Journal of Agricultural Science (2011), **149**, 197–204. © *Cambridge University Press* 2010
doi:10.1017/S0021859610000596

Intra-cultivar variation in cotton: response to single-plant yield selection at low density

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(*Revised MS received 8 May 2010; Accepted 11 May 2010; First published online 12 August 2010*)

SUMMARY

In a 5-year study (2004–2008), the possibility of exploiting intra-cultivar variation in cotton (*Gossypium hirsutum* L.) was investigated. Honeycomb single-plant selection for seedcotton yield was employed within three cultivars at a low density of 1·15 plants/m^2. First- and second-generation progeny lines (1GPLs and 2GPLs) were evaluated for seedcotton yield at low density at three sites, whereas third-generation progeny lines (3GPLs) were tested at the crop density of 10 plants/m^2 across two sites and 2 years. Significant differentiation for seedcotton yield was discovered within cultivar (cvar) *Christina* and cvar *Corona* at both low and crop densities, and within cvar *Flora* at low density. In addition, significant intra-cultivar heterogeneity for fibre quality properties was found at crop density. The 1GPLs and 2GPLs grown at low density showed increases in seedcotton yield of 16 and 19%, respectively, in cvar *Christina*, and of 2·6 and 3·7%, respectively, in cvar *Corona*. In cvar *Flora*, the 1GPLs and 2GPLs yielded 10 and 3·3% lower than the mother cultivar, respectively. When grown at standard crop density, across sites and years, 12 and 5·2% higher yield was obtained by the *Christina*-derived 3GPLs and the *Corona*-derived 3GPLs, respectively, when compared with the original cultivars. These results provide evidence that elite cultivars are not homogeneous but rather heterogeneous material, within which selections can be made to maintain or improve uniformity and further improve desirable agronomic traits.

INTRODUCTION

The development of improved cultivars is time consuming and costly. Thus, the way breeders' seed is treated is of importance for the maintenance of varietal uniformity and the avoidance of degeneration. Elite cultivars have long been believed to be relatively permanent with limited or no important genetic variation. Numerous studies in several crops, however, have indicated significant intra-cultivar genetic variation, based upon either phenotypic differentiation (e.g. Sprague *et al.* 1960; Byth & Weber 1968; Grogan & Francis 1972; Tokatlidis *et al.* 2006; Fasoula & Boerma 2007) or on molecular techniques (Zhang *et al.* 1995; Olufowote *et al.* 1997; Gethi *et al.* 2002). However, the genome undergoes constant renewal, remodelling and restructuring. The processes of intragenic recombination, unequal crossing-over, DNA methylation, excision or insertion of transposable elements and gene duplication, contribute to newly developed genetic variation (McClintock 1984; Rasmusson & Phillips 1997; Cullis 2005; Morgante *et al.* 2005). Moreover, during the whole process of cultivar maintenance and multiplication, contaminating and degrading forces such as outcrossing, mixing, volunteer plants, natural selection and seedborne diseases all change the gene pool for the worse, and it is even possible for a complete loss of the improved features of the cultivar to occur (Laverack 1994; Parlevliet 2007). Improved cultivars may lose their identity and healthiness because of the aforementioned molecular and mechanical parameters. Peng *et al.* (1999) found that the maximum yield of the famous rice cultivar IR8 in the Philippines reduced by 20% over a period of 30 years. On the other hand,

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https://doi.org/10.1017/S0021859610000596 Published online by Cambridge University Press

measures that reduce the risks of these negative forces, together with a proper maintenance selection, can neutralize these adverse effects on the gene pool or even turn them into positive ones by selecting sufficiently strongly (Parlevliet 2007).

Despite its importance, exploitation of intra-cultivar variation has not been widely investigated. Such a possibility in cotton could be useful for maintaining high standards of uniformity in the long term, and improving desirable agronomic characteristics. Undoubtedly, the magnitude of intra-cultivar genetic variation is likely to be limited, and so phenotypically recognizable only under optimal conditions that maximize phenotypic expression and differentiation. Such a condition is met when plants are grown at a very low density that reduces plant stress, allows maximum seedcotton production per plant and optimizes single-plant heritability by increasing genetic variance in proportion to environmental variance (Fasoula & Fasoula 1997, 2002; Tokatlidis *et al.* 2010). Single-plant selection at very low density has been effectively employed to reveal exploitable intra-cultivar variation in maize (*Zea mays* L.) (Tokatlidis 2000), bread wheat (*Triticum aestivum* L.) (Fasoula 1990; Tokatlidis *et al.* 2006) and soybean (*Glycine max* (L) Merr.) (Fasoula & Boerma 2007). In cotton, Fasoulas (2000) reported exploitable genetic variation within a cultivar for seedcotton yield and resistance to *Verticillium* wilt. In recent research (Tokatlidis *et al.* 2008; Tsialtas *et al.* 2008), single-plant selection within three elite cotton cultivars at the low density of 1·15 plants/m^2 revealed significant variation for seedcotton yield, leaf carbon isotope discrimination, ash and K content and fibre length and micronaire scores. Subsequent to these findings, the main objective of the present study was to explore the response to intra-cultivar selection and whether this variation is exploitable to upgrade the cultivar's agronomic performance. For this purpose, in addition to first- and second-generation progeny lines (1GPLs and 2GPLs) tested at three locations at the same low density under which selection was performed, third-generation progeny lines (3GPLs) were tested at the standard commercial crop density over two locations and 2 years.

MATERIALS AND METHODS

Source material and experimental treatments

Source material consisted of three elite cotton cultivars, released in Greece by commercial companies: '*Christina*', released in 1997 by Bios Agrosystems; '*Corona*', released in 1992 by Delta and Pine Land; '*Flora*', released in 2000 by Bayer Crop Science. These cultivars were chosen due to good adaptation and wide cultivation in northern Greece, where the selection process and progeny testing were conducted.

Experimentation was carried out during five successive growing seasons under irrigated conditions and in three locations representing the major cotton-producing areas of northern Greece. The first location (Site 1) was in Alexandroupolis region (40°51′N, 23°17′E, 9 m asl, a sandy clay loam soil with pH 7·3, organic matter 15·1 g/kg, N-NO_3 41·8 mg/kg, P-Olsen 52 mg/kg and K 437 mg/kg). The second location (Site 2) was at the Aristotle University Farm of Thessaloniki (40°32′N, 22°59′E, 6 m asl, a clay loam soil with pH 7·6, organic matter 25·8 g/kg, N-NO_3 17·9 mg/kg, P-Olsen 25 mg/kg and K 109 mg/kg). The third location (Site 3) was in the Larissa region (39°35′N, 22°36′E, 51 m asl, a clay loam soil with pH 7·9, organic matter 23·4 g/kg, N-NO_3 15 mg/kg, P-Olsen 41 mg/kg and K 455 mg/kg). The selection was performed in the first location with seed of the selected plants obtained from flowers covered with paper bags to ensure self-pollination, while progeny testing was practised at low density at the three locations and at crop density at Site 1 and Site 2.

Selection and progeny (1GPLs and 2GPLs) testing at low density

Details have been presented in a previous paper (Tokatlidis *et al.* 2008) and are summarized here briefly. The honeycomb methodology of selection and evaluation (Fasoulas & Fasoula 1995; Fasoula & Fasoula 1997, 2002) was applied at low density. For selection purposes, seedcotton yield of each plant, being at the centre of a circle of a given size, was expressed as a proportion of the mean yield of the surrounding plants in the circle (Batzios & Roupakias 1997), and plants with the highest relative value were selected.

On 17 April 2004, a honeycomb experiment incorporating three entries (R-3) was established at Site 1, involving a total of 550 plants of each cultivar, with plants being 1·0 m apart. The six plants per cultivar having the highest relative seedcotton yields compared to the mean yield of 61 surrounding plants were selected. The seed of each selected plant constituted a separate 1GPL.

In the next growing season, the 18 1GPLs (six from each cultivar), along with the original cultivars as controls, were established in R-21 honeycomb layouts at Site 1 (15 April 2005), Site 2 (20 April 2005) and Site 3 (14 April 2005) with a distance of 1·0 m between plants. The 21 entries were divided into three sets of seven, each set being the control cultivar and the six respective 1GPLs selected from it. Entries were coded in such a way that 60 replicates of each set were evenly distributed across the entire experimental area to ensure comparable conditions for each set. At Site 1, 10 plants with the highest yields on the basis of the average yield of 42 surrounding plants were selected from each 1GPL. Mixed ginned seed from

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the 10 selected plants within each 1GPL formed the respective 2GPL. As for the 1GPLs, the 2GPLs were evaluated in 2006 (sowing date 14 April for Site 1, 25 April for Site 2 and 15 April for Site 3) and the same selection technique led to 3GPLs.

Progeny (3GPLs) testing at the crop density

The 3GPLs were evaluated for seedcotton yield under standard crop density of 10 plants/m^2. The experimental design was randomized complete blocks with three replications of single-row plots, 5·0 × 1·0 m, and 0·1 m between plants. Each set of a given cultivar and its 3GPLs constituted a randomized sub-block including marginal rows. Trials were planted on 30 and 20 April 2007, and again on 16 and 23 April 2008 at Site 1 and Site 2, respectively. Each plot was harvested separately and data on seedcotton weights adjusted to yield/ha.

One sample of the uppermost fully expanded leaves from both sites in 2007 was collected from each plot to estimate the physiological traits of carbon isotope discrimination (Δ), ash and K content. Fibre quality traits (length, uniformity, strength and micronaire score) were estimated in one sample per plot in Site 1 in both years, via high-volume instrumentation. Strength expresses the force required to break a bundle of fibres one tex unit in size (a tex unit is equal to the weight (g) of 1000 m of fibre). Micronaire is a measure of airflow through a compressed specimen of fibre that relates to fibre fineness and maturity. The techniques for assessment of physiological and fibre quality traits were consistent with the respective applied at low density (Tokatlidis *et al.* 2008).

Data analysis

Mean yields per plant at low density were compared using the S.E.D., and coefficient of variation (CV) associated with particular means was calculated as well as the information provided previously (Tokatlidis *et al.* 2008).

At crop density, yields were expressed on a plot basis and subjected to analysis of variance, where environment (location and/or year) effects were considered random and the cultivar effect was fixed.

RESULTS

Evaluation of 3GPLs at crop density for seedcotton yield

Analysis of variance for genotypes derived from cvar *Christina* indicated that year of test and genotype were highly significant ($P < 0·001$). Genotype × site interaction was also highly significant (Table 1), while genotype × year and genotype × site × year interactions were not significant. Thus, for comparison purposes, seedcotton yields were combined across years. The mean yields of the control cvar *Christina* were 5·61 t/ha in Site 1 and 4·97 t/ha in Site 2. The six 3GPLs yielded 0·87–1·18 (average 1·05) of the control mean yield in Site 1 and 1·07–1·31 (average 1·19) of the control mean yield in Site 2. Two and four of the six 3GPLs were significantly higher yielding than the control in Site 1 (i.e. Chr-2 and Chr-8) and Site 2 (i.e. Chr-3, Chr-6, Chr-8 and Chr-12), respectively.

In genotypes related to *Corona*, the main effects of test year and genotype were significant. The genotype × site × year interaction was also significant and thus data from the four trials are given separately in Table 1. At Site 1 during 2007, yields of the 3GPLs ranged from 0·99 to 1·17 of *Corona*, and two lines differed significantly (Crn-5 *v.* Crn-17). In 2008, yields of the six 3GPLs ranged from 0·94 to 1·08 of the control yield, with lines Crn-4, Crn-5 and Crn-17 significantly higher yielding than line Crn-1. At Site 2 during 2007, the six lines averaged 5·01 t/ha, 9·0% higher than the control (4·60 t/ha), and line Crn-17 significantly out-yielded *Corona* (17%). In 2008, lines Crn-5 and Crn-16 were significantly lower (12%) and higher (13%), respectively, in seedcotton yield than the original cultivar.

In selections from cvar *Flora*, only the main effects of test year were significant. Pooled data over sites and years resulted in line yield ranging from 0·95 to 1·04 of the mean yield of *Flora* (6·16 t/ha), while the 3GPL overall yield was 6·12 t/ha (Table 1).

Response to selection for seedcotton yield

The overall response to selection in each generation is given in Fig. 1, with yield combined over lines, sites and years and expressed relative to the respective average control yield. In cvar *Christina* compared to the control, 1GPLs and the 2GPLs (tested at low density) produced 16 and 19% significantly higher yield respectively, with the 3GPLs tested at crop density giving 12% significantly higher yield. The yields of the 1GPLs and 3GPLS of cvar *Corona* did not differ significantly from the control, while the 2GPLs were 3·7% significantly higher yielding. In cvar *Flora*, the 1GPLs were 10% significantly lower yielding, while the 2GPLs and 3GPLs were similar to the original cultivar.

Intra-cultivar variation for physiological and fibre properties at crop density

The analysis of variance did not depict significant genotype effects regarding the three physiological traits (data not shown). For the fibre quality properties, the genotype effects were significant for micronaire reading in cvar *Christina*, for length and micronaire reading in cvar *Flora*, and for length, strength and micronaire reading in cvar *Corona*. The

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Table 1. *Mean seedcotton yield as a proportion of the control mean of the 3GPLs derived from cvars* Christina, Corona *and* Flora *evaluated at crop density (data across two sites and 2 years)*

Christina			*Corona*					*Flora*	
				Site1		Site2			
Genotype	Site1	Site2	Genotype	2007	2008	2007	2008	Genotype	Over site
Chr-2	1·18	1·07	Crn-1	1·03	0·94	1·15	0·98	Flr-9	0·95
Chr-3	0·96	1·18	Crn-4	1·11	1·07	1·07	0·95	Flr-10	1·00
Chr-6	1·12	1·31	Crn-5	0·99	1·08	1·05	0·88	Flr-13	0·96
Chr-8	1·16	1·22	Crn-16	1·10	1·01	1·08	1·13	Flr-15	0·99
Chr-11	1·01	1·14	Crn-17	1·17	1·08	1·17	0·99	Flr-18	1·03
Chr-12	0·87	1·25	Crn-20	1·09	1·06	1·02	1·06	Flr-19	1·04
Over lines	1·05	1·19		1·08	1·04	1·09	1·00		0·99
Control yield (t/ha)	5·61	4·97		4·35	5·93	4·60	6·14		6·16
S.E.D. (error D.F. = 48)*	0·371			0·372				0·274	
CV	0·11			0·08				0·11	
Significant interactions	Genotype × site ($P < 0·001$)			Genotype × site × year ($P < 0·05$)				ns	

* Standard error difference to compare genotype yields across and within columns.
ns: not significant.

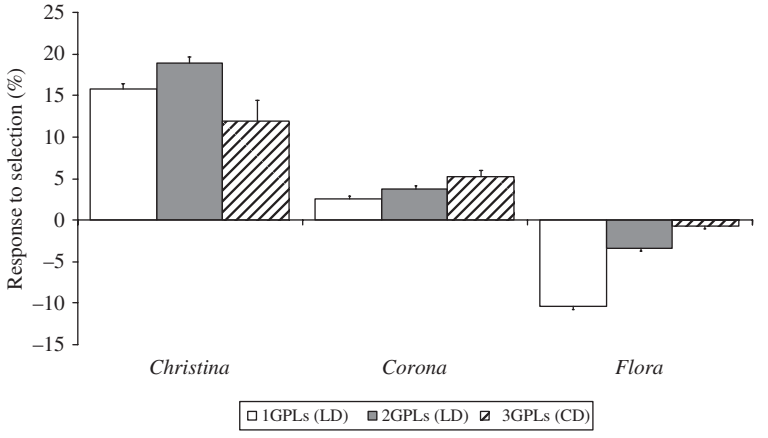


Fig. 1. Average response to selection within cvars *Christina, Corona* and *Flora* estimated as average % yield deviation from the original cultivar of the 1GPLs and the 2GPLs at low density [pooled data over three sites adapted from Tokatlidis *et al.* (2008)], and of the 3GPLs at crop density (pooled data over two sites and 2 years). (Bar on each column indicates the respective S.E.D. with the control value).

genotype × year interactions were not significant. In the lines derived from cvar *Christina*, Chr-2 and Chr-11 had significantly higher micronaire values than Chr-8 and Chr-12 (Table 2). The cvar *Flora*-derived genotype Flr-19 had significantly longer fibre compared with three 3GPLs and the control. Line Flr-10 had the highest micronaire value and line Flr-18 had the lowest micronaire value, both significantly different than four and three of the remaining genotypes, respectively, whereas the micronaire value of Flr-18 was significantly lower than the control mean. Line Crn-5 was numerically the shortest in fibre length and significantly different from four Crn-3GPLs and *Corona*, while the numerically longest in fibre length was line Crn-20 which differed significantly from three 3GPLs. Line Crn-16 produced fibre significantly stronger than three of the Crn-3GPLs and *Corona*, whereas lines Crn-5 and Crn-20 had significantly greater micronaire values compared with lines Crn-1, Crn-4 and Crn-16.

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Table 2. *Mean values for fibre quality properties of the 3GPLs derived from cvars* Christina, Corona *and* Flora *evaluated at crop density (data from Site 1 across 2 years)*

Christina		*Corona*				*Flora*		
Genotype	Micronaire	Genotype	Length (mm)	Strength (g/tex)	Micronaire	Genotype	Length (mm)	Micronaire
Chr-2	4·60	Crn-1	29·8	31·9	3·93	Flr-9	30·9	4·28
Chr-3	4·37	Crn-4	29·8	29·0	4·07	Flr-10	30·5	4·85
Chr-6	4·40	Crn-5	29·6	28·0	4·68	Flr-13	31·1	4·33
Chr-8	4·15	Crn-16	30·6	34·3	4·10	Flr-15	31·9	4·48
Chr-11	4·58	Crn-17	30·4	33·0	4·23	Flr-18	31·1	3·85
Chr-12	4·08	Crn-20	31·3	29·0	4·60	Flr-19	32·1	4·18
Control	4·28		30·8	30·0	4·40		30·8	4·65
S.E.D. (Error D.F. = 24)*	0·188		0·538	1·917	0·232		0·717	0·240
CV	0·08		0·03	0·11	0·09		0·04	0·10

* Standard error difference to compare the genotype means.

DISCUSSION

Intra-cultivar variation

At crop density, the magnitude of intra-cultivar variation (i.e. the relative superiority of the top over the bottom genotype) for seedcotton yield across sites and years averaged 0·22 for the 3GPLs of *Christina* (Chr-6 *v*. control), and 0·10 for the 3GPLs of *Corona* (Crn-17 *v*. Crn-5 and control) (Table 1). Significant differences among the 3GPLs at crop density were also discovered for fibre micronaire within the three cultivars, for fibre length within *Corona* and *Flora*, and for fibre strength within *Corona* (Table 2), but not for any of the three physiological traits.

There are important comparisons between these findings for the 3GPLs which were tested at crop density and the findings obtained at low density using 1GPLs and 2GPLs (Tokatlidis *et al.* 2008). At low density, greater variability was observed. For instance, the magnitude of intra-cultivar variation for seedcotton yield averaged 1·25 (1GPLs) and 1·28 (2GPLs) for cvar *Christina* and 1·14 (1GPLs) and 1·26 (2GPLs) for cvar *Corona*. Moreover, at low density, significant heterogeneity was found for seedcotton yield within cvar *Flora*, for fibre length within cvar *Christina*, and for three physiological traits within the three cultivars (Tokatlidis *et al*. 2008). This supports the hypothesis that very low densities accentuate phenotypic differentiation (Fasoula & Fasoula 1997, 2002; Tokatlidis *et al*. 2006, 2010), thus allowing even limited genetic variability to be identified. These findings are in agreement with those found in other studies (Fasoula 1990; Fasoulas 2000; Tokatlidis 2000; Tokatlidis *et al*. 2006; Fasoula & Boerma 2007). Because of the accumulated reports of intra-cultivar variation, continued selection under very low density has been proposed (Fasoula & Fasoula 2002). In contrast to the typical maintenance stewardship, in which, elite cultivars are treated as permanent records and usually plants of 'incorrect' type are removed (Parlevliet 2007), the suggested technique is more a selection rather than a rouging process. It may progressively lead to new registration by the improvement of important agronomic traits, the required time for which is hard to be answered depending on both crop and cultivar.

Response to intra-cultivar selection

Averaged over sites, the cvar *Christina*- and cvar *Corona*-derived 1GPLs outperformed the original cultivars by 16 and 2·6%, respectively (Fig. 1). Even though more favourable environmental conditions prevailed when the 1GPLs were evaluated (Tokatlidis *et al*. 2008), the advanced self-pollination of 2GPLs achieved greater increase in seedcotton yield over the controls (19% for *Christina* and 3·7% for *Corona*). With a few exceptions, both 1GPLs and 2GPLs exhibited lower CV for single-plant yields than their original parents (Tokatlidis *et al.* 2008), suggesting homozygosity or homogeneity within the selected lines, and indicating that the selection imposed narrowed existing genetic variation within these two gene pools which had been assumed to be fairly homogeneous. By contrast, selection within cvar *Flora* resulted in 1GPLs with 10% lower average yield than the original cultivar but this decreased to 3·3% in the second generation (Fig. 1), and one possible cause was a considerable degree of heterozygosity in the original cultivar (Tokatlidis *et al.* 2008). Across sites, the 1GPLs averaged a CV value 11% higher than cvar *Flora*, but the 2GPLs and the control averaged a

https://doi.org/10.1017/S0021859610000596 Published online by Cambridge University Press

similar CV value, suggesting variability due to heterozygosity. Whether the hypothesis is valid, repeated selfing is required to fix homozygosity and find out whether this variability is exploitable.

The positive response to selection within cvars *Christina* and *Corona* was verified at crop density. Over sites and years, their 3GPLs yielded 12 and 5·2% higher than the original cultivars respectively (Fig. 1). It has been suggested that phenotypic differentiation decreases at high densities due to increased interplant competition (Fasoula 1990; Fasoula & Fasoula 1997, 2002; Tokatlidis *et al*. 2006, 2010). This constitutes a reasonable explanation to the fact that in cvar *Christina* the 19% overall superiority for the 2GPLs at low density decreased to 12% for the 3GPLs at crop density (Fig. 1). A similar influence of low- *v*. commercial crop-density conditions on the magnitude of entry differentiation has been reported in bread wheat (Fasoula 1990; Tokatlidis *et al*. 2006). Selection for seedcotton yield may not exclude cultivar improvement for other important agronomic traits either, and the case of high yielding line Crn-16 (Table 1) with the strongest fibre (Table 2) is instanced. Line Chr-6 that out-yielded cvar *Christina* by 22% at crop density (Table 1) was also superior for fibre length at low density (Tokatlidis *et al.* 2008). Leaf carbon isotope discrimination, Δ, which reflects plant discrimination against $^{13}CO_2$ and the assimilation of $^{12}CO_2$ during photosynthesis, has been suggested as an indirect assessment of water use efficiency, since the two traits are negatively associated (Saranga *et al.* 2004; Tsialtas *et al.* 2008). Lines Chr-2, Chr-3 and Chr-8, as well as Crn-20, had significantly lower Δ values than the controls at low density (Tokatlidis *et al.* 2008), showing a possible advantage under drought stress conditions. Honeycomb single-plant selection for seedcotton yield within the susceptible cvar *Sindos 80* led to the resistant cvar *Macedonia* in terms of *Verticillium* wilt (Fasoulas 2000). No progress from intra-cultivar selection was found in cvar *Flora* based on the 3GPLs performance at crop density (Fig. 1). Although cotton is a self-pollinated species, natural crossing may range from 0 to almost 0·5 (Xanthopoulos & Kechagia 2000). If the cross-pollination hypothesis is real in cvar *Flora*, it cannot be excluded that continuous selection at low density may lead to new lines capable of out-yielding cvar *Flora*, and leading essentially to a new cultivar. In tomato (*Lycopersicon esculentum* L.), exploitable genetic variation for yield was uncovered in advanced generations, after the point of achieving theoretical homozygosity, i.e. F_7 generation (Christakis & Fasoulas 2002).

Results from molecular studies indicate that the genome undergoes constant remodelling and restructuring, suggesting that it is more flexible and plastic than previously assumed (McClintock 1984; Zhang *et al*. 1995; Olufowote *et al*. 1997; Rasmusson & Phillips 1997; Gethi *et al*. 2002; Cullis 2005; Morgante *et al*. 2005). If that is the case, then the observed response to selection within cotton cultivars may have important implications for cultivar maintenance and deserves further consideration. Laverack & Turner (1995) stated that to maintain a cultivar true-to-type and healthy, removing off-type plants is an imprecise method. Gethi *et al.* (2002) pointed out that breeders should take into account the within inbred line genetic variation in maize, particularly when sampling early released materials. The selection method of 1GPLs and 2GPLs at low density and the final performance test of the 3GPLs at crop density open up new prospects for similar studies with other cultivars and crops to determine how universal this rule may be. The practice of advancing generations or maintaining cultivars at commercial densities, may favour degeneration by increasing the relative contribution from strong competitor plants with low seed yields to the next generation compared to that of weak competitor plants with high seed yields (Fasoula 1990; Fasoula & Fasoula 1997, 2002; Tokatlidis *et al*. 2006, 2010). On the other hand, cultivar improvement may result if selection continues at low density after the release of cultivars, covering an adequate number of sites. By this procedure, undesirable mutations or segregates may be removed, while exploiting those affecting yield, stability and other desirable traits. For instance, mote frequencies that represent a loss in yield and cause imperfections in yarn and cloth quality (Bolek 2006), or leaf morphology that affects yield, quality, maturity, water-use efficiency, etc. (Hao *et al.* 2008) might be improvable via intra-cultivar selection at very low density. By improving important agronomic traits and yield capacity for the farming-density conditions, a new registration may result. Intra-cultivar honeycomb selection at very low density resulted in progeny lines that, at farming-density, outperformed the cultivar by 8·0% (Fasoula 1990) and 22% (Tokatlidis *et al*. 2006) in bread wheat and by 10% in cotton (Fasoulas 2000). Additionally, Tokatlidis *et al.* (2006) reported improvement of cultivar stability. Rasmusson & Phillips (1997) reported incremental genetic gains for several traits in a narrow gene pool of barley (*Hordeum vulgare* L.) and hypothesized that gain from selection within elite gene pools occurs because of variation present in the original gene pool as well as *de novo* generated variation. In maize, long-term selection studies for modified oil and protein showed that meaningful variation exists after 90 generations of selection (Dudley & Lambert 2004).

In conclusion, the data reported herein suggest exploitable intra-cultivar variation in cotton. This variation may be due to latent variation, or epigenetic variation in response to environmental changes. Results of the present study and numerous supporting reports imply that the genomes of 'pure line' cultivars

https://doi.org/10.1017/S0021859610000596 Published online by Cambridge University Press

may not be as fixed as previously assumed. Continuous selection within elite cotton cultivars may prove to be a useful technique either to upgrade or to avoid gradual degeneration of genetic background and to maintain uniformity over longer periods of time. Low density, by accentuating the limited intra-cultivar variation, appears ideal to identify the existing and newly developed genetic differences, and to remove inferior plants and identify the superior ones. Hence, prospective analogous research in cotton and other crops may verify that the selection within elite cultivars at low densities as a maintenance of improvement process is a beneficial technique to secure optimal quality of breeder's seed.

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