

## The development of highly-performing open-pollinated maize lines via single-plant selection in the absence of competition

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### Abstract

Selfing and high selection pressures under ultra-low plant densities that ensure absence of competition have been recommended as a means to exploit the additive genetic effects and develop maize (*Zea mays* L.) lines approaching the yield performance of hybrids. Additionally, low plant densities could promote self-pollination due to the better synchronization of pollen and silk emergence. To address these hypotheses selection was applied for three generations using as starting material the F<sub>2</sub> of the maize hybrid 'Costanza' under open-pollination conditions and a density of 0.74 plants m<sup>-2</sup>. The selection was based on two equations: one to estimate the yield genetic potential of single plants and the other the yield genetic potential of entries/ lines. The results were promising in terms of enhanced gene fixation and exploitation of additive gene action. The high selection pressures applied led to fairly homogeneous half-sib lines that approximate the productivity and stability levels of 'Costanza'. Utilization of such lines in the production of hybrids is expected to increase the crop yield potential, while progressive line improvement could ultimately reduce the line-to-hybrid gap. Concluding, progressive replacement of less by more favorable additive alleles is accomplishable when ultra-low plant densities are used so as to maximize pollen production and diminish the anthesis-to-silking interval, thus promoting selfing. The application of the procedure in commercially successful hybrids is recommended in order to develop highly homogeneous lines of superior performance; the hybridization between such lines is bound to lead to less heterogeneous hybrids and reduced seed cost.

**Keywords:** additive gene action, homeostasis, line-to-hybrid gap, plant yield potential, selection intensity, ultra-low density.

**Abbreviations:** ASG, anthesis-to-silking gap; EBV, entry B value, the outcome of equation B; EHI, entry-homeostasis index; EYI, entry-yield index; HS, half-sib; PAV, plant A value, the outcome of equation A; PYI, plant-yield index.

### Introduction

Combining ability and heterosis are the commonly employed selection criteria in maize due to the belief that overdominance is the prevalent type of gene action that determines yield (Fasoula and Fasoula, 2005). Tollenaar and Lee (2002), though, pointed out that the maize yield improvement accomplished so far is associated neither with heterosis nor with yield potential *per se*. Instead, mounting evidence in maize indicate that additive genetic variance is the predominant type of gene action in the expression of yield and similar quantitative traits (Hallauer and Miranda, 1981; Crow, 2000; Butruille et al., 2004; Troyer and Wellin, 2009). Selection for heterosis and combining ability is mainly responsible for the retention of a huge productivity gap between inbred lines and hybrids (Tokatlidis et al., 1999; Fasoula and Tollenaar, 2005). In order to bridge this gap, Fasoula and Fasoula (2002, 2005) suggested that maize improvement should be focused on line productivity *per se* in combination with stability, so as to effectively exploit the additive genetic variation. Encouraging results supporting such a breeding strategy have been presented (Fasoulas, 1997; Tokatlidis et al., 1998, 2008; Vafias and Ipsilantis, 2005). Selection for combining ability was proposed as a secondary task after inbred lines of high productivity have been achieved (Tokatlidis et al., 1998; Fasoula and Fasoula, 2005).

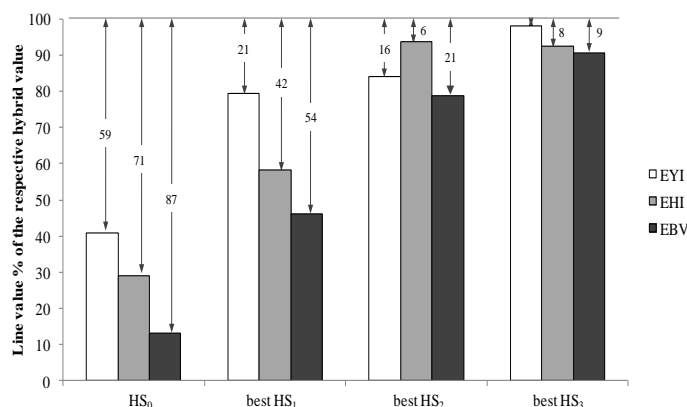
Selection under low plant density was found necessary to overcome the plant yield potential stagnation (Fasoulas, 1993; Duvick, 1997, 2005; Tokatlidis et al., 1998, 2008). Selection in the absence of competition is one of the main principles that distinguish the honeycomb selection methodology from other breeding schemes (Fasoulas, 1993; Fasoula and Fasoula, 1997, 2000, 2002; Fasoula and Tokatlidis, 2012). In the absence of competition, whole-genome phenotype analysis is accomplished by dividing the crop yield potential into three components, namely the plant yield potential, the tolerance to stresses and the responsiveness to inputs (Fasoula and Fasoula, 2000, 2002, 2005; Fasoula and Tokatlidis, 2012). For selection purposes among entries/lines and among plants within each entry/line at the honeycomb experimental layouts (Fasoulas and Fasoula, 1995) the aforementioned crop yield potential components have been incorporated into two novel equations (Fasoula, 2008; Fasoula and Tokatlidis, 2012): equation A for single-plant selection,  $A = (x/\bar{x}_r)^2 \cdot (\bar{x}/s)^2$ , and equation B for entry selection,  $B = (\bar{x}/\bar{x}_t)^2 \cdot (\bar{x}/s)^2$ , where  $x$  is the yield of a particular plant under consideration,  $\bar{x}_r$  is the average yield of the surrounding plants within a moving

**Table 1.** The results of the calculation of the unitless Equation B parameters for HS<sub>1</sub> lines. The entries are arranged in descending order according to the Equation B outcome (EBV). Nine HS<sub>1</sub> lines are presented in groups of three representing the best, middle and worst performing of the total 29 HS<sub>1</sub> lines selected. The respective values for the original hybrid (F<sub>1</sub>) and HS<sub>0</sub> generation as well as the mean HS<sub>1</sub> values are also given. All the values are expressed as % of the respective absolute hybrid (i.e. 100%) values.

Site 1					Site 2				
Entry†	EBV	EYI	EHI	$\bar{x}$	Entry†	EBV	EYI	EHI	$\bar{x}$
9 <sup>(2)</sup>	62.3	79.1	78.80	89.0	9 <sup>(2)</sup>	30.0	79.6	37.6	89.3
1 <sup>(3)</sup>	46.4	77.3	60.02	87.9	4 <sup>(3)</sup>	22.5	70.4	32.0	83.9
18 <sup>(4)</sup>	44.6	62.7	71.14	79.2	16 <sup>(4)</sup>	22.2	79.9	27.7	89.4
.....					.....				
15 <sup>(11)</sup>	28.5	79.1	36.0	89.0	8 <sup>(14)</sup>	13.4	76.1	17.6	87.3
.....					.....				
12 <sup>(15)</sup>	20.9	62.8	33.4	79.2	24 <sup>(15)</sup>	13.4	51.5	25.9	71.8
25 <sup>(16)</sup>	19.9	57.3	34.7	75.7	1 <sup>(16)</sup>	13.3	67.0	19.8	81.8
29 <sup>(17)</sup>	19.8	61.4	32.2	78.3	.....				
.....					10 <sup>(18)</sup>	9.20	65.3	14.1	80.8
HS <sub>0</sub> <sup>(22)</sup>	7.66	32.5	23.6	57.0	HS <sub>0</sub> <sup>(23)</sup>	3.64	44.4	8.19	66.6
.....					.....				
26 <sup>(29)</sup>	3.98	31.8	12.5	56.4	21 <sup>(29)</sup>	0.94	27.0	3.50	52.0
23 <sup>(30)</sup>	1.35	14.2	9.47	37.7	23 <sup>(30)</sup>	0.29	14.3	2.04	37.8
11 <sup>(31)</sup>	0.76	13.9	5.44	37.3	11 <sup>(31)</sup>	0.02	4.36	0.56	20.9
HS <sub>1s</sub> average	22.9	55.1	37.9	73.8	HS <sub>1s</sub> average	11.3	57.3	17.3	74.0
F1(100%) <sup>(1)</sup>	40.0	1.82	22.0	705 g	F1(100%) <sup>(1)</sup>	100	1.76	56.9	569 g

† In parenthesis the entry's rank position. • EBV= entry B value, • EYI= entry-yield index, • EHI= entry-homeostasis index, •  $\bar{x}$  =entry mean yield, • HS<sub>0</sub>= half-sib generation 0 (the starting material), • HS<sub>1s</sub>= half-sib generation 1 (after one selection cycle), • F1= original hybrid 'Constanza', • gray cells indicate the HS<sub>1</sub> lines that produced the final HS<sub>3</sub> lines.

circle of a chosen size,  $\bar{x}_t$  is the overall experimental mean yield and finally,  $\bar{x}$  and  $s$  are the entry mean yield and standard deviation. In the equations, the unitless parameters  $(x/\bar{x}_t)$ ,  $(\bar{x}/\bar{x}_t)^2$  and  $(\bar{x}/s)^2$  measure in that order: the plant yield potential, called plant-yield index (PYI), the entry/line yield potential, called entry-yield index (EYI) and the entry stability, called entry-homeostasis index (EHI), which is common in both equations. The outcome of the equation A, termed plant A value or PAV, ensures the evaluation of all the plants in the experimental design with the same accuracy, the conversion of the plant yield potential into crop yield potential and the application of high selection pressures that maximize genetic gain. The outcome of the equation B, termed entry B value or EBV, ensures the reliable comparison of the entries for crop yield potential, since the first parameter of the equation evaluates the yield potential and the second one assesses the entry's stability of performance. In order to accomplish the goal of bridging the productivity gap between inbred lines and hybrids, Fasoula and Fasoula (2005) suggested that maize should ideally be treated as an inbreeding species and breeding take place under ultra-low plant density conditions that ensure absence of competition, then apply extremely high selection pressures using commercially-successful hybrids as starting material. Their approach places emphasis on the improvement of the plant yield potential of each line along with the improvement of their individual buffering. Lower densities in maize, however, cause earliness of both pollen shedding and silk emergence, the latter to an especially high degree, resulting in a decreased anthesis-to-silking gap (ASG) (Sangoi et al., 2002; Tokatlidis et al., 2005; Uribebarrea et al., 2008). Borrás et al. (2007) noted that under conditions that inhibit plant growth, female flower development is delayed relative to that of the male flowers resulting in an increase in the ASG, while Uribebarrea et al. (2008) reported a density of 3 plants m<sup>-2</sup> as low enough for this gap to be zero. Therefore, ultra-low



**Fig 1.** The average performance of the HS<sub>0</sub> and of the best performing HS<sub>1</sub>, HS<sub>2</sub> and HS<sub>3</sub> lines over the two sites, based on the entry-yield index (EYI), entry-homeostasis index (EHI) and the outcome of equation B (EBV). All the values are expressed as percentage of the respective hybrid values. The number and arrows above each bar denote the line-to-hybrid gap for the respective parameter.

densities may facilitate enhanced self-pollination as the outcome of the better synchronization of pollen and silk emergence, as well as the production of more pollen and longer shedding in comparison with dense stand conditions (Tokatlidis et al., 2005). In this study honeycomb breeding was employed in order to explore the possibility to develop open-pollinated half-sib (HS) lines of high yield performance. Towards this objective, the aforementioned novel equations for individual plant and progeny line evaluation were applied using F<sub>2</sub> seed from a commercially grown hybrid as starting material. To our knowledge, this is the first direct attempt to address such an issue.

**Table 2.** The results of the calculation of the unitless Equation B parameters for HS<sub>2</sub> lines. The entries are arranged in descending order according to the Equation B outcome (EBV). Nine HS<sub>2</sub> lines are presented in groups of three representing the best, middle and worst performing of the total 19 HS<sub>2</sub> lines selected. The respective values of the original hybrid (F<sub>1</sub>) and HS<sub>0</sub> generation as well as the mean HS<sub>2</sub> values are also given. All the values are expressed as % of the respective absolute hybrid (i.e. 100%) values.

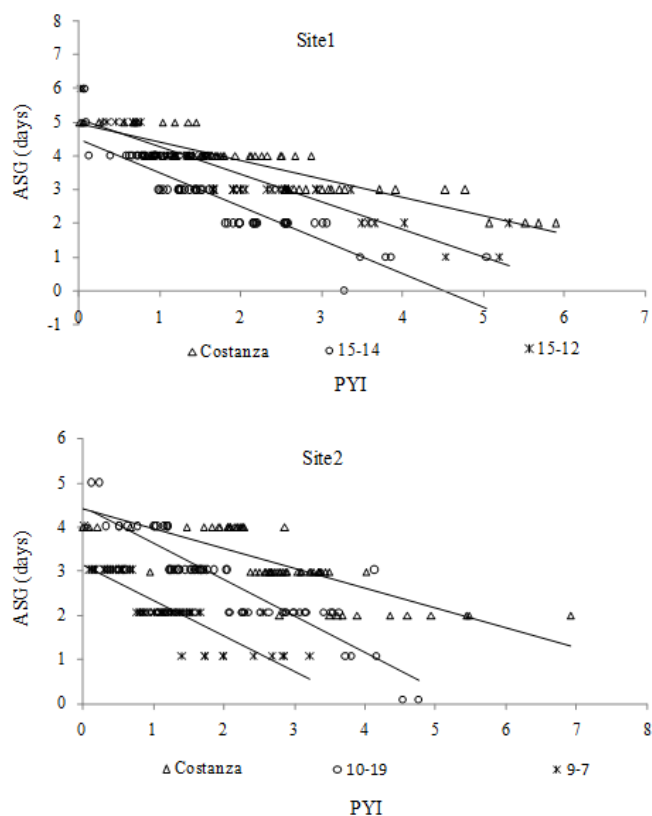
Site 1					Site 2				
Entry†	EBV	EYI	EHI	$\bar{X}$	Entry†	EBV	EYI	EHI	$\bar{X}$
15-14 <sup>(2)</sup>	78.4	83.0	94.4	91.1	10-19 <sup>(2)</sup>	79.3	85.2	93.0	92.3
9-3 <sup>(3)</sup>	77.4	58.8	131	76.7	9-1 <sup>(3)</sup>	46.3	53.0	87.4	72.8
15-12 <sup>(4)</sup>	69.5	87.1	79.8	93.3	2-17 <sup>(4)</sup>	42.1	50.9	82.7	71.3
••••••••••					••••••••••				
2-11 <sup>(10)</sup>	42.4	69.7	60.8	83.5	9-6 <sup>(10)</sup>	20.0	37.1	54.1	60.9
9-8 <sup>(11)</sup>	38.1	49.8	76.5	70.6	24-15 <sup>(11)</sup>	18.9	38.3	49.4	61.9
9-1 <sup>(12)</sup>	37.1	50.9	72.9	71.4	HS <sub>0</sub> <sup>(12)</sup>	18.9	50.3	37.6	70.9
••••••••••					••••••••••				
9-2 <sup>(18)</sup>	25.0	45.6	54.9	67.5	9-9 <sup>(13)</sup>	18.6	40.8	45.6	63.9
2-16 <sup>(19)</sup>	23.7	42.1	56.4	64.8	••••••••••				
HS <sub>0</sub> <sup>(20)</sup>	22.3	36.7	46.2	60.6	4-10 <sup>(19)</sup>	7.5	31.0	24.2	55.7
9-4 <sup>(21)</sup>	10.3	28.4	36.3	53.3	28-14 <sup>(20)</sup>	7.1	25.8	27.7	50.8
HS <sub>2s</sub> average	44.6	56.8	58.0	74.6	9-2 <sup>(21)</sup>	4.8	25.1	19.2	50.1
F1(100%) <sup>(1)</sup>	14.3	1.83	7.83	664 g	HS <sub>2s</sub> average	24.3	43.8	50.4	65.5
					F1(100%) <sup>(1)</sup>	26.3	2.20	11.9	528 g

† In parenthesis the entry's rank position. • EBV= entry B value, • EYI= entry-yield index, • EHI= entry-homeostasis index, •  $\bar{X}$  = entry mean yield, • HS<sub>0</sub>= half-sib generation 0 (the starting material), • HS<sub>2</sub>= half-sib generation 2 (after two selection cycles), • F1= original hybrid 'Constanza', • gray cells indicate the HS<sub>2</sub> lines that produced the final HS<sub>3</sub> lines

## Results

### Performance of the HS<sub>1</sub> → HS<sub>3</sub> lines

The results for the HS<sub>1</sub> lines performance are summarized in Table 1. The hybrid used as control yielded 705 and 569 g plant<sup>-1</sup> at the Site 1 and Site 2, respectively. The mean yield per plant for the HS<sub>1</sub> lines in relation to the hybrid mean ranged from 37.3 to 89% in the Site 1 and from 20.9 to 89.3% in the Site 2. The HS<sub>1</sub> lines averaged 73.8 and 74.0% of the hybrid mean yield in the Site 1 and Site 2, respectively, displaying an average yield gap between hybrid and HS<sub>1</sub> lines of about 26% in both sites. In terms of the equation B and its components, HS<sub>1</sub> lines averaged 22.9% EBV, 55.1% EYI and 37.9% EHI of the respective hybrid values in the Site 1, while the respective values in the Site 2 were 11.3, 57.3 and 17.3%. Out of the 29 HS<sub>1</sub> lines, line 9 performed best in both sites, with its EBV, EYI and EHI corresponding to 62.3, 79.1, and 78.8% of the respective hybrid values in the Site 1, and 30.0, 79.6 and 37.6% in the Site 2. Regarding the HS<sub>2</sub> lines, they averaged mean yields per plant corresponding to 74.6 and 65.5% to that of the hybrid in the Site 1 and Site 2, respectively (Table 2). In relation to the hybrid the HS<sub>2</sub> lines averaged 44.6 and 24.3% EBV, 56.8 and 43.8% EYI, and 58.0 and 50.4% EHI in the Site 1 and Site 2, respectively. It is worth mentioning that in the Site 1 the line 9-3 exhibited higher EHI by 31% compared to the hybrid (Table 2), while two additional HS<sub>2</sub> lines also had higher EHI by 15 and 5% compared to the hybrid (data not shown). The lines 15-14 and 10-19 performed best in the Site 1 and Site 2, respectively, averaging 79, 84, and 94% of the hybrid for the EBV, EYI and EHI (Fig. 1). Selection within these two lines based on the PAV produced the next generation's progeny lines. The mean yield per plant for the final five HS<sub>3</sub> lines in the Site 1 ranged from 92.9 to 98.6% (95.4% on average) in comparison to the hybrid and likewise those in the Site 2 reached 92.7 to 99.3% (95.0% on average) of the hybrid yield (Table 4). The first top line in each site, i.e. 15-14-4, 10-19-2, did not differ significantly from the original hybrid. The mother 15-14 HS<sub>2</sub> line in the Site 1 performed similarly to the previous season in terms of yield, equation B values and its elements and the same was observed for the respective 10-19



**Fig 2.** The linear correlation between anthesis-to-silking gap (ASG) and the plant-yield-index (PYI) of the individual plants for the hybrid 'Constanza' and the HS<sub>2</sub> lines 15-14 and 15-12 from the Site 1 (the respective *r* values are -0.89, -0.90, and -0.92, *P*<0.001), as well as for the hybrid 'Constanza' and the HS<sub>2</sub> lines 10-19 and 9-7 from the Site 2 (the respective *r* values are -0.78, -0.90, and -0.83, *P*<0.001).

**Table 3.** The linear correlation values of the anthesis-to-silking gap with the equation B parameters of 21 entries. The entries included the 19 selected HS<sub>2</sub> lines in the Site 1 and Site 2, the original hybrid and its HS<sub>0</sub>.

ASG	Site 1			Site 2		
	EBV	EYI	EHI	EBV	EYI	EHI
	-0.54 *	-0.22 ns	-0.84 **	-0.65 **	-0.59 **	-0.83 **

• ASG= anthesis-to-silking gap, •EBV= entry B value, • EYI= entry-yield index, • EHI= entry-homeostasis index, • ns= non significant differences • \*= P<0.01, • \*\*= P<0.001.

**Table 4.** The results of the calculation of the unitless Equation B parameters for HS<sub>3</sub> lines. The entries are arranged in descending order according to the Equation B outcome (EBV). All the five HS<sub>3</sub> lines evaluated in each site are presented together with their respective HS<sub>2</sub> progenitor line. The respective values of the original hybrid (F<sub>1</sub>) as well as the mean HS<sub>3</sub> values are also given. All the values are expressed as % of the respective absolute hybrid (i.e. 100%) values..

Site 1					Site 2				
Entry	EBV	EYI	EHI	$\bar{x}$	Entry	EBV	EYI	EHI	$\bar{x}$
15-14-4	89.4	97.3	91.9	98.6*	10-19-2	91.8	98.7	93.0	99.3*
15-14-5	88.0	94.4	93.2	97.2	10-19-5	87.7	91.1	96.2	95.4
15-14-1	84.6	84.7	99.9	92.0	10-19-3	84.7	86.9	97.5	93.2
15-14-3	83.6	84.3	99.2	91.8	10-19	78.9	84.4	93.5	91.9
15-14	77.5	84.6	91.6	92.0	10-19-4	76.3	79.8	95.5	89.4
15-14-2	73.0	86.3	84.7	92.9	10-19-1	70.1	86.0	81.5	92.7
HS <sub>3s</sub> average	86.4	91.2	94.8	95.4	HS <sub>3s</sub> average	85.1	90.4	94.0	95.0
F1(100%)	17.1	1.11	15.5	648 g	F1(100%)	38.2	1.12	34.2	538 g

\* it does not differ significantly compared to the 100% value (z-test, P<0.05). • EBV= entry B value, • EYI= entry-yield index, • EHI= entry-homeostasis index, •  $\bar{x}$  = entry mean yield, • HS<sub>3s</sub>= half-sib generation 3, • F1= original hybrid 'Constanza', • gray cells indicate the respective HS<sub>2</sub> progenitor line for each of the HS<sub>3</sub> line

HS<sub>2</sub> line in the Site 2. Overall, the B values for the HS<sub>3</sub> lines were 86.4 and 85.1% of the corresponding hybrid values in the Site 1 and Site 2, respectively. Compared to yield and homeostasis indexes of the hybrid, the HS<sub>3</sub> lines reached a 91.2 and 94.8% respectively in the Site 1 and a 90.4 and 94.0% in the Site 2, respectively. The best performance among the HS<sub>3</sub> lines based on the equation B values and yield and homeostasis indexes was recorded for the line 15-14-4 in the Site 1 (the respective values were 89.4, 97.3 and 91.9% of that of the hybrid) and for the line 10-19-2 in the Site 2 (the respective values were 91.8, 98.7 and 93.0% of that of the hybrid).

#### Synchronization of pollen and silk emergence in relation to yield performance

The calculation of the interval between pollen and silk emergence of individual plants in each line revealed that in the Site 1, the mean ASG for the HS<sub>2</sub> lines varied from 2.4 days (in the line 9-3) up to 5.7 days (in the line 9-4) and the best performing line 15-14 (Table 2) exhibited a 2.9-day interval; the hybrid and its HS<sub>0</sub> showed an intermediate 3.8-day gap (data not shown). In same sense in the Site 2, the mean ASG for the HS<sub>2</sub> lines ranged from 2.2 up to 5.3 days, while for the hybrid and HS<sub>0</sub> silk emergence occurred 3.2 and 4.7 days after anthesis, respectively. As far as the three best performing HS<sub>2</sub> lines (10-19, 9-1 and 2-17) are concerned, the ASG was 2.8, 2.6 and 2.4 days, respectively, whereas the three worst performing HS<sub>2</sub> lines (4-10, 28-14 and 9-2) displayed an ASG of 5.3, 4.2 and 5.1 days, respectively. In general, a negative correlation was observed between the ASG and any of the equation B elements with the exception of the EYI in the Site 1 (Table 3). On a single-plant basis, the ASG varied from 0 to 7 days in both sites, with this interval showing high negative correlation with the PYI. The correlation *r* values varied from -0.72 up to -0.93 (P<0.001) partly shown in Fig. 2. The only relatively low correlation value was that of the HS<sub>0</sub> in the Site 2 (r=-0.47), though, this value was also significant at the 0.001 level.

#### Discussion

On the whole, the application of a two-step selection procedure that initially ranks the progeny lines based on their equation B values, then within those lines highlights the outstanding according to their equation A values individual plants, ultimately resulted in HS<sub>3</sub> lines that closely resemble the performance of the commercial hybrid. Notably, two top HS<sub>3</sub> lines, the 15-14-4 in the Site1 and 10-19-2 in the Site 2 (Table 4), displayed an average EYI of 98% compared to that of the hybrid (Fig. 1). An 8% lag behind the hybrid in the EHI could probably explain why the HS<sub>3</sub> lines also lagged by 9% in the EBV. The fact that in essence the EHI represents the reverse value of the coefficient of variation (CV) means that it reflects the ability of an entry to withstand environmental forces (i.e., its genetic buffering) as well as its genetic homogeneity (Fasoula and Fasoula, 1997; Tokatlidis et al., 1999, 2008; Fasoula and Fasoula, 2005; Fasoula and Tokatlidis, 2012). In this case, the EHI values of the two top HS<sub>3</sub> lines represent an average CV of just 22 versus 21% of that of the hybrid. Such low CVs reflect genetic homogeneity and imply that actually the HS lines approximate the hybrid at least in terms of genetic buffering. As demonstrated in Fig. 1, these lines have in effect reached high levels of homeostasis even in the previous generation. The occurrence of three HS<sub>2</sub> lines with higher EHI value, thus lower CVs, than the hybrid was an unexpected but probably not fortuitous event. Another important point emerges by considering the ancestry of the top HS<sub>3</sub> lines. They originated from the HS<sub>1</sub> lines coded 15 and 10, which were not among the highest scoring for the EBV HS<sub>1</sub> lines primarily due to their low EHI (Table 1). However, in terms of their EYI the line 15 was equivalent to the best performing HS<sub>1</sub> line, while the line 10 was 14% above the average HS<sub>1</sub> line performance. This suggests that at an early generation more emphasis should be placed on the first component in both equations, so that the outstanding for the PYI plants within the best according to the EYI progeny lines are selected regardless of

their EHI values. Undoubtedly, within a highly heterogeneous line the presence of a particular plant whose genotype might evolve to an exceptional pure line cannot be excluded. In this sense, a strict employment of the two equations is recommended at later generations, when adequate homogeneity has been reached hence the EHI reflects mainly the environmental influence. In an extended review paper, Crow (2000) pointed out that the high performance in maize hybrids is largely due to additive and dominance effects, while Butruille et al (2004) reported that after a six full-sib reciprocal recurrent selection in maize populations most of the selection response for grain yield was attributed to additive genetic effects. Similarly, Vafias and Ipsilantis (2005) demonstrated that high and stable yield in maize depended on dominant and additive gene action whereas Wardyn et al. (2007) observed that dominance variance constitutes a significant portion of the total genetic variance affecting grain yield. In this study, a successful exploitation of additive genetic actions in the HS lines is demonstrated by bridging of the gap between the HS lines and hybrid yield performance (Fig. 1), the reduced ASG that was associated with the improvement of the three components of the equation B (Table 3) and particularly some remarkable single-plant yields (Fig. 2). Interestingly, Monneveux et al. (2006) found kernel abortion to be positively correlated with ASG, while Menkir and Kling (2007) have previously reported a strong negative relationship between the ASG and grain yield. In this sense, Cárcova et al. (2000) recommended synchronous pollination to improve kernel set and likewise Bolaños and Edmpades (1993) as well as Westgate (1997) proposed selection for decreased ASG to improve grain yield. It is self-evident that a reduced interval between pollen shedding and silking emergence increases the chance of self-pollination. Thus, the inverse connection between the ASG and PYI (Fig. 2) indicates that the selection of high yielding plants essentially identifies those with a relatively high degree of self-fertilization. Theoretically, the open-pollination conditions adopted in the study favor cross pollination, nevertheless the wide interplant distance used and the apparently small difference in the CV between the genetically homogeneous hybrid and some of the HS lines definitely point towards the latter's genetic homogeneity. The application of extremely high selection intensities (1.46-0.43%) combined with the strong negative correlation between the ASG and PYI effectively supported homozygosity and ultimately promoted additive genetic effects through the selection process employed. According to the general equation of the expected response to selection such high selection intensities enhance the selection efficiency (Falconer, 1989); however, they are applicable only in the absence of competition, because in dense stand conditions the negative relationship between yielding and competitive ability obstructs the identification of high yielding genotypes (Fasoulas, 1993; Fasoula and Fasoula, 1997, 2002, 2005; Tokatlidis et al., 2010; Fasoula and Tokatlidis, 2012). The application of the honeycomb breeding in the absence of competition to exploit additive genetic effects towards an improved potential yield per plant was documented by Ipsilantis and Koutsika (2000) and Tokatlidis et al. (1999, 2008). In the latter case, a procedure that involved the recycling of a commercial hybrid was used and resulted not only in the improvement of the plant yield potential but also in a reduced load of deleterious genes. In conclusion, single-plant selection in the absence of competition based on the application of the two equations described could facilitate the bridging of the productivity gap between inbred lines and hybrids in maize. Especially for the

outcome of the equation B, EBV, two very recent studies revealed its additional capacity to effectively estimate cultivars' performance at farming-density conditions. In other words, maize hybrids improved for the EBV use resources more effectively particularly in the case of dryland maize production and perform as density-neutral cultivars; this means that they exhibit a wide range of optimum density, which is an agronomic trait that enhances a hybrid's consistency of performance (Tokatlidis et al., 2011). Berzsenyi and Tokatlidis (2012) found density-neutral hybrids to be the most adaptable ones across variable environments ranging from marginal to exceptionally favorable. Likewise, in a study involving dry bean (*Phaseolus vulgaris* L.) genotypes, the EBV could be grouped alongside statistical measurements used as criteria for evaluating agronomic stability, which again points towards more effective resource-use efficiency in favorable environments (Papadopoulos and Tokatlidis, 2011). In any case, improving the productivity of maize inbred lines will allow cheaper hybrid seed production and reduced cost per unit of yield gain. More importantly however, a progressive line improvement could lead to a reduction in the productivity gap between inbred lines and hybrids, so that ultimately hybrid development may no longer be cost effective. Towards such a goal, the two equations employed as selection criteria in this study present uniquely valuable tools.

## Materials and methods

### Experimental management

The experimentation was consistently conducted under open pollination conditions at the density of 0.74 plants m<sup>-2</sup> according to the honeycomb experimental designs (Fasoulas and Fasoula, 1995). Moreover, isolated field trials were chosen to avoid contamination with foreign pollen and individual plants were harvested separately. According to Bos and Caligari (2008) open pollination results in HS lines, which consist of plants that are each other's half sibs since they descend from the same maternal parent but possibly from different paternal parents. The experiments were established in two regions of Greece where maize is commercially produced. The first, named Site 1, was in the farm of the Technological Education Institute of Western Macedonia in Florina (40°46'N, 21°22'E, 705 m elevation) and the second, named Site 2, was in the region of Trikala (39°55'N, 21°64'E, 120 m elevation). Two- or three-seed sown hills were thinned to the desired single-plant hills at the two-leaf stage. Nitrogen and P fertilizer (element level) were applied at the rate of 150 and 75 kg ha<sup>-1</sup>, respectively, at the sowing dates, while additional N (135 kg ha<sup>-1</sup>) was applied when the plants reached 50 cm in height. Complete weed control was obtained by tilling and manual hoeing. The trials were regularly irrigated to avoid drought stress. Regarding grain yield per plant, to compare two means the z-test was used for independent samples and different standard deviations. In addition, the aforementioned two equations were employed for the selection of favorable genotypes. In other words, the equation A (PAV = PYI • EHI) was used to apply single-plant selection within the outstanding, according to the equation B (EBV = EYI • EHI), progeny lines (Fasoula, 2008; Fasoula and Tokatlidis, 2012). It should be noted that for individual plant selection based on PAV the size of the moving circle used to calculate the average yield of the surrounding plants ( $\bar{x}_r$ ) was constantly 31.

### **Source material and selection of the HS<sub>1</sub> lines**

The starting material was F<sub>2</sub> (HS<sub>0</sub>) generation seed from the commercial maize hybrid 'Costanza'. In 2007, 2,300 F<sub>2</sub> plants were grown at the Site 1 according to the non-replicated honeycomb arrangement (NR-0). Of the 2,165 plants that were individually harvested the 29 that showed the highest PYI values were selected, so that the selection intensity was 1.34%; these formed the 29 HS<sub>1</sub> progeny lines. It should be noted that in this case the second component of the equation A, EHI, is immaterial because all the plants have the same value.

### **Evaluation of the HS<sub>1</sub> lines and selection of the HS<sub>2</sub> lines**

The following season (2008), the 29 HS<sub>1</sub> progeny lines along with the original hybrid (F<sub>1</sub>) and HS<sub>0</sub> were tested in both experimentation sites according to the replicated-31 (R-31) honeycomb design, including 50 plants per entry (i.e. line) in each site. About 1,300 HS<sub>1</sub> plants were harvested individually in each site. Then, out of the HS<sub>1</sub> lines exhibiting the highest EBV 19 plants were selected based on their PAV, which corresponds to a selection intensity of 1.46%; these formed the 19 HS<sub>2</sub> lines.

### **Evaluation of the HS<sub>2</sub> lines and selection of the HS<sub>3</sub> lines**

During 2009, the 19 HS<sub>2</sub> lines were grown in both sites in R-21 honeycomb trials, along with the original hybrid (F<sub>1</sub>) and HS<sub>0</sub>. The number of the replications was 70 plants per entry in each site. Eventually, five plants were selected from each site; they were the ones that displayed the highest PAVs among those belonging to the top for the EBV HS<sub>2</sub> line. These came out of 1,128 and 1,177 individually harvested plants from the Site 1 and Site 2, respectively, so the average selection intensity was 0.43%.

Additionally, the dates of pollen shedding emergence (anthesis) and silking emergence were recorded in order to measure the ASG.

### **Evaluation of the HS<sub>3</sub> lines**

During 2010 the 5 HS<sub>3</sub> lines from each experimentation site along with their mother HS<sub>2</sub> line and the original F<sub>1</sub> hybrid were tested in an R-7 honeycomb trial in each site, including 100 plants per entry.

### **Conclusions**

The results demonstrate that the constant replacement of less favorable additive alleles with more favorable ones is accomplishable through the honeycomb breeding procedure when ultra-low plant densities are used so as to maximize pollen production and diminish the ASG. However, due to the high genetic heterogeneity of the HS<sub>1</sub> progeny lines the selection at such an early generation should be based more on the PYI than EHI value in order to avoid the risk of overlooking an exceptionally performing genotype within that highly heterogeneous progeny line. The selection strategy employing the equations A and B focuses on the improvement of the lines' grain yield per plant along with the improvement of their individual buffering. The findings presented could have a significant impact initially on the cost of hybrid-seed production and ultimately on a future prospect of replacing hybrids with open-pollinating lines. Thus, the application of this procedure within commercially successful hybrids is recommended as a means to develop highly

homogeneous inbred lines of superior performance; the hybridization between such lines is bound to produce less heterogeneous hybrids as well as reduce the hybrid-seed cost. It would be intriguing to investigate the possibility that repeated cycles of the procedure described could ultimately reduce the line-to-hybrid gap so that hybrid development may no longer be cost effective.

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### **References**

- Berzsenyi Z, Tokatlidis IS (2012) Density-dependence rather maturity determines hybrid selection in dryland maize production. *Agron J* 104: 331-336.
- Bolaños J, Edmeades GO (1993) Eight cycles of selection for drought tolerance in lowland tropical maize. I. Responses in grain yield, biomass and radiation utilization. *Field Crops Res* 31: 233-252.
- Borrás L, Westgate ME, Astini JP, Echarte L (2007) Coupling time to silking with plant growth rate in maize. *Field Crops Res* 102: 73-78.
- Bos I, Caligari P (2008) *Selection Methods in Plant Breeding*, 2<sup>nd</sup> edn. Springer P.O. Box 17, 3300 AA Dordrecht, the Netherlands.
- Butruille DV, Silva HD, Kaeppler SM, Coors JG (2004) Response to selection and genetic drift in three populations derived from the Golden Glow maize population. *Crop Sci* 44: 1527-1534.
- Cárcova J, Uribebarrea M, Borrás L, Otegui ME, Westgate ME (2000) Synchronous pollination within and between ears improves kernel set in maize. *Crop Sci* 40: 1056-1061.
- Crow JF (2000) The rise and the fall of overdominance. *Plant Breed Rev* 17: 225-257.
- Duvick DN (1997) What is yield?, *Developing Drought and Low N-tolerant maize* (Eds Edmeades GO et al.), CIMMYT, EL Batán, Mexico, pp. 332-335.
- Duvick DN (2005) The Contribution of breeding to yield advances in maize (*Zea mays* L.). *Adv Agron* 86: 83-145.
- Falconer DS (1989) *Introduction to Quantitative Genetics*, 3<sup>rd</sup> edn. Longman Scientific and Terminal, Wiley, New York.
- Fasoula VA (2008) Two novel whole-plant field phenotyping equations maximize selection efficiency, in: Prohens J., Badenes M.L. (Eds), *Modern Cultivar Breeding for Present and Future Needs*, Proc. 18th Eucarpia General Congress, 9-12 September 2008, Valencia, Spain, pp. 361-365.
- Fasoula DA, Fasoula VA (1997). Competitive ability and plant breeding. *Plant Breed Rev* 14:89-138.
- Fasoula VA, Fasoula DA (2000) Honeycomb breeding: principles and applications. *Plant Breed Rev* 18:177-250
- Fasoula VA, Fasoula DA (2002) Principles underlying genetic improvement for high and stable crop yield potential. *Field Crops Res* 75: 191-209.
- Fasoula DA, Fasoula VA (2005) Bridging the productivity gap between maize inbreds and hybrids by replacing gene and genome dichotomization with gene and genome integration. *Maydica* 50: 49-61.

- Fasoula VA, Tollenaar M (2005) The impact of plant population density on crop yield and response to selection in maize. *Maydica* 50: 39–48.
- Fasoula VA, Tokatlidis IS (2012) Development of crop cultivars by honeycomb breeding. A review. *Agron Sustain Dev* 32: 161–180.
- Fasoulas AC (1993). Principles of crop breeding, (Ed Fasoulas AC), P.O. Box 1555, GR-54006, Thessaloniki 17, Greece.
- Fasoulas AC (1997) Overcoming inbred line stagnation for productivity and stability in maize breeding, *Proceedings of the XVIIth Conference on Genetics, Biotechnology, and Breeding of Maize and Sorghum* (Ed Tsaftaris AS), Aristotelian University of Thessaloniki, Greece). Cambridge: The Royal Society of Chemistry.
- Fasoulas AC, Fasoula VA (1995) Honeycomb selection designs. *Plant Breed Rev* 13: 87–139.
- Hallauer AR, Miranda JB (1981) Quantitative genetics in maize breeding. Iowa State University Press, Ames, IA, USA, 1981.
- Ipsilandis CG, Koutsika-Sotiriou M (2000) The combining ability of recombinant S-lines developed from an F-2 maize population. *J Agric Sci* 134: 191–198.
- Menkir A, Kling JG (2007) Response to recurrent selection for resistance to *Striga hermonthica* (Del.) Benth in a tropical maize population. *Crop Sci* 47: 74–684.
- Monneveux P, Sánchez C, Beck D, Edmeades GO (2006) Drought tolerance improvement in tropical maize source populations: Evidence of progress. *Crop Sci* 46: 180–191.
- Papadopoulos II, Tokatlidis IS (2011) A novel statistic estimated in the absence of competition to foresee genotype performance at the farming conditions parallels the agronomic concept of stability. *Aust J of Crop Sci* 5: 822–830.
- Sangoi L, Gracietti MA, Rampazzo C, Bianchetti P (2002) Response of Brazilian maize hybrids from different eras to changes in plant population. *Field Crops Res* 79: pp. 39–51, 2002.
- Tokatlidis IS, Koutsika-Sotiriou M, Fasoulas AC, Tsaftaris AS (1998) Improving maize hybrids for potential yield per plant. *Maydica* 43: 123–129.
- Tokatlidis IS, Koutsika-Sotiriou M, Fasoulas AC (1999) Load of deleterious genes and stability of performance in maize. *Maydica* 44: 127–132.
- Tokatlidis IS, Koutsika-Sotiriou M, Pessios E (2008) Beneficial exploitation of additive genetic effects to improve yield potential per plant in maize. *Cereal Res Commun* 36: 461–470.
- Tokatlidis IS, Koutsika-Sotiriou M, Tamoutsidis E (2005) Benefits from using maize density-independent hybrids. *Maydica* 50: 9–17.
- Tokatlidis IS, Has V, Mylonas I, Has I, Evgenidis G, Melidis V, Copandean A, Ninou E (2010) Density effects on environmental variance and expected response to selection in maize (*Zea mays* L.). *Euphytica* 174: 283–291.
- Tokatlidis IS, Has V, Melidis V, Has I, Mylonas I, Evgenidis G, Copandean A, Ninou E, Fasoula VA (2011) Maize hybrids less dependent on high plant densities improve resource-use efficiency in rainfed and irrigated conditions. *Field Crops Res* 120: 345–351.
- Tollenaar M, Lee EA (2002) Yield potential, yield stability and stress tolerance in maize. *Field Crops Res* 75: 161–169.
- Troyer AF, Wellin JE (2009) Heterosis decreasing in hybrids: yield test inbreds. *Crop Sci* 49: 1969–1976.
- Uribelarrea M, Cárcova J, Borrás L, Otegui M.E. (2008) Enhanced kernel set promoted by synchronous pollination determines a tradeoff between kernel number and kernel weight in temperate maize hybrids. *Field Crops Res*, 105: 172–181.
- Vafias BN, Ipsilandis CG (2005) Combining ability, gene action and yielding performance in maize, *Asian J Plant Sci* 4: 50–55.
- Wardyn BM, Edwards JW, Lamkey KR (2007) The genetic structure of a maize population: The role of dominance. *Crop Sci* 47: 465–474.
- Westgate ME (1997) Physiology of flowering in maize: identifying avenues to improve kernel set during drought, *Developing Drought and Low N-tolerant maize* (Eds Edmeades GO et al.), CIMMYT, EL Batán, Mexico, pp. 136–141.