

Repeatability and stability analysis for fiber traits in upland cotton (*Gossypium hirsutum* L.)**Dimitrios Baxevanos^{1*}, Ioannis T. Tsialtas² and Christos Goulas³**¹ELGO-“Demetra”, Forage and Pasture Plants Institute, 412 23, Larissa, Greece²Aristotle University of Thessaloniki, Faculty of Agriculture, Lab. of Agronomy, 541 24 Thessaloniki, Greece³Aristotle University of Thessaloniki, School of Forestry, Lab. of Forest Genetics and Breeding, 541 24 Thessaloniki, Greece***Corresponding author: baxevano@gmail.com****Abstract**

Cotton fiber quality is defined by several traits, which are affected by genotype×environment interaction (GEI). The objectives of this work were to study GEI of fiber traits, the repeatability and the effect of the size of testing environments in detecting cultivar genetic differences by High Volume Instrument (HVI), and the usefulness of two GEI derived stability indices, σ^2_i and GGE Instability (GGEIN), and one G+GEI derived index, GGE Distance (GGED). A five-year balanced dataset (“5-yr”), included 56 trials, planted in Greece, was divided in one-year (“1-yr”) and two-year (“2-yr”) datasets for each of ten fiber quality traits and lint yield (LY) to estimate repeatability in ranking cultivars using Spearman’s rank correlations along with heritability on broad sense (H). In conclusion, the HVI system applied in two replications and two years represented by 20-24 environments was effective in discriminating cotton cultivars for the majority of quality traits. In particular, lint percentage (L%) and fiber elongation were the most repeatable traits and they could be estimated precisely using 10-12 environments (“1-yr” data). Fiber upper half mean length, strength, uniformity, yellowness (+b), reflectance (Rd) and LY could precisely be estimated by 20-24 environments (“2-yr” data). Fiber grade, micronaire and short fiber index (SFI) were moderately repeatable using “2-yr” data. Neither Shukla stability variance (σ^2_i) nor GGEIN were repeatable in ranking for stability, whereas GGED index was similar repeatable to mean values but superior in consistency and of higher repeatability for quality traits with high GEI.

Keywords: cotton fiber; cotton stability; GGEbiplots; GGE Distance; HVI.**Abbreviations:** +b - yellowness; E - environment; G - genotype; GEI - genotype×environment interaction; GGED - GGE Distance stability index; GGEIN - GGE Instability Index; H - heritability on broad sense or repeatability (σ^2_g/σ^2_p); L% - lint percentage; MV - mean value; Rd - reflectance; σ^2_p , σ^2_g , and σ^2_{ge} - phenotypic, genotypic and GEI variance components; σ^2_i - Shukla stability index; SFI - Short Fiber Index.**Introduction**

Cotton fiber is defined by a suite of traits, which have a major impact on fiber selling price. Several fiber quality parameters, such as fiber length, length uniformity, strength and elongation, are genetically controlled, mainly but other traits like micronaire, color reflectance (Rd) and yellowness (+b), though genetically controlled, are impacted, to a greater degree by environmental conditions (Meredith, 1984; Krieg and Hequet, 2005; Saha et al., 2008). Many environmental factors, including temperature, cultural practices, water availability, soil properties (e.g. fertility), harvest time, and equipment can impose significant variability and stress the importance of the growing environments and the GEI (Kelley and Boman, 2005; Johnson et al., 2002; Gipson and Joham, 1969). Moreover, High Volume Instrument (HVI), the commercially accepted quality measurement system, has been questioned for its sensitivity or consistency for the effective cultivar discrimination (Kelly et al., 2012). HVI was not effective in detecting small genetic differences for fiber strength (May and Jividen, 1999; Green and Culp, 1990). On the contrary, heritability estimates of 2.5 span length based on HVI were similar to those obtained using fibrograph (May and Jividen, 1999). The previous brief account stresses the importance of coping with GEI. Numerous methods have been proposed to measure the response of genotypes to environmental changes. Shukla’s stability variance (σ^2_i ;

Shukla, 1972) is based on partitioning the GEI sum of squares into components by each genotype. However, σ^2_i confounds GEI signal and GEI noise making no distinction among them (Yan and Kang, 2003). Another GEI derived index is the GGE Instability (GGEIN) index, which is based on GGE Biplot model, and decomposes G plus GE effects, through the singular value decomposition (SVD), into two or more principal components, thereby removes the noise caused by E (Yan and Kang, 2003). A commercially successful commercial cultivar should combine both yield and stability, for this to be realized in a single index, two G+GE derived indices have been proposed, YS_i stability parameter and more recently GGE Distance (GGED) (Kang, 1993; Yan and Kang, 2003). Both indices are dependent and proportional to the mean performance and GEI (Yan and Kang, 2003; Baxevanos et al., 2007). The power of each of these indices to rank genotypes effectively is crucial for breeders and agronomists. The σ^2_i was found low to moderate repeatable for various crops, cotton included (Helms, 1993; Sneller et al., 1997; Baxevanos et al., 2007); the same also holds true for GGEIN (Baxevanos et al. 2007). GGED was found to be highly correlated with yield and more effective in ranking genotypes when GEI was high and yield repeatability was low (Baxevanos et al., 2007). Analogous data have not been reported for fiber quality traits. In Greece, upland cotton

Table 1. Results of combined (“5-yr” dataset) analysis of variance of quality parameters and lint yield (LY).

Source of variation	df	Mean squares										
		^a L%	Micronaire	^b Length (mm)	Strength (gram/tex)	Uniformity (%)	Elongation	^c SFI	^d +b	^e Rd	Grade	^f LY (kg 0.1 ha ⁻¹)
Genotypes (G)	5	342.8**	2.23**	0.037**	148.96**	47.82**	175.66**	22.21**	5.04**	100.45**	235.75**	2677485**
Environments (E)	55	25.52**	2.03**	0.01**	70.18**	14.89**	11.96**	48.62**	4.57**	160.66**	880.85**	3335540**
Replications/E	56	1.17 ^{ns}	0.11 ^{ns}	0.0005 ^{ns}	3.48 ^{ns}	2.46 ^{ns}	0.61 ^{ns}	1.49 ^{ns}	0.23 ^{ns}	3.02 ^{ns}	43.85**	54290**
Interactions (GEI)	275	1.73**	0.14**	0.001**	4.53**	3.65**	0.83 ^{ns}	2.14 ^{ns}	2.7 ^{ns}	3.92**	33.6 ^{ns}	91318**
Polled error	277	1.00	0.08	0.0005	3.27	2.83	0.71	1.61	0.22	2.81	28.19	28230

* and ** significance at P<0.05 and 0.01, respectively, ^aLint percentage, ^bUpper half mean length, ^cShort Fiber Index, ^dyellowness, ^ereflectance index, ^fLint yield

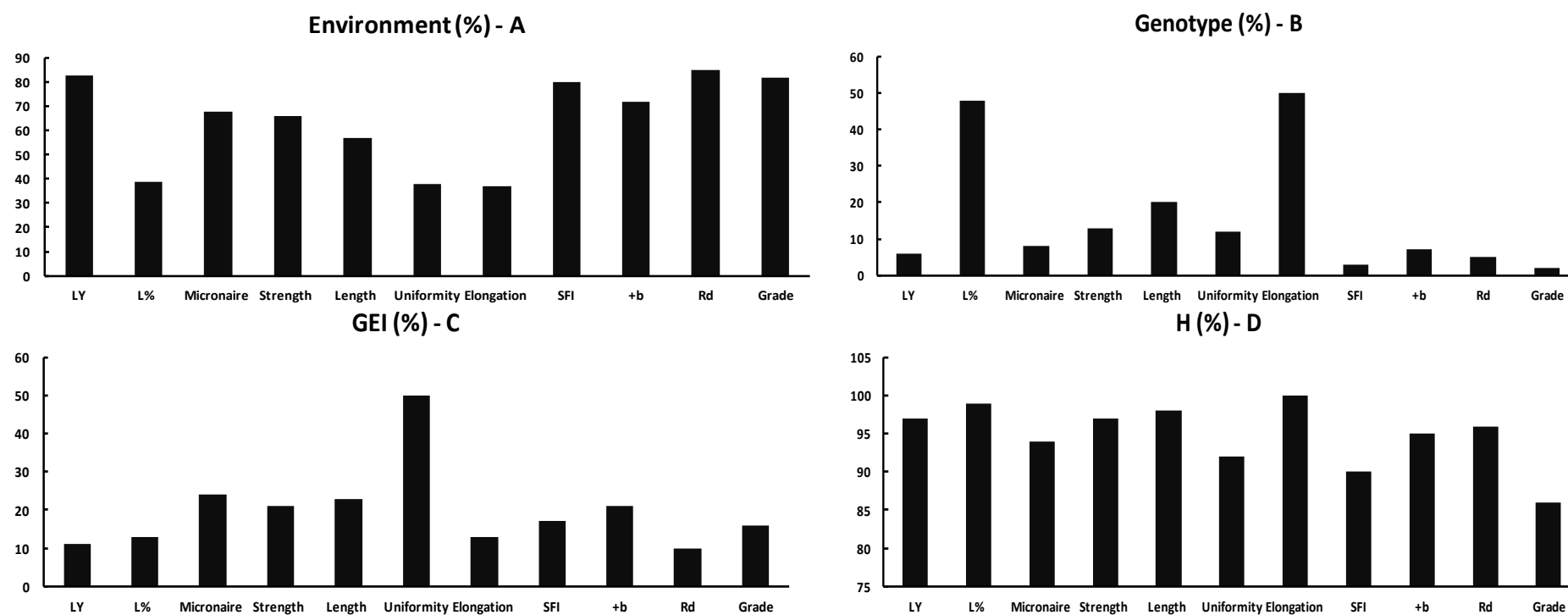


Fig 1. Average (%) environment, genotype, GEI contribution to SS_{TRTMT} and Heritability on broad sense (H) for cotton quality traits and lint yield based on “5-yr” datasets. Where: LY- lint yield, L% - lint percentage, SFI – Short Fiber Index, +b – yellowness, Rd – reflectance.

grows under various climatic and soil conditions; in southern and central Greece, cotton is grown under water shortages along with high temperatures, in the north, cotton is watered more efficiently and temperatures are milder, however more growth season is shorter (Kalivas and Kollias, 2001). Quality is also strongly affected by the temperature and water shortages as well the weathering during harvest can cause significant losses (Tsaliki, 2005). A GGE Biplot model investigation has proved that Greece constitutes a complex cotton yield mega-environment since no repeatable pattern in grouping among locations was identified and for this reason, along with mean performance, stability analysis is of value (Baxevasos et al., 2006). Breeders face great difficulties in their effort to improve fiber traits while maintaining yield (Meredith, 1984). This challenge might be tackled by the extension of stability analysis to fiber traits and the estimation of heritability of HVI to discriminate cotton genotypes effectively. The objectives of this work were to study: i) GEI and the effect of the testing environment sample set on acquiring accurate HVI estimates, and ii) the inter-relationship and the repeatability between three stability indices based on quality traits and lint yield (LY), in the context of a cotton cultivar evaluation program.

Results and Discussion

Analysis of variance

Lint yield and quality traits were significantly affected by G and E main effects (Table 1). Interaction was also significant for the quality traits with the exception of elongation, +b and grade (Table 1). However, while statistical significance of GEI is important for estimating various genetic and environmental parameters, it is the relative size of the GEI signal to the G variation that should be considered (Yan and Kang, 2003). The percentages of the treatment sum of squares accounted for by G, E and GEI have been used as an indicator of the total variation attributable to each component. The partitioning of Sum of Squares (SS) to G, E and GEI, as percentages of SS_{TRTMT} on across the respective datasets, is presented on Table 2 and Figure 1. Based on “5-yr” combined data, the less controlled traits by E were L%, length, length uniformity and elongation (37-57%). Elongation and L% were controlled, mainly, by G (48-50%), whereas LY, micronaire, Short Fiber Index (SFI), and the fiber color parameters were the least G controlled parameters (2-8%). These data agree with other studies on cotton fiber and yield (Meredith, 1984; Balanche, 2005) but fiber strength was more affected by E (66%) being higher than previous reports of 34% (Meredith, 1984) and 48% (Kerby et al., 2001). Summarizing, they were three groups regarding the dependence on G: L% and elongation were strongly controlled by G; strength, length, uniformity were moderately controlled; whereas LY, micronaire, SFI and the fiber color indices were the least controlled by G (Figure 1A-B). The percentage of GEI contributions to quality traits in the “5-yr” combined dataset ranged from 13% to 50% (Table 2, Figure 1C). The size of GEI/G ratio in “5-yr” data was 1.6-8.0 folds the size of G for the quality traits except length that was approximately equal, and L%, elongation that is smaller (Table 2). Particularly in the case of micronaire, uniformity, SFI, +b, was 3.0-5.7 folds and for grade, eight folds higher. The high GEI of quality suggests further study on the influence of G and GEI to the phenotypic variation with the analysis of the heritability (H) on broad sense (Mohammadi et al., 2010). The analysis of variance for each of the datasets (“1-yr”, “2-yr”, “5-yr”) was used to calculate H and its ratios

(σ_{ge}^2/σ_p^2 , σ_e^2/σ_p^2) in order to investigate whether low H was due to GEI or noise (Table 2). In “1-yr” datasets that represented 10-12 environments with years averaged, the H was low for all the parameters. As the number of environments increased up to 20-24 in “2-yr” and up to 56 environments in “5-yr” datasets, H increased because of the σ_e^2 and σ_{ge}^2 reductions since they were divided by higher degrees of freedom, resulting in more powerful *F*-tests. In the combined “5-yr” datasets, LY and quality parameters were highly heritable, with elongation and L% having the highest values of 100% and 99%, respectively (Table 2, Figure 1D). Micronaire, uniformity, SFI, and color grade were ranked at the bottom for H (Figure 1D). The lower H within “1-yr” trials was related with high σ_e^2 or σ_{ge}^2 . Particularly, regarding uniformity, it could be explained by the high GEI (GEI=50% for “5-yr” data, three folds higher than G), and the high error σ_e^2 ratio in “1-yr” and “2-yr” datasets ($7\% \leq \sigma_e^2/\sigma_p^2 \leq 22\%$) (Table 2). This is in agreement with Meredith (2003) who reported large GEI for uniformity with an impact on SFI, which was related with uniformity and was also high in σ_e^2 ratio in “1-yr” and “2-yr” ($16\% \leq \sigma_e^2/\sigma_p^2 \leq 21\%$). Thus, he suggested further investigation since the textile industry is expected to focus on SFI, since reducing it, weight losses in the spinning process will be reduced. Regarding the three color parameters (+b, Rd, grade) the overall picture is that, despite the moderate to high σ_e^2 ratio in “1-yr” and “2-yr” ($7\% \leq \sigma_e^2/\sigma_p^2 \leq 23\%$), H values in “2-yr” data were sufficient (>70%). H value for grade was the lowest (57% in “1-yr”) and with the higher σ_e^2 ratio whereas, Rd index had the highest H in the “1-yr” (82%) and “2-yr” (96%) datasets compared with the rest color indices. Summarizing, micronaire, uniformity, SFI, and grade traits were moderately heritable within “1-yr” datasets and might need “2-yr” datasets for more effective cultivar differentiation. Finally, LY had excellent heritability ratio and low σ_{ge}^2 and σ_e^2 indicating that the environments were homogeneous within a mega-environment (Baxevasos et al., 2007).

Correlations between stability indices per se and with quality means

Rank correlations between parameter mean values (MV) and the three stability indices were calculated for the combined “5-yr” datasets (Table 3). Mean values were highly correlated with GGED (Table 3). GGED is a composite index, proportional to MV and GEI (Balanche, 2005; Baxevasos et al., 2007); the lower the GEI/G ratio the higher the correlation. Short Fiber Index showed low correlation with GGED ($r = -0.71$) and grade ($r = -0.89$), both having high GEI/G ratio, 5.7-8.0, respectively (Table 2). σ_i^2 was correlated moderately to GGEIN for quality traits and LY ($0.42 \leq r \leq 0.71$) (Table 3). There was no significant correlation between MV, GGED versus σ_i^2 and GGEIN, as it was being expected (Baxevasos et al., 2007). Furthermore, it is essential to investigate whether these indices are repeatable in ranking cotton cultivar.

Repeatability of rank correlation

In the context of a cultivar evaluation program, it is important for the agronomists or breeders to quantify the number of evaluation trials necessary for repeatable cultivar ranking. The average repeatability of MV and GGED for “1-yr” and “2-yr” datasets, estimated by Spearman’s rank correlation, is presented in Table 4. As the number of environments increased from “1-yr” to “2-yr” datasets, the repeatability of any MV increased as well its consistency indicated by the

Table 2. Averages of sum squares treatment partitioning for genotype (G), environment (E), interaction (GEI), heritability on broad sense (H) and the heritability ratios (σ_{ge}^2/σ_p^2 , σ_e^2/σ_p^2) of lint yield (LY) and quality traits derived from “1-yr”, “2-yr” and “5 yr” datasets.

Traits	Datasets	Genotype (%)	Environment (%)	GEI (%)	G/E ^a	H (%)	σ_{ge}^2/σ_p^2 (%)	σ_e^2/σ_p^2 (%)
LY	1-yr	11	74	15	1,4	85	10	5
	2-yr	9	79	12	1,3	90	8	2
	5-yr	6	83	11	1,8	97	2	1
^b L%	1-yr	57	29	14	0,2	94	4	2
	2-yr	51	35	14	0,3	97	2	1
	5-yr	48	39	13	0,3	99	0	1
Micronaire	1-yr	12	63	25	2,1	78	7	15
	2-yr	9	67	24	2,7	86	4	10
	5-yr	8	68	24	3,0	94	2	4
Strength	1-yr	32	41	27	0,8	90	2	8
	2-yr	19	58	23	1,2	93	1	6
	5-yr	13	66	21	1,6	97	0	3
^c Length	1-yr	32	41	27	0,8	89	2	9
	2-yr	24	51	25	1,0	95	1	4
	5-yrs	20	57	23	1,2	98	0	2
Uniformity	1-yr	19	39	42	2,2	72	6	22
	2-yr	15	42	43	2,9	82	3	15
	5-yr	12	38	50	4,2	92	1	7
Elongation	1-yr	75	9	16	0,2	97	0	3
	2-yr	61	26	13	0,2	99	0	1
	5-yr	50	37	13	0,3	100	0	0
^d SFI	1-yr	19	40	41	2,2	73	6	21
	2-yr	33	38	29	0,9	80	4	16
	5-yr	3	80	17	5,7	90	2	8
Yellowness (+b)	1-yr	12	64	24	2,0	74	3	23
	2-yr	9	69	22	2,4	87	1	12
	5-yr	7	72	21	3,0	95	0	5
Reflectance (Rd)	1-yr	11	72	17	1,5	82	3	15
	2-yr	6	81	13	2,2	91	2	7
	5-yr	5	85	10	2,0	96	1	3
Grade	1-yr	7	65	28	4,0	57	8	35
	2-yr	4	76	20	5,0	70	4	26
	5-yr	2	82	16	8,0	86	2	12

^aGenotype (%) / Environment (%) ratio, ^bLint percentage, ^cUpper half mean length.

^dShort Fiber Index

lower coefficient of variation (CV, %) in “2-yr” data. However, the improvement in MV for L% and elongation were small and using only “1-yr” datasets (10-12 environments) produced high repeatability ($r \geq 0.88$); both traits controlled mainly by G (Figure 1B). This was also evident by their low CVs. The less repeatable indices (“2-yr” datasets) were SFI ($r = 0.55$), micronaire ($r = 0.62$) and grade ($r = 0.58$). All these indices were affected by E and had high error ratios. Repeatability of +b increased from 0.61 in “1-yr” datasets to 0.78 in “2-yr” ones. Length and strength were also highly repeatable in “2-yr” datasets ($r \geq 0.85$). In summary, L% and elongation were adequately measured using “1-yr” datasets (10-12 environments), whereas length, uniformity, strength, +b, and LY required “2-yr” data (20-24 environments). SFI, micronaire, Rd and grade were moderately repeatable using 20-24 environments, in agreement with the findings previously discussed. GGED was slightly lower repeatable than MV regarding the “1-yr” data (average 0.54), however repeatability improved using “2-yr” datasets to become similar to MV (average 0.77) (Table 4). One interesting point is that repeatability of GGED for micronaire, uniformity, SFI and grade (all traits with high GE/G ratio 3 – 8 fold) was higher than MV. This is in agreement with Baxevanos et al. (2007), who working on a large cotton dataset from Greece, Spain and Turkey, found

that GGED was more repeatable and consistent than MV of lint yield in years with high GEI. In that dataset, trial locations consisted a mega-environment and biplot analysis conformed to the best practices that impose the use of stability within the mega-environment (Gauch, 2006; Yang et al., 2009). However, with quality like the ones with high GE/G GGED can be useful. Moreover, GGED was more consistent in genotypic ranking in comparison with MV because the CV of each trait was lower (Table 4). On average, the CV of GGED was 26.01% and 11.67%, compared with 32.65% and 24.43%, respectively, for MV. σ_i^2 and GGEIN “1-yr” and “2-yr” averages are presented on Table 5. For all datasets, cultivars were 100% stable according to *F*-probability test applied in σ_i^2 index [except for uniformity (93% and 76% in “1-yr” and “2-yr” datasets, respectively) and LY (96% in “1-yr” datasets and 75% in “2-yr” datasets) – data not shown]. σ_i^2 index was not repeatable in any dataset or quality index. GGEIN was low to moderate repeatable in the same traits, lint yield included but still too low. Both indices exhibited high CV for both “1-yr” and “2-yr” datasets. This again implies that within this particular mega-environment, the use of GE derived indices was not useful. Limitations of this research were the small number of cultivars (six) belonging in the same company with narrow genetic base (Zhang et al., 2005).

Table 3. Pairwise Spearman's correlations among mean values (MV) and stability indices based on "5-yr" datasets.

Variable	by Variable	^a L%	Micronaire	^b Length	Strength	Uniformity	Elongation	^c SFI	Yellowness(+b)	Reflectance (Rd)	Grade	^d LY (kg 0.1 ha ⁻¹)
GGEIN	σ_i^2	0.62	0.65	0.67	0.69	0.58	0.65	0.59	0.53	0.42	0.53	0.71*
GGED	σ_i^2	-0.37	-0.66	0.72	0.26	-0.20	-0.49	-0.09	-0.43	0.09	0.37	-0.48
GGED	GGEIN	0.24	-0.22	0.12	0.11	0.30	-0.14	0.34	0.22	0.23	-0.12	-0.34
^e MV	σ_i^2	0.31	0.77	-0.66	-0.09	0.20	0.49	0.03	0.37	-0.09	-0.49	0.25
MV	GGEIN	0.25	0.21	0.10	-0.12	-0.14	0.15	0.17	0.21	-0.14	-0.21	-0.23
MV	GGED	-0.94**	-0.94**	-0.99**	-0.94**	-1.00**	-1.00**	-0.71	-0.94**	-1.00**	-0.89*	-0.94**

* and ** significance at P < 0.05 and 0.01, respectively, ^aLint percentage, ^bUpper half mean length, ^cShort Fiber Index, ^dLint yield, ^eMean value.

Table 4. Average repeatability derived from "1-yr" and "2-yr" datasets of mean values (MV) and GGE Distance (GGED) applied on cotton traits.

Traits	"1-yr"MV	CV(%)	"2-yr"MV	CV(%)	"1-yr"GGED	CV(%)	"2-yr"GGED	CV(%)
^a L%	0.86	9.0	0.88	8.6	0.56	6.1	0.82	5.5
Micronaire	0.51	49.3	0.62	26.5	0.36	38.3	0.69	15.4
^b Length	0.73	23.3	0.88	28.5	0.71	17.3	0.75	3.8
Strength	0.66	44.3	0.85	17.7	0.69	38.1	0.76	8.8
Uniformity	0.70	29.7	0.69	18.6	0.46	21.1	0.78	7.7
Elongation	0.96	3.1	0.97	7.9	0.93	2.2	0.95	3.9
^c SFI	0.43	60.6	0.55	50.1	0.31	56.2	0.61	28.3
+b	0.61	40.8	0.78	18.7	0.05	32.1	0.72	9.3
Rd	0.64	24.3	0.74	19.1	0.66	18.4	0.73	9.8
Grade	0.48	51.3	0.58	58.2	0.48	38.1	0.68	23.9
Lint Yield	0.76	23.4	0.78	14.8	0.76	18.2	0.72	11.6
Average	0.67	32.65	0.76	24.43	0.54	26.01	0.77	11.64

^aLint percentage, ^bUpper half mean length, ^cShort Fiber Index.

Table 5. Average repeatability derived from "1-yr" and "2-yr" datasets of σ_i^2 and GGE Instability (GGEIN) indices applied on cotton traits.

Traits	"1-yr" σ_i^2	CV (%)	"2-yr" σ_i^2	CV (%)	"1-yr" GGEIN	CV (%)	"2-yr" GGEIN	CV (%)
^a L%	0.18	82.0	0.21	66.2	0.10	34.9	0.25	25.6
Micronaire	0.03	115.0	0.20	85.5	0.22	67.9	0.31	89.5
^b Length	0.12	54.4	0.38	22.6	0.32	86.6	0.22	75.3
Strength	0.23	89.7	0.13	66.0	0.10	87.9	0.25	65.2
Uniformity	0.00	74.5	0.10	65.3	0.03	89.9	0.21	57.2
Elongation	0.22	55.5	0.21	68.1	0.13	78.7	0.12	69.4
SFI	-0.24	78.6	-0.14	65.2	0.12	76.5	0.28	22.1
+b	-0.11	95.8	0.12	51.2	0.04	98.8	-0.14	78.2
Rd	0.11	69.7	0.03	32.5	0.18	34.7	0.13	65.6
Grade	-0.04	55.6	0.12	56.5	0.18	68.3	0.36	56.8
Lint Yield	-0.05	72.1	0.23	66.2	0.34	56.2	0.25	32.7
Average	0.04	76.63	0.14	58.94	0.15	70.95	0.25	49.99

^aLint percentage, ^bUpper half mean length, ^cShort Fiber Index.

Table 6. The “1-yr” and “2-yr” datasets and any possible combinations based on “1-yr” and “2-yr” datasets for estimating repeatability by Spearman’s rank correlation between the respective combinations.

“1-yr” subsets	“1-yr” combinations	“2-yr” subsets	“2-yr” combinations
2001	2002 vs 2001	2001/2002	2001/2002 vs 2003/2004
2002	2003 vs 2001	2001/2003	2001/2002 vs 2003/2005
2003	2003 vs 2002	2001/2004	2001/2002 vs 2004/2005
2004	2004 vs 2001	2001/2005	2001/2003 vs 2002/2004
2005	2004 vs 2002	2002/2003	2001/2003 vs 2002/2005
	2004 vs 2003	2002/2004	2001/2003 vs 2004/2005
	2005 vs 2001	2002/2005	2001/2004 vs 2002/2003
	2005 vs 2002	2003/2004	2001/2004 vs 2002/2005
	2005 vs 2003	2003/2005	2001/2004 vs 2003/2005
	2005 vs 2004	2004/2005	2001/2005 vs 2002/2003
	10 combinations		2001/2005 vs 2002/2004
			2001/2005 vs 2003/2004
			2002/2003 vs 2004/2005
			2003/2004 vs 2002/2005
			2003/2005 vs 2002/2004
			15 combinations

Materials and methods

Plant materials

A balanced dataset from the Delta and Pine Land International Agronomic Services (Delta & Pine Land Co is a brand of Monsanto Co) Cultivar Evaluation Program in Greece was used. Six commercial cultivars (DP 388, DP 493, DP 5111, Delta Opal, SG 96, Sicala 40), five proprietary of Monsanto Co and one (Sicala 40) proprietary of Bayer Crop Science, were evaluated in common for five seasons (2001-2005) at 10-12 locations each year, comprising a total of 56 environments. Trial locations were distributed like follows: central Greece 6-8 locations, northern Greece 3-4, southern Greece one location. Trial cultivars were registered after compared with commercial checks for fiber length ≥ 28 mm, strength ≥ 27 gram/tex, and $3.5 \leq$ micronaire ≤ 4.7 . The experimental design was the Randomized Complete Block design with plots of four rows, 0.95 m apart and 10 m long, and four replications at each site. Standard cultural practices applied to the official cotton cultivar evaluation trials were followed throughout the growing season. The two inner rows of each plot were hand-picked twice and the seedcotton yield was recorded. The first pick started when the latest maturing cultivar had 70% open bolls whereas the second pick was conducted when the latest bolls (depending on the cultivar) were open. A random subsample of seedcotton (1 kg) was selected from two replications of the first pick for fiber quality determinations. Samples were ginned on a gin and the lint percentage (L%) calculated as the ratio of lint weight to the total seed plus lint weight. Fiber quality traits were evaluated at the Delta and Pine Land Company (Scott Mississippi) using an HVI 900 Semi-Automatic system (Uster Technologies AG, Uster, Switzerland). Quality traits were micronaire, fiber length as the upper half mean length (mm), strength (gram/tex), uniformity as the ratio of mean fiber length to upper-half mean length expressed as a percentage (%), elongation, short fiber index (SFI), yellowness (+b), reflectance (Rd) and grade recorded as the first two digits of color grade determined by the intersection point of +b and Rd parameters on the Universal Standard for Grade of American Upland Chart.

Data sets and statistical analysis

Three distinct subsets were constructed (Table 6). The first set, which will be referred herein as set “1-yr”, included five one-year subsets. The second set, which will be referred from

now as set “2-yr” included ten two-year subsets. The third dataset, which will be referred herein as set “5-yr”, included the combined locations across the five year period. In the analysis of variance, location \times year combinations were considered as environments. Cultivars were considered as fixed and environments as random effects, respectively (mixed model). The ANOVA treatment sum of squares ($SS_{TRTMT} = SS_G + SS_E + SS_{GEI}$), including the cultivar (G), environment (E) and their interaction (GEI), was partitioned into its components SS_G , SS_E , SS_{GEI} (Sneller et al., 1997). Thus, it was possible to estimate the contribution of each main effect (G, E, and GEI) to the variability of each trait studied. Furthermore, the contribution of G, E, GEI, and pooled (error) to among genotypes phenotypic variance (σ_p^2) was calculated based on the expected mean squares applicable to the appropriate mixed model (McIntosh, 1983). The σ_g^2/σ_p^2 ratio was calculated as heritability on broad sense (H) or repeatability trial index (Guillen-Portal et al., 2004). This parameter was used as a criterion of the effectiveness of each dataset to differentiate among cultivars. Three indices were estimated for each cultivar within each dataset calculated by the GGE Biplot Pattern Explorer software (Yan, 2001). Two indices that approximates the genotype’s contribution to the GEI: Shukla’s stability variance index (σ_p^2) appropriately tested for significance (Kang and Magari, 1996), and GGE Biplot derived GGE Instability (GGEIN) (Yan, 2001), and an index depended on GE and mean value (G), GGE Distance (GGED) (Yan, 2001). A cultivar with low σ_p^2 or GGEIN is considered stable and with low GGED, stable and high yielding (Yan and Kang, 2003). The pairwise interrelationships between means of each variable (LY, L% and quality traits) with their respective stability indices were calculated using Spearman’s rank correlation for the combined “5-yr” dataset. Spearman’s rank correlation is considered more reliable as compared to Pearson’s for traits where the assumption of normal distribution is not fulfilled (Annicchiarico et al., 2000). In the same manner, Spearman’s rank correlation coefficients were calculated in order to study the repeatability of stability statistics and mean value of each variable *per se* across one or two consecutive years, pertinent to the “1-yr” and “2-yr” datasets. The “1-yr” dataset produced ten combinations and the “2-yr” dataset, a total of 15 combinations (Table 6). This analysis allowed studying the repeatability as means of reliable cultivar ranking, based on either indices or mean value (MV) *per se*, using data of different combinations across testing years. Deviations from unity correlation coefficient, i.e. the lack of complete

repeatability could be due to interaction or experimental errors (Annicchiarico et al., 2000). Finally, for each variable, the measurements summarized by the calculation of averages and their coefficient of variation (CV, %) was used as an index of variability.

Conclusions

Elongation and L% were the most repeatable traits and could be estimated precisely using “1-yr datasets” (10-12 environments). Lint yield, strength, length, uniformity, +b and Rd were also heritable but could be accurately estimated by analyzing “2-yr” datasets (20-24 environments). Micronaire, SFI, and grade were moderately repeatable even with “2-yr” data. Mean values were more repeatable with “1-yr” data and equally repeatable with “2-yr” datasets in comparison with GGED. However, GGED was more consistent in ranking cultivars and slightly more repeatable for traits with high GEI. GGED can be complementary to MV, especially when taking into account that GGE Biplot model offers the capacity for a simultaneous evaluation of environments, genotypes or even multiple traits as far as the model diagnosis conforms to best practices. Neither σ^2_i nor GGEIN were repeatable in ranking cultivars for stability.

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