

## Biometrical Identification of Gene Actions and QTLs Linked to Agronomic and Fiber Quality Traits in Cotton

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**T**HE MAJORITY of cotton fiber quality and agronomy traits are quantitatively inherited traits that jointly influence the utility of cotton. Identification of mode of inheritance and loci of quantitative traits for agronomic and fiber traits in Egyptian cottons and their allelic association would be of great interest to both cotton breeder and molecular biologist. This study employed several biometrical models to analyze the effects and mode of gene action in several populations derived from the cross between two cotton lines, *i.e.*, Egyptian cotton G89 (*Gossypium barbadense*) used as female parent and TAMCOT (*Gossypium hirsutum*) as male parent. The study also analyzed QTLs associated with agronomic and fiber traits across 103 F<sub>2</sub> plant families. The implemented statistical models provided estimates of additive and dominance genetic effects as well as the direction of the effects of alleles from both parents and predicted gain. With the current set of probes, the results identified eighteen QTLs controlling fiber quality properties closer to designated markers. Fiber length, strength, elongation, fineness, uniformity and yellowness were influenced by 3, 3, 3, 4, 3, and 2 QTLs, respectively. Cotton yield traits showed very limited insignificant QTLs, Maximum likelihood locations such as those obtained in this study do not necessarily represent physical distances. The results indicated more dominance than additive effect in gene mode of action. It could be concluded that selection procedures based on breeding backcrossing populations proved to be effective in shifting favorable gene frequency under the current gene action and genetic variation and would be successful in improving these traits. Investigating the correlation between traits and DNA markers linked to specific yield and/or fiber quality QTLs will facilitate marker-assisted selection in cotton breeding programs as well as for cloning genes for transformation. Locating the used markers that proved to be diagnostic for identification the assigned traits with such a physical map linkage groups will be, practically, very useful for cotton breeder and producer.

**Keywords:** Egyptian cottons, QTLs, Gene actions, Variances partition, Heritabilities, MAS, Genetic gain .

Egypt is producing the extra-fine cottons, including both the long and extra long staple varieties. The extra-fine cotton is the type which are used to spin yarns of 50 or higher count. These cottons are produced in perhaps 15 countries out of 80 countries producing some kind of cottons. Egypt is accounting for nearly half of the total world production of extra-fine cotton (Lawrence, 1998). In the past few years, Egyptian cotton production is confronted with many problems in cost of field production, marketing policy, and maintaining or improving fiber quality to meet the production and industrial worldwide challenges with other cottons. Moreover, farmers forced to plant cotton which became a burden instead of being their major bread earner. Consequently, Egyptian production fell from more than 12 million canters to an average of six million canters, forcing ginning mills to work under strained circumstances, never fully utilizing their capacity (Abaza, 1998). Most likely, these six million canters are reduced to less than five million due to the gradual reduction in the planted area. To overcome this dilemma is perhaps requiring making rapid and precision genetic changes in cultivar development to increase yielding capacity, or otherwise help cotton breeder in selection methodology either using the regular conventional analysis or employ the new advent of biotechnology like markers assisted plant breeding, the current research outcomes will shed light on those remedy steps.

The majority of cotton yield and quality characters are quantitatively inherited traits. This type of traits are affected to a great extent by environmental conditions that may lead to lack of stable genetic condition that in turn lead to lack of uniform breeding methods. In this context the genetic markers technology can help in avoiding the effect of environmental variable during breeding steps.

Genetic information based on molecular devises of crop genome is usually presented in the structure of a genetic linkage map (Abdalla, 2006). To my knowledge, the implementation of molecular markers and QTLs identification in cotton genetic analysis was not demonstrated until Shappley (1994) and Reinisch *et al.* (1994), separately, provided the first cotton linkage maps. Subsequently, several cotton QTLs has been identified. For example, QTL for agronomic and fiber traits using RFLP markers have been identified (Shappley *et al.*, 1998), for leaf morphology using RFLP markers (Jiang *et al.*, 2000), for stomatal conductance using RAPD and SSR markers (Ulloa *et al.*, 2000), for agronomic traits using RAPD and AFLP markers (Khan *et al.*, 1998), for density of leaf and stem trichomes using RFLP markers (Wright *et al.*, 1999).

Many statistical packages articulated to analyze quantitative traits and identify QTLs. Models of generation mean analysis proposed by Gamble (1962) and model of cracking variance components proposed by Mather & Jinks (1982) were very famous in the analysis quantitative traits. It was very early in the last century, when Sax (1923) reported a positive association between seed size and seed coat pigmentation in beans. He concluded that the association was a linkage of a single gene controlling the seed colour with gene(s) controlling the seed coat. This work was a core of the idea of single-marker analysis (SMA) for identifying QTLs. This method is then investigates the association between

trait(s) and one marker at a time. In SMA, the mapping population is partitioned into different genotypic classes that reflect genotypes at the marker locus. If the tested phenotypes, by ANOVA, are differed significantly, a gene(s) affecting the trait is said to be linked to the marker locus used to subdivide the population (Tanskley, 1993). Although SMA captures the basic idea of QTL mapping, Lander & Botstein (1989), however, stated several drawbacks of this method like the need to a considerable large number of tested progeny especially when the trait does not lie at the marker locus and also the suggested false positive rate of  $\alpha = 0.05$  neglects the fact that many markers are being tested. This denotes, while the chance of a false positive at any given marker is only 5%, the chance that at least one false positive will occur somewhere in the genome is much higher. On the other hand, since SMA does not require a linkage map, it is the analysis of choice whenever information about linkage maps is not available or unlinked markers. This fact also explains why SMA was widely employed in earlier studies (Soller *et al.*, 1976).

Interval-marker analysis (IMA), interval mapping (IM) and composite interval mapping (CIM) procedures are very reliable when information is available for several genetic markers. IM is based on an Expectation Maximization (EM) algorithm (Dempster *et al.*, 1977) that maximizes the likelihood ratio tests of a single QTL by averaging it across the possible states of the unknown genotype at flanking markers. The log likelihood ratio (LOD score) is comparing the hypothesis of the presence of a single QTL at any locus to the null hypothesis of no segregating QTL at that locus. LOD is then scan against linkage groups and compare to a threshold, usually set to a value of two, to ensure a 0.05 overall false positive error rate (Lander & Botstein, 1989). A one or two LOD support interval is used as an interval estimate for QTL location. There are, however, some problems with IM. It is not efficient to use only two markers at a time to do the test, as the information from other markers is not utilized (Zeng & Weir, 1996). CIM (Jiang & Zeng, 1995) is analogy to IM, since it is evaluating the presence of a putative QTL at flanking markers. However, CIM uses the multiple regression method. In multiple regression, the partial regression coefficient of a trait on a marker is expected to depend only on those QTL that are located on the interval bracketed by the two neighboring markers and to be independent of any other marker. The main problem in this method is the number of regressor markers (background markers) where using too many background markers will increase the variance of the LOD score, and thus will decrease the power for detecting QTLs (Zeng & Weir, 1996).

One more point, interaction of such a quantitative trait and environment has been discussed in many studies. With cotton plant that cultivated with a wide range of environments, large numbers of genes needs to be manipulated to confer adequate quality under these environments conditions. This will reduce the expected rate of genetic gain, (Paterson *et al.*, 2003). These difficulties may be partially ameliorating by efficiencies gained through identification and use of diagnostic DNA markers. Under the same markers conditions, in ongoing

researches my team is investigating the QTLs X Site based on a previous work of Abdalla *et al.* (2005) and QTLs X planting date interaction.

Implementing the integration between conventional breeding and recent biotechnology, the present study was planned to identify QTLs and investigate presence mode of inheritance in diverse biometrical schemes by employing an interspecific cross between Egyptian cotton G89 (*Gossypium barbadense*) and (*Gossypium hirsutum*). The implication in plant breeding was discussed too.

## Materials and Methods

### *Genetic resources*

Field trials were conducted in the summer seasons of the years 2003 through 2005 at Cairo University labs and experimental farm, located in Giza, Egypt. Parents exploited in this study were the Egyptian cotton G89 (*Gossypium barbadense*) used as female parent and Tamcot (*Gossypium hirsutum*) as male parent. The unique high-quality fiber properties of the G89 cotton and the high productivity of Upland cotton, led to our choice of the mapping parents. Following the artificial hybridization of inter-specific cross between pure lines of these two parental cottons, we got the fertile  $F_1$ 's seed, 2003. In 2004, the  $F_1$  plants were selfed breed to produce the  $F_2$ s population. The  $F_1$  plants are back crossed to both  $P_1$  and  $P_2$  to produce the  $BC_1$  and  $BC_2$ , 2004. In that year, hybridization was repeated again between parental materials to get some more  $F_1$  seeds. Since cotton plant is a prolific-multi-flower plant, the  $F_1$ s and segregating generation plants were able to produce enough seed for obtaining enough plants and lint. In April 6, 2005; Parents, filial generations, and first backcrosses were grown in RCBD with four replicates, 2005. Each replicate consists of four plots. Parental materials and non-segregants were grown in one row per plot. Segregants, however, were grown in two rows per plot. Each raw was 5m long, spaced 60cm apart with seed sown by hand, 30cm apart. Directly after complete emergence, the plants were thinned in one plant per hill. Fertilizers, weed control, irrigation, and insect control were standard practices for production of Egyptian cotton. Data were recorded on a single guarded plant basis for the above mentioned populations. Living specimens of these populations are presently maintained in the green house to produce seed, fiber, and leaf tissue for this mapping project and other further studies. For QTLs identification, cotton DNA of each parent,  $F_1$  and  $F_2$  was isolated from fresh young leaves harvested as a bulk sample from 4 to 5 plants (parents,  $F_1$ ) and from individual 103  $F_2$  plants. The  $F_2$  plants used as a polymorphic mapping population were labeled for data recording among the other  $F_2$  plants tagged for data collecting. The genotyping steps including cotton DNA isolation, markers used and constructing the genetic linkage maps were described in details in Abdalla (2006).

### *Data recorded*

Data on cotton yield and fiber traits were recorded for the constructed populations on individual guarded plants. For cotton yield the following characters were measured; (SCY) seed cotton yield per plant (gm) as the sum of a two hand harvested times, (LCY) lint cotton per plant (gm), number of bolls per  
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plant (NB) determined by dividing total seed cotton weight per plant by average weight of boll, boll weight (BW) in gm, lint percentage (L%) as ratio of lint to seed cotton expressed as percentage, seed index (SI) determined by the weight in grams of 100 seeds, earliness index (EI) as a percent of first picking to the total of the two picking times and plant height (PH) in cm. For quality traits, high volume instrument (HVI) was done by Cotton Research Institute, Agriculture Research Centre at Giza. The measurements comprehensively included the following dependent variables: lint moisture, colour index, mean length (ML) cm, uniformity (U), strength (ST), micronaire (M), HVI Reflectance or whiteness (Rd), HVI yellowness (+b)

### *Biometrical procedures*

MAPMAKER/EXP and MAPMAKER/QTL (Lincoln *et al.*, 1993) version 3 was employed to express the genetic maps exploited in the current research (Abdalla *et al.*, 2006). MAPMAKER/QTL used to compute "QTL likelihood" covering the entire genome. It uses interval mapping to detect regions in the genome which are likely to contain putative QTLs, test the strength of the data supporting the hypotheses that particular QTLs exist, and locate the likely position of these putative QTLs. It is worth to mention the logic's of the program used to generate QTL likelihood positions works as follows: The program iteratively "steps" along the genome and at each point calculates a "maximum likelihood QTL map". That is, at each point, MAPMAKER/QTL asks the question, assuming there is a QTL right here, what is the maximally likely manner in which it's inheritance affects the trait? What is the strength of the data supporting this hypothesis? These answers resulted are expressed as a number of real valued parameters, including: 1. The effect of the QTL on the trait, expressed in terms of additive and "dominance" effects for the population used. 2. The fraction of the total variation in the trait across the population explained by the QTL (this is equivalent to an  $R^2$  value calculated by linear regression). 3. The mean and standard deviation of the variation in the trait not controlled by the QTL. 4. A LOD score, also called a log-likelihood, indicating the strength of the data supporting this hypothesis. As DNA data were collected from an  $F_2$  intercross, each individual will have one of three possible genotypes at any QTL: A/A (back to parent 1), A/B (hybrid), or B/B ( $P_2$ ). Reinisch *et al.* (1994) discussed three scenarios that the  $F_2$  DNA polymorphism can fit. While investigating the current polymorphism we adopted the scenario of only one of the two homozygous parental lines had one distinguished fragment. In principle, the two fragments could be allelic, or could represent polymorphisms at two different loci. MAPMAKER/QTL expresses the effect of these QTL genotypes on the trait using the "additive and dominance" method, with the QTL effects measured in terms of the amount that alleles derived from the A or B parent, individually or together, contribute to the phenotypes of individuals with an otherwise A/A background. The thresholds suggested for most traits LOD = 2 corresponded to about 0.25 (after accounting for multiple comparisons). Higher thresholds were suggested for some traits like lint length, uniformity and yellowness as stated by Paterson *et al.* (2003).

On the other hand, the phenotypic data of the aforementioned populations ( $P_1$ ,  $P_2$ ,  $F_1$ ,  $F_2$ ,  $BC_1$  and  $BC_2$ ) were statistically analysed to get estimates of population parameters like mean, and variances. The generations mean analysis in terms of additive (a), dominance (d) was calculated according to Gamble (1962). Types of epistatic effects including additive additive (aa), additive dominance (ad) and dominance dominance (dd) were calculated using the same model. Significance of these effects were tested against "T" test where  $\pm t = \text{Effect} / (\text{Variance of the effect})^{1/2}$ . The variances components in terms of additive (D) and dominance (H) genetic variances as well as the within-plot and between plots variances were calculated according to Mather & Jinks, (1982). The genetical parameters of mid parents heterosis (H %), inbreeding depression (ID %), heritability in narrow sense ( $h^2_n$ ) and predicted genetic advance from selection (Gs) were calculated as following:  $H\% = ((F_1 - MP)/(MP)) \times 100$ ,  $ID\% = ((F_2 - F_1)/F_1) \times 100$ ,  $h^2_n = \frac{1}{2}D / (\frac{1}{2}D + \frac{1}{4}H + E)$ , and  $Gs = 2.06 \times \sigma_A \times h^2_n$ , where, 2.06 is a constant denotes the selection differential, its value equal 2.06 for selection intensity of 5% of  $F_2$  plants.  $\sigma_A =$  phenotypic slandered deviation of  $F_2$ .  $Gs\% = (Gs/\mu_{F_2}) \times 100$ .

## Results and Discussion

### *Fitting data to the statistical model used (Model Test)*

The basic methodology for mapping QTLs in the current research involves arranging a cross between two inbred strains differing substantially in quantitative traits as it shown in Table 1. The mean performance of the traits presented in Table 1 indicated that most quantitative traits contributed by the G89 parent ( $P_1$ ) conditioned by low yield of fibers that were long and strong while those from TAMCOT ( $P_2$ ) imparted high yield of short and weak fibers. After that, the progeny of any segregating population (the  $F_2$  plants in the current work) are scored for the assigned traits and for a number of RFLP genetic markers, (Abdalla, 2006). This leads to three types of data. 1- A marker map which gives numbers, names and positions of molecular markers on chromosomes or chromosome segment. 2- Marker data for a set of progeny from the cross. 3- Measurement data on phenotypic traits for the same progeny.

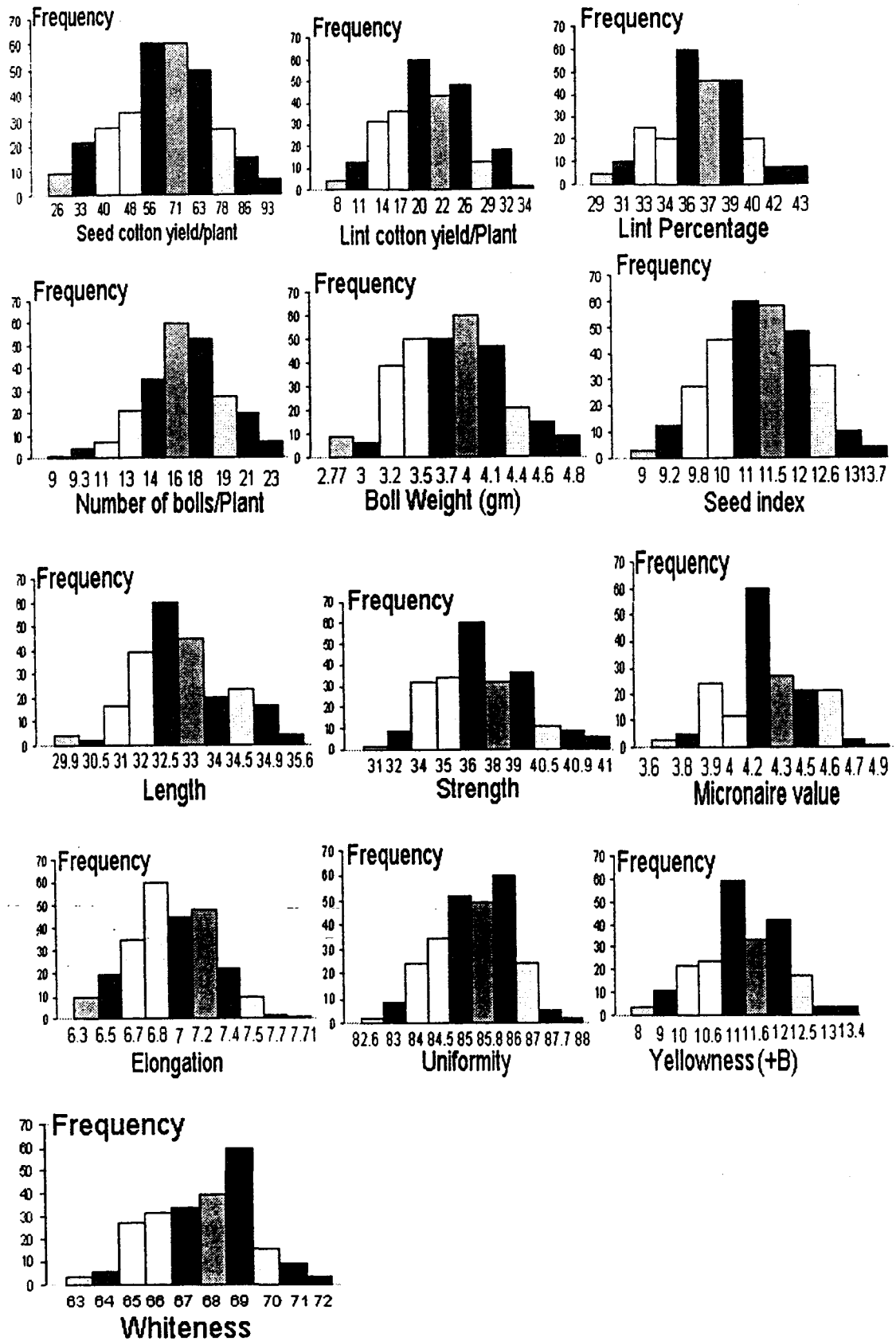
On the other hand, the factors classifying the phenotypic data for each marker or marker pair are: blocks or replications, markers and genotypes within a marker. The current materials of this work would rather to consider blocks as a fixed effect, since we are not interested in estimating responses over a population of possible blocks or other allocated environments. The markers are fixed too, since there is no question of their being a sample of genes of fixed part of cotton genome. The plant genotypes are random since they represent a sample of  $F_2$  of the possible gamete genotypes derived from the  $F_1$  cross through recombination and we are interested in the effects of any QTL over the population of such genotype. Moreover, analysis of such a QTL located in a specific position of the genome can be done under an assumption that the phenotype can be explained by a single QTL located at the position being tested together with normally distributed noise (errors) (Lincoln *et al.*, 1993). This is the same assumptions that control the majority of ANOVAs conditions used for classical quantitative traits analysis. Obviously, the analysis can be affected by deviations from

these assumptions. The deviation from normality for any QTLs exploited trait as it showed in Table 2 or Fig. 1 can be managed by some kinds of transformation. Table 2 showed the phenotypic distributions for each trait and estimates of normality tests including values of the mean ( $\mu$ ), standard deviation ( $\sigma^2$ ), kurtosis, skewness, and quartile ratio of the trait data, as well as the fraction of the individuals whose phenotypes fall within  $0.25 \sigma^2$  of the mean,  $0.5 \sigma^2$ ,  $1\sigma^2$ ,  $2\sigma^2$  and  $3\sigma^2$ . Under these assumptions we will be able to compare the genetic parameters calculated based on the integration with molecular data with those obtained from conventional data based on populations' analysis. Accordingly, both of Table 2 and histograms presented in Fig. 1 shed light on the frequency distributions of the  $F_2$  data and testing the fitting of QTL model to normality. For the considered agronomic and fibers traits, the frequency plots revealed by traits figure showed a continuous normal distribution for all traits except boll weight, whereas for quality characters, the uniformity, yellowness and whiteness were not normally distributed. The log transformation was used to normalize these traits. The results, collectively, pointed to the adequacy of  $F_2$  data in the analysis of QTL.

**TABLE 1 . Mean performance, type of gene actions, heterosis (H %), and inbreeding depression (ID) for the studied populations.**

Characters	Generation mean analysis (Gamble's Model)											H%	ID
	Non-segregating populations			Segregating populations			Type of gene actions§						
	P <sub>1</sub>	P <sub>2</sub>	F <sub>1</sub>	F <sub>2</sub>	BC <sub>1</sub>	BC <sub>2</sub>	a	d	aa	ad	dd		
PH(cm)	102.04	79.12	103.20	90.40	100.50	97.33	-3.17	46.68**	34.06*	-8.29	-42.16**	1393**	-1240
SCY/P(gm)	43.20	59.21	65.06	55.16	51.04	57.33	6.29**	9.96**	-3.90**	1.72*	19.69**	2706**	-1522
LCY/P(gm)	14.00	15.90	15.00	12.40	20.00	20.34	0.34	31.13*	31.08**	0.61*	-51.86**	0.33	-17.33
NB/P	15.82	13.31	15.56	12.26	15.37	14.16	-1.22	11.03**	10.03**	-0.04	-8.83	686	-2125
BW(gm)	2.73	4.45	4.18	4.50	3.32	4.05	0.73**	-2.67	-3.26	0.13**	4.06**	1643**	7.66
L%	36.87	35.60	36.00	35.54	35.12	35.48	0.36**	-1.19	-0.96	-0.99*	4.23**	-0.65	-1.28
SI(gm)	9.15	10.82	11.77	10.75	10.75	11.00	0.25**	2.29	0.50	0.59	-0.49	1788**	-8.67
EI(gm)	40.30	67.89	59.20	58.40	46.80	68.50	21.70**	2.10	-3.00**	-7.91	-1.01	944**	-1.35
ML	32.85	26.02	32.00	28.90	33.00	30.00	-3.00	12.97**	10.40**	-0.41	-13.53**	871**	-9.69
EL	6.89	6.16	6.94	6.85	6.95	6.02	-0.93*	-1.0**5	-1.46**	0.57	2.45	636	-1.30
ST	36.65	31.58	36.54	36.08	37.09	36.85	-0.24	5.99**	3.56**	-2.29	-10.13	711**	-1.26
MIC	3.52	4.03	3.5	3.8	3.48	4	0.52**	-0.51*	-0.24	-0.265	-0.17	-728**	8.57
U	86.56	83.09	88.43	87.67	87.00	87.12	0.12	1.17	-2.44	-1.86	0.71	425	-0.86
Rd	64.57	72.91	66.29	65.03	65.59	69.44	3.85**	7.49**	9.94**	0.32	-9.94	-356	-1.90
Plus B	11.62	9.52	11.90	12.01	12.00	11.44	-0.56	0.17**	-1.16	-0.49	-0.78	1258**	0.92

§ Significance of gene effects were tested against "T" test where  $\pm t = \text{Effect} / (\text{Variance of the effect})^{1/2}$ . SCY(gm); seed cotton yield per plant, LCY(gm); lint cotton per plant, NB/P; number of bolls per plant; BW (gm); boll weight, L% : lint percentage, SI; seed index, EI; earliness index, PH; plant height in cm, ML; mean length, U; uniformity, ST, strength; M; micronaire value; HVI Reflectance or whiteness (Rd), HVI yellowness (+b). a=additive, d = dominance and aa, ad, dd were epistatic effects.



**Fig. 1. Frequency distributions for agronomic and fiber traits as constructed by Mapmaker/ QTL for cotton agronomic and quality fiber traits in the F<sub>2</sub> population.**



**TABLE 2.** The phenotypic distributions calculated for some agronomic and fiber traits in interspecific F<sub>2</sub> progeny.

	F <sub>2</sub> distribution				quartile ratio	Fraction within n deviations				
	mean	sigma	skewness	kurtosis		0.25	0.5	1	2	3
SCY/P(gm)	55.6	14.97	-0.13	-0.57	1.12	0.15	0.36	0.66	0.95	1
LCY(gm)	19.93	5.82	-0.02	-0.69	1.21	0.17	0.33	0.69	0.97	1
L%	35.74	3.16	-0.22	0.26	1	0.24	0.43	0.69	0.93	1
BW (gm)	3.69	0.46	0.13	-0.41	1.02	0.18	0.36	0.68	0.94	1
Log (BW (gm))	0.56	0.05	-0.17	-0.33	1.01	0.18	0.36	0.68	0.95	1
BAD NB/P	16.07	3.39	0.66	2.07	0.9	0.29	0.47	0.73	0.96	0.98
SI (gm)	10.9	1.13	-0.15	0.08	1.08	0.17	0.39	0.69	0.95	1
EL	6.82	0.35	0.29	2.62	0.85	0.3	0.41	0.73	0.97	0.98
ML	32.46	1.26	0	0.29	0.88	0.16	0.45	0.7	0.94	1
MIC	4.2	0.28	0.25	0.41	1.08	0.15	0.47	0.65	0.95	0.99
ST	36.36	2.78	0.57	-0.03	1.09	0.16	0.39	0.69	0.95	1
U	85.16	1.26	-0.51	1.08	0.94	0.19	0.39	0.73	0.95	0.98
Rd	66.99	2.19	-0.27	-0.22	1.15	0.15	0.31	0.7	0.95	0.99
+B	11.11	0.93	0.1	0.14	0.95	0.21	0.41	0.7	0.94	1

SCY(gm); seed cotton yield per plant, LCY(gm); lint cotton per plant, NB/P; number of bolls per plant, BW (gm); boll weight, L%; lint percentage, SI; seed index, EI; earliness index, ML; mean length, U; uniformity, ST; strength, M; micronaire value, HVI Reflectance or whiteness (Rd), HVI yellowness (+b)

Based on the F<sub>2</sub> data, the correlation coefficients for the basic agronomic and fiber traits are given in Table 3 with the significance of all traits studied at P value less than 0.05. The correlation analysis for fiber quality, however, showed four different correlation coefficients were highly significant. The significant correlation coefficient was between length and uniformity, between length and strength, between length and micronaire, and between uniformity and strength. These results are similar to those reported by many investigators locally and internationally as well, of them, (Ulloa & Meredith Jr., 2000). Such a positive correlation among traits would be of beneficial implementation for marker- assisted selection in plant breeding as well as for cloning genes for transformation.

#### *Type of gene action and genetic parameters*

The classical genetic models expressed for investigating quantitative traits help identify the presence of genes that influence expression of specific traits. The main idea is that the morphological characteristics of an individual parent have been used to predict the characteristics of its progeny through mean and variances. Populations mean performance analysis presented in Table 1, and variances components partitioning in Table 4 for yield and fiber traits of the studied traits pointed out that the variances of F<sub>2</sub> were more dispersion than the

other generations and was significant for all traits studied. Different types of gene action showed various significant effects, (Table 1). Dominance type of epistasis showed significant effects for all traits. The Mather's Model was used for partitioning the genetic variance into the additive (D) and dominance (H) genetic variance, (Table 4). Since the relative magnitude of the significant gene effects determines its importance in the inheritance of the character, Results revealed that the dominance genetic effects (Table 1) and genetic variances effects (Table 4) were significant and larger in magnitude than additive for all traits except the whiteness (Rd), a trait of colour. From these findings, it could be concluded that the additive, non-additive gene effects and epistasis were affecting the nature of gene action for the characters study.

**TABLE 3. Correlations among cotton fiber quality traits, lint yield and yield components calculated for the F<sub>2</sub> progeny of *G. barbadense* × *G. hirsutum* population.**

Trait	ML	ST	U	MIC	EL
ST	0.34**	-----			
U	0.77**	-0.55*	-----		
MIC	-0.13**	-----	-0.92*	-----	
EL	-0.13*	-0.4*	0.45*	0.47*	-----
Trait	SCY	LCY	BW	BN/P	L%
LCY	0.76*	-----			
BW	0.35*	0.80*	-----		
BN	0.71*	0.69*	-0.03*	-----	
L%	0.09*	0.64*	0.03*	0.15*	-----
SI	0.08*	-0.20*	0.06*	-0.23*	-0.72*

\*, \*\* were represented the significant levels at  $P < 0.05$ ,  $0.005$ , respectively. SCY(gm); seed cotton yield per plant, LCY(gm); lint cotton per plant, NB/P; number of bolls per plant, BW (gm); boll weight, L%; lint percentage, ML; mean length, U; uniformity, ST; strength, M; micronaire value.

Agronomy and fibers traits had recorded mid-parent heterotic and significant effects for yield traits except lint percentage (Table 1). Heterosis for fiber length, strength and finance were evident. Negative ID was obtained for all traits studied except Boll weight and yellowness, (Table 1). The negative values of ID associated with all traits except BW and +b suggesting that heterosis in the F<sub>1</sub> will not be followed by an appreciable reduction in the successive generations performance.

Data in Table 4 showed that heritability estimates of cotton yield components and fiber properties were small to moderate for all traits. Heritability estimates ranged from as low as 12.52 to relatively high 57.78 for seed index (Table 4), with moderate heritabilities with the other traits. Many published works showed that heritability estimates of cotton yield components and fiber properties were moderate to high (approximately 50 to 80%), (Abdalla, 2001; Abdalla *et al.*, *Egypt. J. Agron.* 29, No. 2 (2007)

1999; Meredith & Bridge, 1984 and May, 1999) . The previous findings of the genetic estimates were reflected in a slight expected gain from selection of highest 5% of F<sub>2</sub> plants except for LCY, BW, and lint traits. From these findings we may conclude that the analysis of a complex trait in early generations is especially appropriate in the case where the trait shows heterotic effects back to a wide genetic base for tested parents associated with relatively high heritability. This indicating, collectively, that these traits can be manipulated in early segregating generations and confidently can be subjected to QTL analysis.

**TABLE 4.** populations' variances, variances components, heritability ( $h^2_n$ ) and the F<sub>2</sub> expected gain from selection (g % (F<sub>2</sub>)).

Characters	Variances Model (Mather's Model)											
	Non segregating populations			Segregating populations			Variance components			Heret-ability $h^2_n$	Predicted gain	
	P1	P2	F1	F2 <sup>§</sup>	BC1	BC2	1/2D	1/4H	E		g	g% (F <sub>2</sub> )
PH(cm)	30.00	21.32	18.89	66.15**	40.10	26.91	65.29	76.25	23.40	39.58	6.63	7.34
SCY/P(gm)	22.90	20.67	18.70	44.75*	18.53	23.14	47.83	44.83	20.76	42.17	5.81	10.54
LCY/P(gm)	25.21	20.23	19.56	50.33**	18.80	20.21	61.65	48.17	21.67	46.89	6.85	55.26
NB/P	1.50	1.80	1.68	5.01**	2.40	2.82	4.80	5.96	1.66	38.65	1.78	14.54
BW(gm)	0.60	0.65	0.49	1.88**	0.66	0.52	2.58	1.89	0.58	51.09	1.44	32.07
L%	6.00	4.98	4.01	11.14**	5.08	6.25	10.95	11.81	5.00	39.45	2.71	7.63
SI(gm)	0.10	0.06	0.06	0.24**	0.01	0.08	0.39	0.21	0.07	57.78	0.58	5.42
EI(gm)	15.64	10.78	11.33	24.66*	14.00	11.90	23.42	25.03	12.58	38.37	3.93	6.72
ML	4.02	3.40	3.66	8.62**	5.03	5.30	6.91	10.09	3.69	33.39	2.02	6.99
EL	0.05	0.04	0.07	0.12*	0.08	0.07	0.09	0.14	0.05	31.58	0.23	3.29
ST	0.09	1.02	2.01	2.40*	1.08	1.20	2.52	2.50	1.04	41.58	1.33	3.68
MIC	0.47	0.54	0.50	0.90**	0.46	0.52	0.82	0.89	0.50	37.10	0.73	19.08
U	25.08	21.62	17.09	56.12*	30.20	24.20	57.84	62.06	21.26	40.97	6.32	7.21
Rd	1.90	1.80	1.20	2.02**	1.60	1.90	0.54	2.14	1.63	12.53	0.37	0.56
Plus B	0.90	1.01	0.60	1.18*	1.00	0.09	1.27	0.89	0.84	42.40	0.95	7.90

<sup>§</sup> F<sub>2</sub> variances significant at 5% and 1% levels. SCY(gm); seed cotton yield per plant, LCY(gm); lint cotton per plant, NB/P; number of bolls per plant, BW (gm); boll weight, L%; lint percentage, SI; seed index, EI; earliness index, PH; plant height in cm, ML; mean length, U; uniformity, ST; strength, M; micronaire value, HVI Reflectance or whiteness (Rd), HVI yellowness (+b). D=additive, H=dominance, E=env. Noise and g%=genetic Gain

### *QTL analysis for cotton fiber quality traits*

As indicated from the results of heritabilities estimated based on classical statistical models and accompanied with each trait in Table 4, we concluded that these traits confidently can be subjected to QTL analysis. Details of the genetic linkage map produced in the current research described by Abdalla (2006). With the current set of probes, a quite large numbers of QTLs were detected. Cotton yield traits, however, showed very limited non-significant QTLs. This may back to the background of the designated set of probes and/or probably needing to tray

more probes or other probes diagnostic to yield traits. Biometrical parameters for the markers that only showed significant traits QTLs position are provided in Table 5. The mode of inheritance of traits is presented too.

Recall, mean performance of the traits presented in Table 1 indicated that most quantitative traits contributed by the G89 parent ( $P_1$ ) conditioned by low yield of fibers that were long, strong, while those from TAMCOT ( $P_2$ ) imparted high yield of short, weak fibers. Thus, results in Table 4 showed that fiber length affected by three QTLs. Increase was conferred by the alleles from the long-fibered parent (*G. barbadense*) at one locus pAR018 by 1.24 mm, while the alleles from the short-fibered parent (*G. hirsutum*) at two QTLs closer loci pAR078 and A116, by 1.88 and 0.27 mm, respectively. The markers mode of gene actions was RA, AD and RD for the markers pAR018, pAR078 and A116, respectively. The locus A118 showed a heterotic effect ( $d/a$  ratio  $> 3$ ), with reduced fiber length conferred by the heterozygote ( $d=1.24$ ). Simply this means the male parents the, *hirsutum*, succeeded to pass alleles of short fibers to the female parent and, thus, affect its quality. However, the recessiveness of the three out of four loci is a positive point for markers aided selection. Regarding the fiber strength one copy of *G. barbadense* alleles of markers A116 and A174 increased fiber strength by 0.17 g/tex and 0.16 g/tex, respectively. On the other hand, the pAR023 decreased fiber strength by 0.05 g/tex. The three significant QTL markers were recessive in their expression (Table 5). While the study presented QTLs for fiber strength, we recommend that they must be interpreted with caution. This back to two reasons; since it recorded a relatively low level of heritability (31.58) and presented non-additive types of gene actions (Tables 1, 3 and 5). The associate recessiveness, however, will help in marks aided plant breeding, (Paterson, 1998). Fiber uniformity presented in Table 5 showed three significant QTLs were detected with statistical significance and collectively revealed 12% of variance explained. Increased fiber uniformity was conferred by the (*G. barbadense*) allele at one locus (pAR054,  $a=0.4$ ); and the *G. hirsutum* allele at one locus (pAR082,  $d=0.5$ ). The heterozygote showed lower fiber uniformity at one locus (G110  $d/a=-1.4$ ).

The statistically significant QTLs of elongation were three and collectively explained about 24% of the phenotypic variance explained in the  $F_2$  population (Table 5). The mode of action accompanied with this trait was recessive dominance for two out of four QTLs detected and was additive the other locus. This reflects the possibility to utilize the crossing between these two parents to improve elongation. Fiber fineness as measured by micronaire value detected four significant QTLs jointly revealed 16% of variance explained.

The heterozygote showed lower fiber fineness at those four loci. Increased fiber fineness (lower Micronaire value) was conferred by the *G. barbadense* G89 allele two loci (pAR07 and A173) and the *G. hirsutum* allele at two loci (pAR023 and pAR088). For fiber colour yellowness, two QTLs were detected with statistical significance (Table 5). Reduced fiber yellowness (better quality) was conferred by the *G. hirsutum* allele at these two loci (G101 and pAR036).

**TABLE 5. Biometrical parameters of significant QTLs affecting yield quality traits of cotton.**

Trait	Code of the nearest marker to QTL locus	QTL effects in the studied data set					LOD
		Percent of variance explained	Additive (a)	Dominance (d)	d/a	Mode of gene action	
ML	pAR018	6.7	1.24	-1.316	-1.061	RA	3.2
	pAR078	5.7	-1.88	-0.85	0.452	AD	2.68
	A118	8.8	-0.268	1.24	-4.626	RD	2.08
ST	A116	5.5	0.171	-1.1	-6.432	R	3.74
	pAR039	8.2	-0.006	0.052	-8.666	R	2.42
	A174	6.7	0.162	-0.218	-1.345	RA	3.02
EL	pAR082	6.5	0.396	-0.32	-0.823	RD	3.04
	G109	7.8	0.245	-0.36	-1.497	RD	2.7
	pAR019	9.7	-0.36	-0.28	0.77	A	3.85
MIC	pAR023	4.5	-0.43	0.15	-0.34	RD	4.04
	A173	5.3	0.39	-0.30	-0.76	RA	5.79
	pAR07	6.8	1.1	-1.6	-1.45	RA	6.48
	pAR088	3.7	-2.66	0.80	-0.30	RD	3.19
U	pR054	4.8	0.39	-0.21	-0.538	RA	3.03
	pAR082	3.4	-0.63	0.53	-0.841	R	3.79
	G110	6.4	0.012	-0.017	-1.416	R	4.04
+b	G101	9.7	0.95	0.13	0.136	A	5.7
	pAR036	4.6	0.024	0.002	0.083	A	4.2

A or a= additive, B or b= Dominance, R= recessive and +b= yellowness. ML; mean length, U; uniformity, ST; strength, M; micronaire value, HVI yellowness (+b)

Types of gene actions calculated based on these markers are in complete accordance with those calculated based on the classical statistical models. The allelic and non-allelic interactions had effect on the nature of gene action. This showed the importance of non-additive part of gene effects in the inheritance of these characters. The published heritability estimates (Meredith & Bridge, 1984 and May, 1999) were supported, up to some extent, by the current results, (Tables 4 and 5). This pointed up that these traits can be manipulated in early segregating generations. Moreover, with a group of markers closer to the markers used in our study Paterson *et al.* (2003) described a base set of marker near to a set of QTLs that are relatively unaffected by environmental parameters. This means that the detected QTLs may account for progress from selection in a wide range of environments that are often employed in mainstream cotton breeding programs. Currently, the author's team investigating this point with different planting dates of proposed populations. A few *G. hirsutum* loci had a negative effect in *G. barbadense*, which may be due to either different genetic backgrounds or interaction of genes between the two cotton species (Table 5). Efforts will continue, however, to improve yield and fibers of Egyptian cottons by hybridization to introgress the desired genes to genotypes that require. DNA

markers used in this study could serve as diagnostic tools for cotton breeders to follow selection for fiber quality at seedling stages in early segregating generations. Moreover, investigating the correlation between traits and DNA markers linked to specific yield and/or fiber quality QTLs will facilitate marker-assisted selection in cotton breeding programs to engineer highly productive Egyptian cottons with superior quality of fiber strength, length, and fineness. Such inferences were also shown by Kohel *et al.* (2001). They demonstrated that the results from characterizing QTLs of fiber quality properties should allow plant geneticists to investigate the origin of fiber quality genes, and the level of their expression in the required cotton genotype backgrounds.

### Conclusion and Future Prospect

The future improvements of cotton yield and fiber quality traits will depend upon the cooperative applications of traditional plant breeding and molecular genetic tools (Collard *et al.*, 2005). It is believed that selection of promising recombination in early generations is necessary to maintain population of practical size. Obviously, the current study was dealing with biometrical genetics of quantitative traits from two points of view. In one hand, the whole genetic constitution including the traits of interests was investigating through conventional breeding. That object we implemented utilizing Gamble's and Mather's biometrical models. On the other hand, dealing with quantitative traits in narrow scale that is normally known as QTLs analysis was done through a block of specific genes (probe sequence) and specific population ( $F_2$ ). Gene action types effects resulted in the present research may support what generally believed, that the dominance gene effect had an important contribution in the inheritance of quantitative characters in cotton. Epistatic gene action had a significant contribution in the inheritance of the studied traits indicating the presence of significant genetic variation in this cross.

The important implementation of analysis of QTLs is the ability to identify specific linkage groups or chromosome regions that affect economic traits, the thing that the current study demonstrated. The QTLs investigation allows breeders to search germplasm for useful genes and ultimately accelerate the breeding progress. Targeting the attempt to map QTLs for yield components and fiber quality traits, a major effort to map the cotton genome is ongoing by several research groups working supplementary by some way in separate American schools (Paterson *et al.*, 2003; Reinisch *et al.*, 1994; Shappley *et al.*, 1998; Ulloa & Meredith 2000 and Yu *et al.*, 1998). As a part of this ongoing study, the current work objectives are to map QTLs of cotton genome through populations obtained from interspecific crosses and to develop a core of markers with more practical application for cotton breeders. Herein we report QTLs that influence cotton yield, yield component characteristics and fiber quality traits using a partial linkage map developed from *G. barbadense* × *G. hirsutum* populations (Abdalla, 2006). After finishing the assaying of a quite numbers of probes with significant QTLs to the mapping population, we will locate the probes data set

from this work to the assigned linkage groups or chromosomes group to join the universal cotton genome framework data set (Saranga *et al.*, 2001).

The basic questions cotton breeders often ask are which upland genes for boll weight, for example as a major yield components, are missing in the extra long stable (ELS) of Egyptian cottons or which ELS genes for fiber quality are missing in the Upland cottons, and whether the ELS genes have the same expression in Upland cottons once they are introgressed. Through investigating QTLs closer or linked to a block of genes control these traits; we will be able to answer this question in very limited time. In this context, however, I would like to emphasize that the majority of these genetic maps have been developed through interspecific hybridization, which currently has little use in a conventional breeding program (Reinisch *et al.*, 1994 and Yu *et al.*, 1998). Although the introgression from *G. hirsutum* has played a major role in the breeding of *G. barbadense* (Wang *et al.*, 1995), the Egyptian cotton breeding school itself has a caution to employ *G. hirsutum* cottons in the cotton breeding for improvement programs. This is in the sake of maintaining our distinct quality characters away from any gene contamination.

We also need to know whether different fiber quality QTLs can complement one another to increase quality or interact to reduce it. While recorded yield improvements, some negative reduction for Egyptian cottons fibers quality had recorded too. For that reason, I preferred to use Egyptian genotype like female parent to keep the majority of the Egyptian genetic constitution in the resultant crosses and practicing the backcrossing as a method of breeding to improve the traits of interest and practicing selection in early segregating generations. If the researcher interest is to analyze QTLs based on markers technology, however, he has to use genomes that differ widely in their genetic background. This point with polyploidy cotton plant especially is more complicated, since a fairly large number of research showed a lack of polymorphism inside each group of *Gossypium* taxa (Abdalla *et al.*, 2001). This reflected in the few number of significant QTLs detected in our study as well as other published researches. Information from detailed mapping efforts, however, would help shed light on gene introgression and transformation of important cotton yield and fiber traits avoiding the majority of wide crossing cautions.

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## التعيين الإحصائي لطرز الفعل الجيني ومواقع الصفات الكمية المرتبطة بالصفات الزراعية والتيلة في القطن المصري

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أغلب الصفات الزراعية والجودة للقطن المصري هي صفات كمية تؤثر مجتمعة في تحديد الاستفادة من نبات القطن. وعلى ذلك فإن تعيين نظام توارث ومواقع تلك الصفات الكمية وتفاعلاتها الأليلية هو غاية عظيمة لكل من مربى القطن خصوصاً وباحث البيولوجيا الجزيئية عموماً. هذه الدراسة وظفت عدة نماذج إحصائية لتحليل تأثيرات وطرق الفعل الجيني في عدة مجتمعات قطنية مشتقة من تهجين سلالة نقية أمومية من صنف القطن المصري جيزة ٨٩ مع سلالة نقية أبوية من القطن الأمريكي تمكوت . هذه الدراسة أيضاً حللت مواقع الصفات الكمية المرتبطة بصفات التيلة لعدد ١٠٣ نبات فردي من نباتات عائلات الجيل الثاني. تحت تلك الظروف تمكنت الدراسة من تعيين تقارب ثمانية عشر موقع للصفات الكمية من الواسمات الوراثية المستخدمة والمتحكمة في صفات التيلة. حيث أظهرت النتائج أن بعض صفات التيلة مثل طول التيلة والمتانة والاستطالة والنعومة والتجانس والاصفرار تأثرت بعدد ٣،٤،٣،٣،٢ و ٢ من مواقع الصفات الكمية علي الترتيب. الإمكان الأعظم للمواقع التي حددتها الدراسة ليس بالضرورة تمثل المواقع الطبيعية أما صفات المحصول فأظهرت عدد قليل وغير معنوي من تلك المواقع . بالنسبة لطرز فعل الجين فإن النماذج المستخدمة اتفقت على أن تأثير السيادة كان أكثر من الإضافة لأغلب الصفات الهامة. ورات الدراسة في مجملها أن الانتخاب المبني على تربية المجتمعات الرجعية يكون مناسباً لزيادة تكرارات الجين المرغوب وبالتالي تحسين تلك الصفات. كما أن دراسة الارتباط بين الصفات المدروسة وواسمات المادة الوراثية المرتبطة بالصفة الكمية وكذلك ارتباط تلك الصفات ببعضها يعتبر أحد أهم معينات الانتخاب في برامج تربية القطن وتوصيف الجينات تمهيداً لهندستها ونقلها. وبالتالي فإن أحلال الواسمات المستخدمة في خرائط مجموعات الارتباط أو الكروموسومات الطبيعية سوف يكون في غاية المنفعة التطبيقية لكل من مربى ومنتج القطن المصري .