.5 cm) which was 8.39% shorter than check variety, while Assiut-1 gave the shortest spike length (12.1 cm) which was 17.46% shorter than check variety (Figure 4).

Table 3. Mean performance of the five new inbred lines and a check variety Giza 168 of wheat over two seasons and three locations.

Lines	Traits						
	Heading Date (day)	Plant Height (cm)	Spike Length (cm)	Spikes/ m ²	No. of grains/Spike	1000 Kernel weight (g)	Grain yield kg/ha
Assiut-1 (103)	77.73	103.20	12.13	470.00	79.63	49.5	8357.1
Assiut-2(1006)	79.93	105.97	12.67	470.17	82.73	49.5	8250.0
Assiut-3 (B-52)	77.63	106.7	13.30	446.93	73.10	47.7	7857.1
Line-95	82.37	106.70	13.07	407.77	68.53	53.8	6964.3
Line-92	80.30	103.67	13.47	400.10	62.00	51.5	6600.0
Giza-168	87.60	90.50	14.70	422.83	68.80	45.7	7071.4
LSD(0.05)	2.61	4.42	0.60	15.13	4.66	7.97	282.1

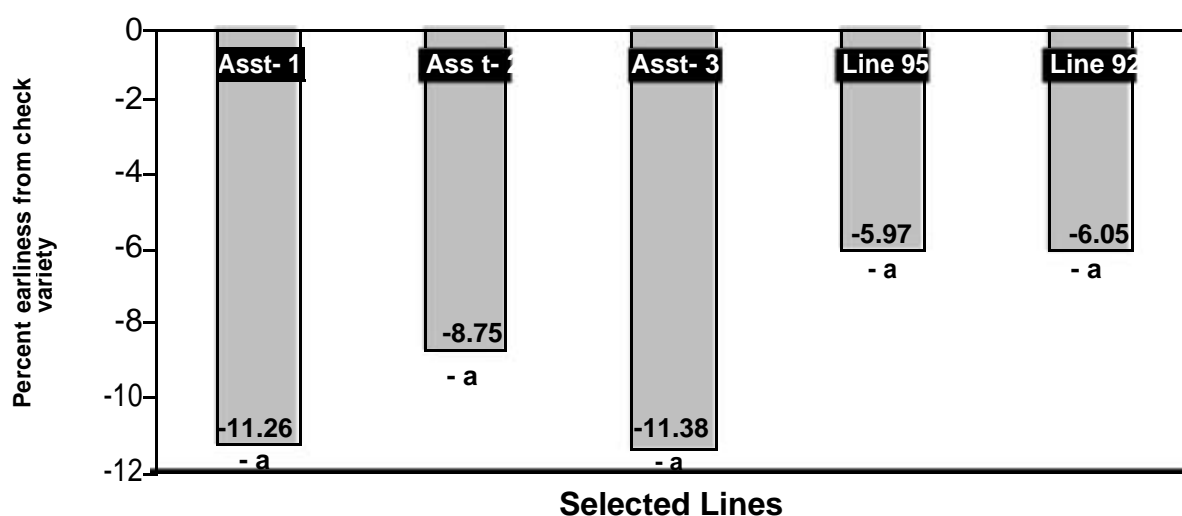
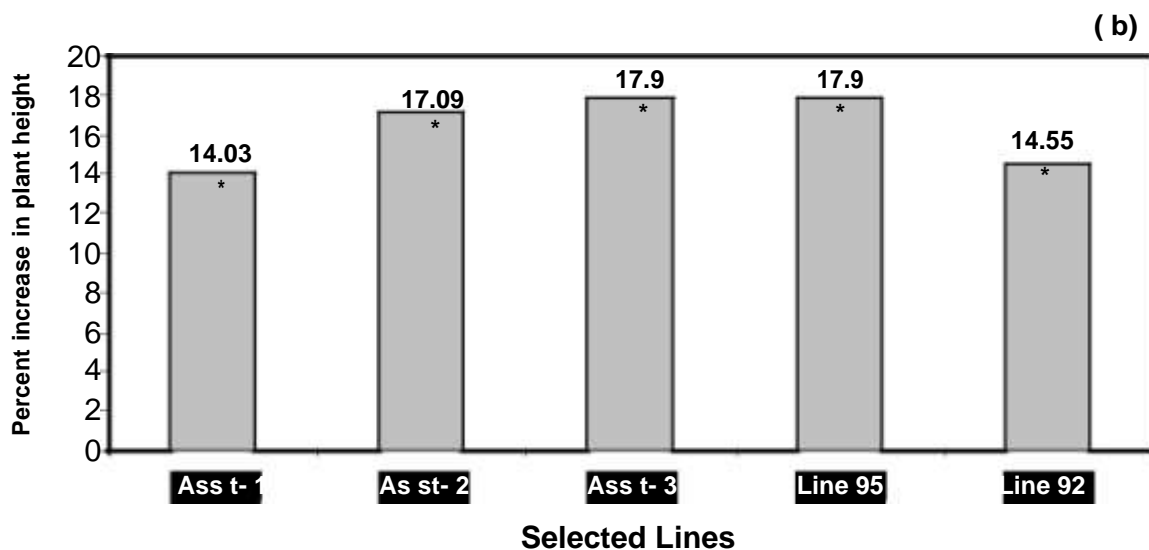
**Figure 2.** Percent decrease in days to heading of the new lines as compared with the check variety Giza-168 over six environments. - a: Significantly lower than Giza 168.**Figure 3.** Percent increase in plant height of the new lines as compared to the check variety Giza- 168 over six environments. *Significantly higher than Giza 168.

Table 4. Mean performance of five new lines and a check variety Giza-168 of wheat over two seasons at Assiut, Qena and Kafr-Elshekh locations.

Trait	Heading days			Plant height (cm)			Spike length (cm)			Spikes/m ²			Grains/spike			1000-Kernel weight (g)			Grain yield kg/ha		
	Assuit	Qena	Kafr-Elsh.	Assuit	Qena	Kafr-Elsh.	Assuit	Qena	Kafr-Elsh.	Assuit	Qena	Kafr-Elsh.	Assuit	Qena	Kafr-Elsh.	Assuit	Qena	Kafr-Elsh.	Assuit	Qena	Kafr-Elsh.
ASST-1(103)	80.6	71.6	81.0	110.1	87.1	112.4	12.9	10.3	13.2	514.5	409.5	486.0	88.2	70.4	80.3	48.8	47.9	51.8	8964.3	7250.0	8821.4
ASST-2(1006)	83.9	73.7	82.2	112.4	92.3	113.2	13.5	10.8	13.7	507.5	406.0	497.0	90.4	71.3	86.5	48.7	48.1	51.6	8892.9	7357.1	8535.7
ASST-3(B-52)	79.5	69.8	83.6	114.5	93.5	112.0	14.5	11.2	14.2	493.5	381.5	465.5	74.5	65.2	79.6	47.0	46.3	49.8	8642.9	6750.0	8178.6
LINE- 95	87.8	76.9	82.4	115.4	98.0	106.7	14.7	11.4	13.1	458.5	357.0	407.8	72.7	64.4	68.5	52.0	48.6	60.7	7571.4	6357.1	6964.3
LINE- 92	89.2	75.4	82.3	113.3	94.0	103.7	14.6	12.3	13.5	441.0	359.2	400.1	65.3	58.7	62.0	50.6	47.5	56.4	7000.0	6000.0	6500.0
G-168CHECK	92.3	79.8	90.7	95.1	80.0	96.4	15.8	12.6	15.7	452.5	362.5	453.5	68.8	63.8	73.8	45.0	44.9	47.2	7785.7	6250.0	7178.6
LSD(0.05)	2.5	1.8	2.4	3.4	3.1	4.1	1.2	0.8	1.3	8.7	6.7	7.9	3.6	3.4	3.2	2.0	2.1	1.8	678.6	464.3	500.0

Regarding number of spikes/m², the data in Table 3 revealed that three inbred lines (Assiut-1, Assiut-2 and Assiut-3) significantly surpassed the check variety. On the other hand, Line 92 and Line 95 gave less number of spikes/m² than the check variety. The highest number of spikes that is 470.2 and 470 were obtained from inbred lines Assiut-2 and Assiut-1, respectively. These lines increased with 11.2 and 11.16% as compared with the check variety, respectively (Figure 5). However, Line 92 and Line 95 decreased by 5.4 and 3.6% in no. of spikes/m² as compared with the check variety Giza-168, respectively. About the variation among various agro-climatic conditions, plants grown at under Assiut conditions gave the highest number of spikes/m² followed by those at Kafer El-shekh, while the lowest number of spikes/m² was obtained from plants grown at Qena. Larger variations in different agronomic traits have been reported in wheat (Porceddu, 1976; Tripathi et al., 1973). Mahmoud and Ahmed (2005) evaluated different wheat genotypes and detected large differences among wheat genotypes in number of spikes/m² under different environments. The selected inbred lines differed significantly in number of grains/spike at all locations (Table 4). Inbred lines

Assiut-1, Assiut-2 and Assiut-3 surpassed significantly the check variety Giza-168 in no. of grains/spike (Table 3).

On the other hand, the check variety surpassed Line 92 significantly in number of grains/spike. At Qena, plants recorded the lowest number of grains/spike, while the highest no. of grains/spike was obtained at Assiut followed by Kafer El-shekh location. Decrease in grains/spike under Qena condition could be attributed to the viability of pollen grain, pollination and fertilization process that were probably harmfully affected by the higher temperature at Qena. The negative effect of high temperature on the number of grains was reported by many researchers including Porter and Gawith (1999) and Spiertz et al. (2006). Figure 6 showed that inbred line Assiut-2 produced 20.25% higher number of grains/spike over the six environments as compared with the check variety Giza-168, while Line 92 produced 9.88% less number of grains/spike over six environments as compared with the check variety. Highly significant differences were observed in seed index of the inbred lines compared to the check variety Giza-168 as indicated by 1000 kernel weight. The lowest 1000-kernel weight (45.7 g) averaged over the six environments was

obtained by the check variety, while Line 95 gave the highest 1000-kernel weight of 53.8 g averaged over the six environments (Table 3). This value was 17.7% higher than that of the check variety (Figure 7). Concerning the different locations, the highest 1000-kernel weight was obtained from plants grown at Kafer El-shekh. These results could be attributed to the relatively low temperature and high humidity at the site during the heading and maturity stages. Thus, the grain filling was good and produced larger size of grains. On the other hand, decrease in 1000 kernel weight at Qena due to the high temperature that led kernels to reach maturity stage before complete filling (Porter and Gawith, 1999; Spiertz et al., 2006; Menshawy, 2007; Al-Otayk, 2010).

The most important component in yield is grain yield per plant. Many complex morphological features and physiological processes of the growth and development of the crop lead to the final grain yield per plant. The grain yield differed significantly among the wheat inbred lines (Table 2). Inbred lines Assiut-1, Assiut-2 and Assiut-3 gave grain yield significantly higher than that of the check variety. On the other hand, two inbred lines (that is Line 95 and Line 92) gave grain yield lower than that of the check variety at all the three

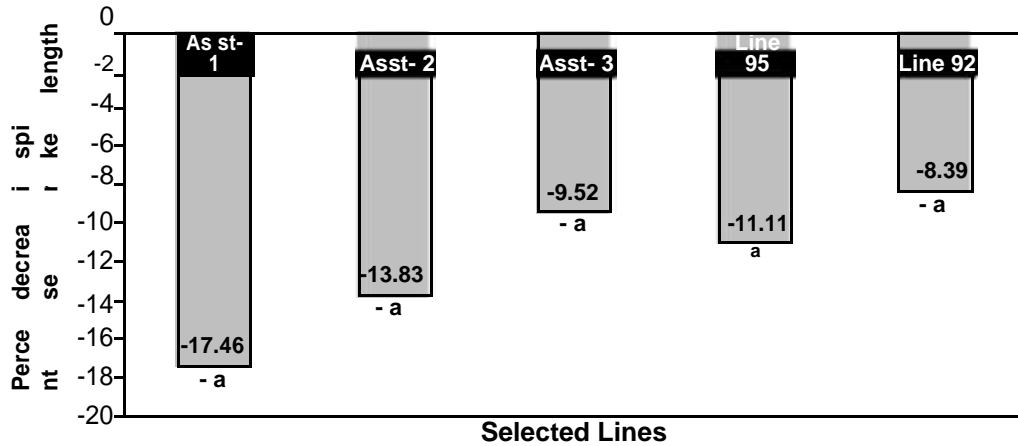


Figure 4. Percent decrease in spike length of the new lines as compared to check variety Giza-168 over six environments.- a: Significantly lower than Giza 168.

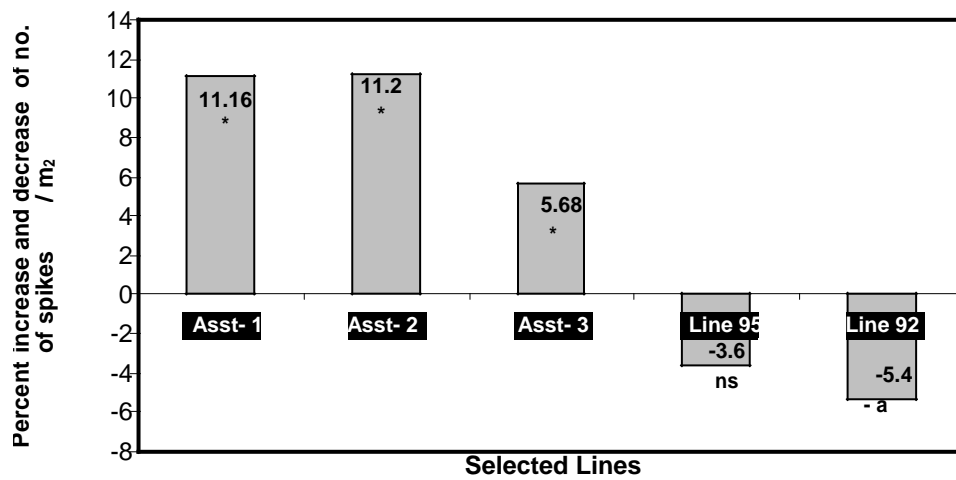


Figure 5. Percent increase and decrease in number of spikes/m² of the new lines as compared with Giza-168 over six environments. *Significantly higher than Giza 168; - a: Significantly lower than Giza 168; ns: Not significant compared to Giza 168.

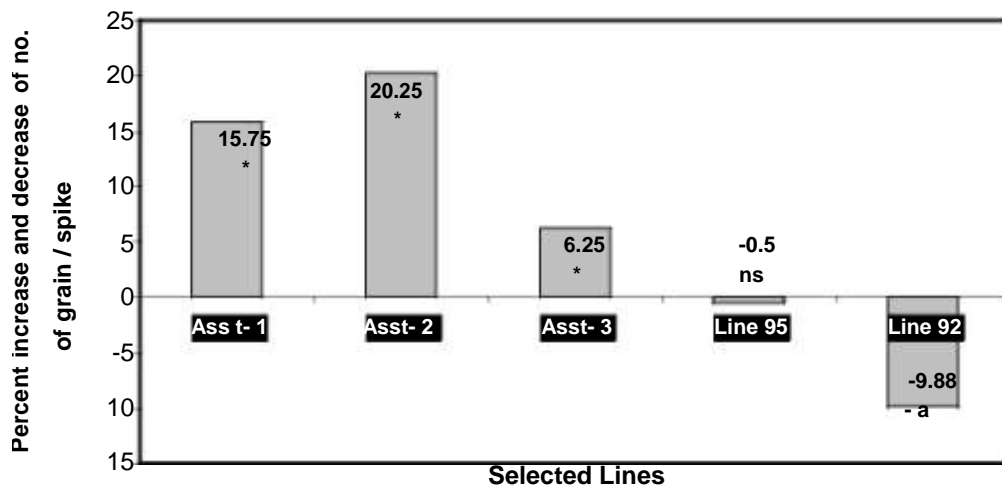


Figure 6. Percent increase and decrease in number of grains/spike of the new lines as compared to the check variety Giza-168 over six environments.

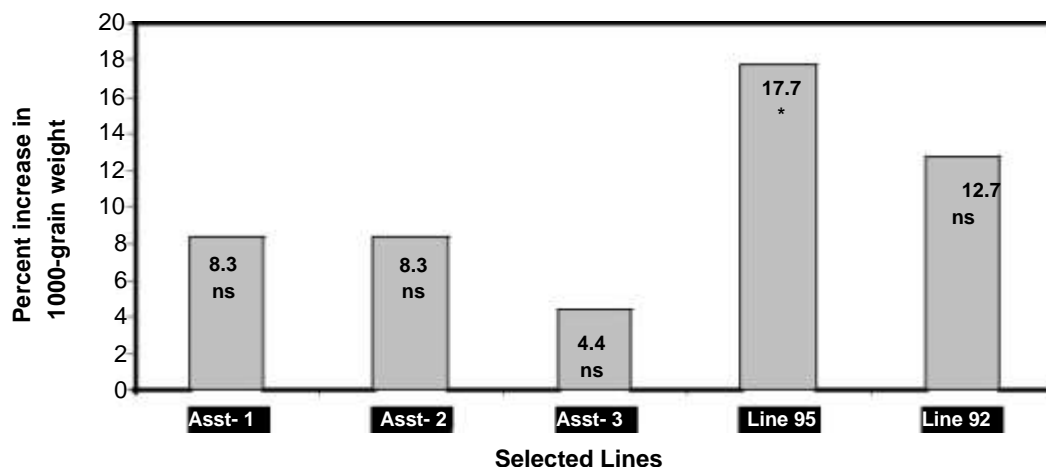


Figure 7. Percent increase in 1000-kernel weight of the new lines as compared to the check variety Giza-168 over six environments.

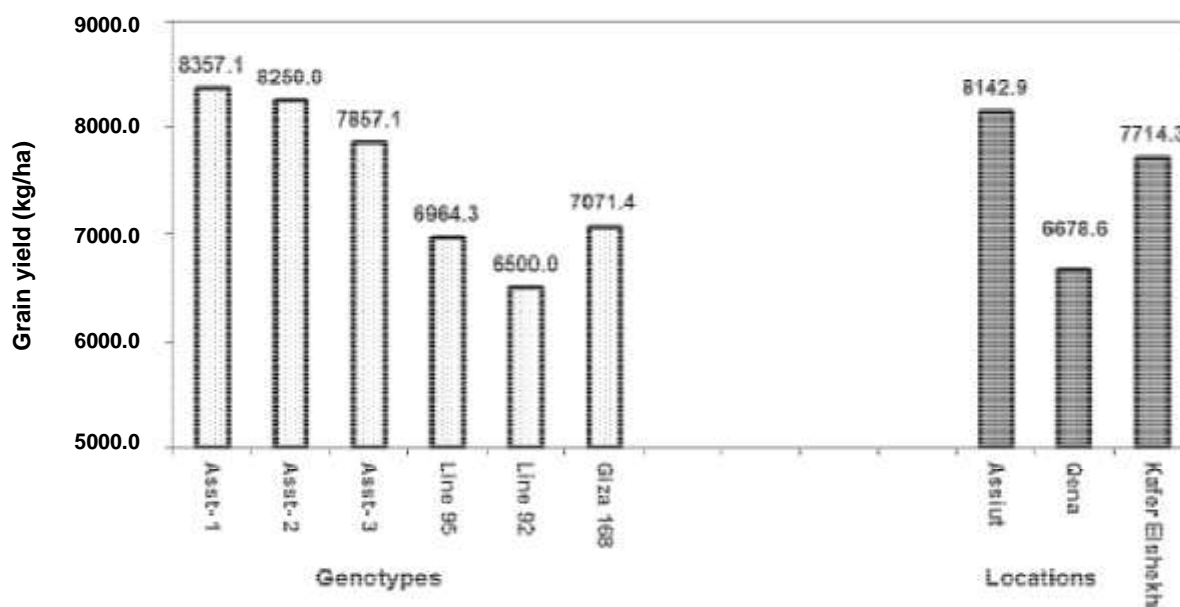


Figure 8. Grain yield kg/ha of wheat genotypes averaged over six environments and locations over genotypes.

locations (Table 4 and Figure 8). Figure 8 showed that inbred line Assiut-1 gave the highest grain yield (8,356 kg/ha) averaged over environments and line Assiut-2 occupied the second place for grain yield (8250 kg/ha). Line 92 recorded the lowest grain yield of 6,500 kg/ha. All the evaluated inbred lines produced the higher grain yield at both Assiut and Kafer ELshekh (that is 8,143 and 7,714 kg/ha, respectively) while at Qena, the lowest grain yield was recorded (Table 4 and Figure 8). Concerning the percentage of either increasing or decreasing of inbred lines grain yield compared to check variety. Figure 8 illustrated that, inbred lines Assiut-1, Assiut-2 and

Assiut-3 recorded significantly higher grain yield compared to the check variety. These lines showed 18.01, 16.84 and 11.11%, respectively, higher grain yield compared to the check variety. On the other side, grain yield of inbred line 92 decreased significantly (8.08%) as compared with the yield of check variety. There were no significant differences between yields of Line 95 and the check variety. These results were in harmony with those obtained by Taghian et al. (1998), Kheiralla et al. (2001), Mahmoud (2006) and Al-Otayk (2010). They found different yield performance of wheat genotypes under different climatic conditions.

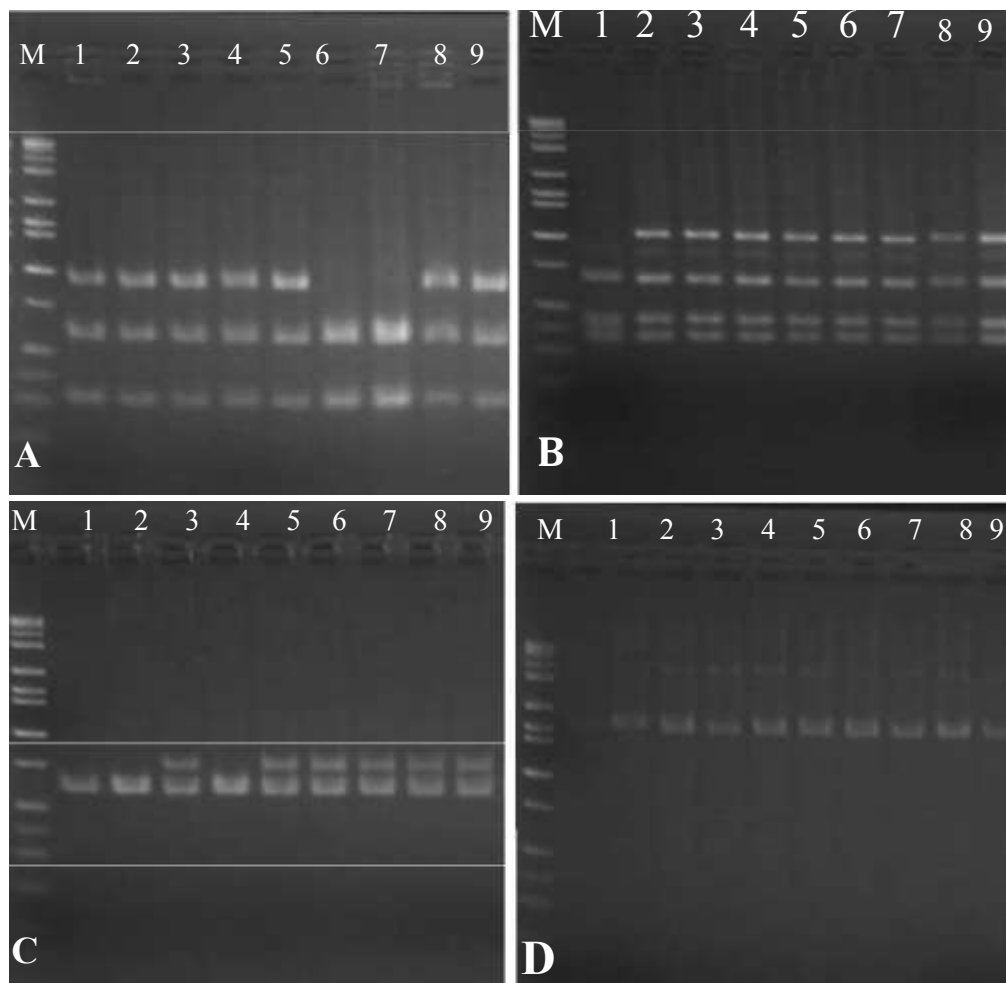


Figure 9. Bands amplified by EST markers (A. P04G09860, B. P04G05030, C. P04G14790 and D. P04G11880) on agarose gel (2% agarose), where M is wide range ladder DNA marker, lanes 1-4 are four parents (Sonora 69, Giza 164, Sakha 69 and Tokowe) and from 5-9 are the five lines (Assiut-1, Assiut-2, Assiut-3, line-95 and line-92). PCR products were digested with two restriction enzymes (*Rsal* and *ApaI*). Arrows indicate the sizes of bands in bp.

Detection of genetic variability using EST marker

Double bands with sizes of 950 and 900 bp, could be amplified by using EST marker P04G09860 for the four parents and the three lines {Assiut-1(103), Line-95 and Line-92}, while two lines {that is Assiut-2(1006) and Assiut-3(B-52)} showed single band with 900 bp size (Figure 9). Then, the PCR products have been digested with two restriction enzymes (that is *Rsal* and *ApaI*). However, the restriction enzymes could cut the smaller band of 900 bp into two fragments with the sizes of 600 and 300 bp, respectively (Figure 9).

In total, after digestion three clear bands could be seen in the four parents Sonora-69, Giza-164, Sakha-69 and Tokowe as well as in the three inbred lines (that is Assiut-1, line-95 and line-92). However, the other two lines that is Assiut-2 and Assiut-3 showed only two clear bands with sizes of 600 and 300 bp. Four bands were obtained

after digesting the PCR products which were amplified by using EST marker P04G05030. Three out of four bands were detected on all parents and all the studied inbred lines, while one band (1100 bp) was missed only on the parent Sonora-69 and appeared on the other three parents and all lines (Figure 2). For the EST marker P04G14790, two fragments were obtained from the digested PCR products with sizes of 750 and 600 bp. The first band of 750 bp was missed on three parents (Sonora-69, Giza-164 and Tokowe) while it appeared in parent Sakha-69 and all the inbred lines.

For the EST marker P04G11880, single band with size of 1600 bp could be amplified and could not be digested with the restriction enzymes. This band was appeared on all parents and studied lines except for the parent Sonora-69 (Figure 9). These results showed that the polymorphic bands appeared among the parents and studied line and that the parents and studied lines have

wide range of genetic variation at molecular DNA marker level. Consequently, the EST analysis could be used to detect the genetic variability among parents and lines.

Conclusion

In spite of the recent advances in modern molecular breeding and genetic engineering, classical breeding is still, and will be the best choice for developing new lines in wheat. Here, we described a long run classical breeding program that was based on crossings followed by selection for nine generations to get early and high yielding bread wheat lines adapted to grow in old Nile river valley lands as well as in the new reclaimed desert lands in Egypt. Five superior lines were obtained that could be termed as new promising cultivars. These lines showed higher stability across different environments and maintained high yielding ability with early maturity. To confirm the genetic variation among the parents and within the new promising lines, four EST markers were used. Band patterns proved that the parents and the new promising lines had wide range of genetic variation at DNA level.

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