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A novel statistic estimated in the absence of competition to foresee genotype performance at the farming conditions parallels the agronomic concept of stability

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## Abstract

Selection in the absence of competition has been proposed as a means to develop new cultivars serving the needs of a sustainable agriculture. Hence, the possibility to foresee genotype performance in agricultural practice via a statistic measurement in the absence of competition is of paramount importance. The study primarily aimed to evaluate the agronomic value of a novel statistic model designed for low density conditions, i.e. relative crop yield potential (*CYP*), to foresee yielding and stability performance under diversifying farming conditions. The *CYP* was estimated from 21 dry bean (*Phaseolus vulgaris* L.) genotypes tested under the ultralow density of 1.15 plants m<sup>2</sup>, and at both greenhouse and open field conditions, deemed to represent heat stress and optimal conditions, respectively. In addition, several known parametric and non-parametric statistics were measured under the commercially used density of 4.8 plants m<sup>-2</sup> across five environments at greenhouse and open field conditions. The results presented highlight the possibility of the *CYP* being a useful tool to serve for cultivar comparisons and selection purposes. The top genotypes on the *CYP* basis were also the best according to the stability statistics of superiority measure ( $P_i$ ), the stratified ranking procedure (T/M/L), the rank-sum index ( $I_i$ ) and the *GGE* biplot pattern of stability. Evidence provided show that *CYP* is correlated with the above statistics, which according to literature present criteria for agronomic sense of stability, implying more effective resource-use efficiency in favourable environments. This model seems useful either for the evaluation of existing cultivars or developing new ones through selection within traditionally cultivated landraces. This makes it ideal for dealing with the problems of cultivar adaptation to the on-going climate changes and the demand for higher quantities of food in the near future.

**Keywords:** dry bean, homeostasis, honeycomb breeding, resource use efficiency, stability statistics, yield potential. **Abbreviations:**  $CV_i$ , coefficient of variation of Francis and Kanenberg (1978);  $W_i^2$ , ecovalence of Wricke (1962); GxE, genotype by environment interaction; GGE, genotype and genotype by environment biplot stability;  $I_i$ , rank-sum index of Kang and Pham (1991); T/M/L, ranking procedure of Fox et al. (1990);  $b_i$ , regression approach of GxE interaction of Finlay and Wilkinson (1963); CYP, relative crop yield potential;  $S_i^{(2)}$  and  $S_i^{(3)}$ , stability parameters of Nassar and Huehn (1987);  $\sigma_i^2$ , stability variance of Shukla (1972);  $P_i$ , superiority measure of Lin and Binns (1988);  $S_{di}^2$ , variance in regression deviations of Eberhart and Russell (1966).

### Introduction

To date, the concept of sustainable agriculture attains special consideration due to several conflicts of conventional agriculture (Malézieux et al., 2009). Because agriculture is one of the sectors most vulnerable to the risk and impacts of global climate change (Tingem et al., 2009), management strategies that highlight flexibility as a major factor of sustainability have been suggested, as a means of adaptation to the on-going environmental changes (Lichtfouse et al., 2009). Consequently, sustainable agriculture is strongly reliant upon the availability of well-adapted cultivars that satisfy its special requirements without compromising productivity, since otherwise its viability is unsound (Fasoula and Tokatlidis, 2011).

Honeycomb methodology has been proposed as a breeding procedure to develop cultivars that entirely meet the needs of sustainable agriculture (Fasoula and Tokatlidis, 2011). From this viewpoint, Vlachostergios et al. (2011) presented some encouraging results regarding organic breeding in lentil (*Lens* 

culinaris Medik.), so did Tokatlidis et al. (2011) in managing to succeed optimal resource-use efficiency in dryland maize (Zea mays L.). The major principles that distinguish this method from the other conventional breeding schemes and experimentation designs include the large interplant distance used to minimize stress and erase genotype by density interaction (i.e. absence of competition), and the systematic entry arrangement to cope with the soil heterogeneity. Selection in the absence of competition optimizes heritability and response to selection by allowing application of high selection pressure, eliminating the confounding effects of the negative relationship between yielding and competitive ability and maximizing the phenotypic expression and differentiation (Fasoula and Fasoula, 2000; 2002; Fasoula and Tokatlidis, 2011). In the honeycomb layouts (Fasoulas and Fasoula, 1995) entries are always allocated evenly across the experimental area, in such a way that every plant of a given entry is consistently surrounded by plants of the

remaining entries forming a complete circular replicate; this systematic instead of a randomized entry arrangement ensures the objective comparison of the entries.

In the absence of competition, whole-genome phenotype is accomplished by partitioning the crop yield potential (i.e. yield per unit ground area) into three components, which are plant yield potential (i.e. yield per plant when any kind of stress is absent), tolerance to stresses, and responsiveness to inputs (Fasoula and Fasoula, 2000; 2002; Tokatlidis and Tsialtas, 2008). Improved plant yield potential and tolerance to stresses extend the lower and the upper limit of optimum plant density, respectively, while genotypes carrying genes for responsiveness to inputs are capable to exploit favourable growing conditions. Tolerance to stresses and responsiveness to inputs tie together plant yield potential with crop yield potential and lead to the development of density-neutral cultivars, i.e. cultivars that yield optimally at a wide range of densities (Fasoula and Fasoula, 2000; 2002; Tokatlidis and Tsialtas, 2008). For comparison purposes in the absence of competition the aforementioned crop yield potential components have been incorporated into a single statistic measure (Fasoula, 2008; Fasoula and Tokatlidis, 2011). To foresee performance of different genotypes at the farming practiced conditions, the relative crop yield potential (CYP) of the  $i_{th}$  genotype is estimated by the equation:

 $CYP_i = (\overline{x}_i / \overline{x}_i)^2 \cdot (\overline{x}_i / s_i)^2$ ,

where  $\bar{x}_i$  is the genotype mean,  $s_i$  its standard deviation and  $\bar{x}_t$  the over genotypes experimental mean. The first parameter,  $(\bar{x}_i/\bar{x}_i)^2$ , measures the yield potential of the genotype in the absence of any plant-to-plant interference. The second parameter,  $(\bar{x}_i/s_i)^2$ , estimates the relative homeostasis, which selects for tolerance to environmental influences. To tie together the plant yield with the crop yield potential, one needs to multiply the two parameters and get the CYP values. Among genotypes evaluated under comparable conditions, those with top CYP values are expected to perform better at farming conditions. Therefore, it is wise to search for the possibility of identifying genotypes on the CYP basis that would have good performance under the commonly applied dense stand. The use of a yield reliability index facilitates the selection and recommendation of the best adapted cultivars, as yield and stability are combined into a unique measure of genotype merit (Annicchiarico, 2002).

To detect the best adapted genotypes the common practice is the multi-environment experimentation (González *et al.*, 2006; Baxevanos *et al.*, 2008; Basu et al., 2009). Genotype by environment (GxE) interactions, however, are not avoidable in agricultural investigations (Sabaghnia et al., 2010), complicating the identification of superior genotypes, but their interpretation can be facilitated by proper statistical measures (Akcura *et al.*, 2006; Sabaghnia *et al.*, 2006; Solomon *et al.*, 2007; Baxevanos *et al.*, 2008; Mohammadi and Amri, 2008; Fikere et al., 2010). Numerous such tools have been proposed, categorized as parametric and nonparametric methods based on their statistical properties. Among the parametric stability models, are the joint regression analysis including the regression coefficient ( $b_i$ ) by Finlay and Wilkinson (1963) and the variance of deviations from regression  $(S_{di}^2)$  by Eberhart and Russell (1966). Some other univariate stability parameters are, the stability variance  $(\sigma_i^2)$  (Shukla, 1972), the ecovalence  $(W_i^2)$ 

(Wricke, 1962), the coefficient of variation ( $CV_i$ ) (Francis

and Kanenberg, 1978), and the superiority measure  $(P_i)$  by Lin and Binns (1988). As far as the non-parametric statistics are concerned, Nassar and Huehn (1987) proposed two stability statistics  $(S_i^{(2)} \text{ and } S_i^{(3)})$  combining yield and stability based on yield ranks in each environment, while Fox et al. (1990) suggested stratified ranking at each environment separately to estimate the proportion of localities at which the genotype occurred in the top, middle and bottom third of the ranks. Kang and Pham (1991) developed the rank-sum index  $(I_i)$  based on yield and  $\sigma_i^2$  ranks, remarking on the preference of agronomists and breeders to assign more weight to yield than to  $\sigma_i^2$ . Actually, the aforementioned statistics may approach stability differently. Generally, there are two contrasting concepts of yield stability, the "biological" and the "agronomic" (Becker and Leon, 1988). A particular genotype is characterized as stable according to the biological concept when it tends to maintain a constant yield across environments, regardless of the environmental yield potential. Agronomic stability implies that the genotype yield response across environments always parallels the average response of the tested genotypes, meaning that its  $b_i$ value equals one and has zero GxE interaction, while

genotypes combining high yield with  $b_i$  value above one are

regarded potent to exhibit particular adaptation to favourable environments.

In a recent work, Tokatlidis et al. (2010) applied the honeycomb breeding procedure within two traditionally cultivated dry bean landraces. A single-generation selection proved adequate to produce progeny lines yielding much higher than the mother landraces (up to 38%) over diversifying conditions. Subsequently, the agronomic value of the CYP statistic designed for the honeycomb procedure merits investigation. The primary object of this work was to address the value of the CYP statistic, obtained from a reduced set of experimental data at ultra-low density under heat stress and optimal conditions, in order to foresee cultivar performance for yield and stability in dense stands. Indeed, Tokatlidis et al. (2011) evaluated two sets of maize hybrids and found the crop yield of the top hybrids in each set to be predicted via CYP. In this study, however, to accomplish a more thorough consideration of the CYP, the aforementioned parametric and non-parametric stability statistics were also calculated using the same experimental data under the dense stand and across diversifying conditions. An additional goal was to investigate interrelationships among all the studied statistics.

### **Results and discussion**

In general, during the growing season temperatures did not differ between the greenhouse and the open field conditions. However, particularly at grain filling stage considerably higher temperatures were recorded at the atypical greenhouse

Genotype	СҮР	t ha <sup>-1</sup>	$b_i$	$S_{di}^2$	$\sigma_i^2$	$W_i^2$	$CV_i$	$P_i$	$S_{i}^{(2)}$	$S_{i}^{(3)}$	T/M/L	$I_1$	$I_2$	$I_3$
A1	5.76 (13)	2.05 (17)	0.67 (11)	0.30 (13)	0.10 (9)	0.41 (10)	0.21 (4)	0.83 (16)	8.30 (7)	2.13 <sup>(3)</sup>	7 (17)	26 (15)	43 (15)	60 (16)
A2	8.70 <sup>(9)</sup>	2.03 (18)	0.67 (12)	0.63 (18)	0.20 (15)	0.74 (15)	0.25 (11)	0.88 (17)	17.7 (12)	4.37 (9)	6 (19)	33 (17)	51 (18)	69 <sup>(18)</sup>
A3	4.06 (17)	1.90 (19)	1.03 (1)	1.24 (21)	0.34 (19)	1.24 (19)	0.40 (21)	0.98 (19)	19.2 (13)	4.47 (10)	7 (18)	38 (20)	57 <sup>(20)</sup>	76 (19)
A4	8.41 (10)	2.14 (15)	$0.06^{(20)}$	1.07 (20)	0.54 (21)	1.99 <sup>(21)</sup>	0.24 (9)	0.98 (18)	29.8 <sup>(19)</sup>	9.46 <sup>(18)</sup>	9 <sup>(13)</sup>	36 (18)	51 <sup>(17)</sup>	66 <sup>(17)</sup>
A5	2.95 (21)	1.76 (21)	0.07 (19)	0.62 (17)	0.41 (20)	1.52 (20)	0.23 (6)	$1.40^{(21)}$	10.8 (9)	$2.48^{(4)}$	6 (21)	41 (21)	62 (21)	83 (21)
A6	3.66 (18)	2.87 (4)	$1.45^{(16)}$	0.29 (12)	0.13 (11)	0.50 (12)	0.28 (17)	0.07 (5)	7.30 (6)	6.35 <sup>(13)</sup>	14 (4)	15 (6)	19 (5)	23 (4)
A7	5.24 (14)	$1.89^{(20)}$	0.61 (14)	0.68 (19)	0.22 (16)	$0.84^{(16)}$	0.27 (16)	1.05 (20)	20.7 (15)	5.11 (12)	6 (20)	36 (19)	56 (19)	76 (20)
A8	22.8 <sup>(1)</sup>	$2.90^{(3)}$	$1.40^{(15)}$	0.27 (11)	$0.11^{(10)}$	0.44 (11)	0.26 (12)	$0.05^{(3)}$	4.30 <sup>(4)</sup>	5.06 <sup>(11)</sup>	$15^{(3)}$	$13^{(4)}$	$16^{(2)}$	19 <sup>(2)</sup>
A9	12.2 (6)	2.32 (12)	$0.68^{(10)}$	0.16 (6)	0.07 (6)	0.27 (6)	0.17 (2)	0.52 (14)	23.2 (16)	7.61 <sup>(14)</sup>	9 <sup>(12)</sup>	$18^{(10)}$	30 (12)	42 (11)
B10	5.06 (15)	2.45 (11)	1.32 (9)	0.26 (10)	0.09 (8)	0.37 (9)	0.30 (19)	0.26 (9)	27.2 (17)	8.50 (15)	$10^{(10)}$	19 <sup>(12)</sup>	30 (11)	41 <sup>(10)</sup>
B11	13.6 <sup>(3)</sup>	2.84 <sup>(5)</sup>	1.30 (8)	0.55 (16)	0.17 (14)	0.64 (14)	0.27 (13)	0.06 (4)	50.8 <sup>(20)</sup>	27.5 <sup>(20)</sup>	13 (5)	19 <sup>(11)</sup>	24 <sup>(8)</sup>	29 <sup>(7)</sup>
B12	10.5 <sup>(7)</sup>	2.48 <sup>(9)</sup>	1.29 (7)	0.01 (1)	0.02 (1)	0.10 (1)	0.27 (14)	0.28 (10)	$12.2^{(10)}$	4.35 (8)	9 <sup>(11)</sup>	$10^{(2)}$	19 <sup>(6)</sup>	28 <sup>(6)</sup>
B13	12.6 (4)	3.01 (2)	1.34 (13)	0.24 (9)	0.08 (7)	0.36 (8)	0.24 (10)	0.03 (2)	3.80 <sup>(2)</sup>	4.22 (7)	15 (2)	9 <sup>(1)</sup>	$11^{(1)}$	13 (1)
B14	6.08 <sup>(12)</sup>	2.83 (6)	1.96 (21)	0.04 (3)	0.27 (18)	$1.00^{(18)}$	0.36 (20)	0.10 (6)	53.3 <sup>(21)</sup>	33.3 <sup>(21)</sup>	13 (6)	$24^{(14)}$	30 (10)	36 <sup>(9)</sup>
B15	12.3 (5)	2.32 (13)	$0.92^{(3)}$	0.12 (5)	0.03 (3)	0.13 (3)	0.22 (5)	0.42 (11)	12.7 <sup>(11)</sup>	3.68 (6)	8 <sup>(14)</sup>	16 <sup>(7)</sup>	29 <sup>(9)</sup>	42 (12)
B16	3.46 (19)	$2.24^{(14)}$	1.16 (6)	0.09 (4)	0.02 (2)	0.11 (2)	0.27 (15)	0.48 (13)	9.70 (8)	2.55 (5)	7 (16)	16 (8)	30 (13)	44 (14)
B17	14.9 <sup>(2)</sup>	3.07 (1)	$1.60^{(18)}$	$0.46^{(15)}$	0.23 (17)	0.85 (17)	0.29 (18)	0.01 (1)	6.20 <sup>(5)</sup>	$8.86^{(16)}$	$15^{(1)}$	$18^{(9)}$	19 <sup>(4)</sup>	20 <sup>(3)</sup>
B18	10.3 (8)	$2.48^{(10)}$	0.52 (17)	0.34 (14)	0.15 (13)	0.58 (13)	0.16 (1)	0.43 (12)	19.8 <sup>(14)</sup>	9.21 <sup>(17)</sup>	11 (8)	23 (13)	33 (14)	43 <sup>(13)</sup>
B19	7.12 (11)	2.57 (7)	0.97 (2)	0.21 (7)	0.05 (4)	0.21 (4)	0.21 (3)	0.23 (7)	27.7 (18)	12.0 (19)	12 (7)	$11^{(3)}$	$18^{(3)}$	25 (5)
В	4.74 (16)	2.53 (8)	1.12 (4)	0.03 (2)	0.06 (5)	0.21 (5)	0.23 (7)	0.24 (8)	3.80 (3)	$0.99^{(2)}$	10 (9)	13 (5)	21 <sup>(7)</sup>	29 <sup>(8)</sup>
Α	2.98 (20)	2.10 <sup>(16)</sup>	0.87 <sup>(5)</sup>	0.23 (8)	0.14 (12)	0.35 (7)	0.24 (8)	0.70 (15)	1.70 <sup>(1)</sup>	0.74 <sup>(1)</sup>	8 <sup>(15)</sup>	28 (16)	44 <sup>(16)</sup>	60 (15)

**Table 1.** Statistic parameters for 21 dry bean genotypes: Relative crop yield potential (*CYP*), mean grain yield (t ha<sup>-1</sup>), parametric measures ( $b_i$ ,  $S_{di}^2$ ,  $\sigma_i^2$ ,  $W_i^2$ ,  $CV_i$ ,  $P_i$ ), and non-parametric measures ( $S_i^{(2)}$ ,  $S_i^{(3)}$ , T/M/L,  $I_1$ ,  $I_2$ ,  $I_3$ ). The *CYP* statistic was estimated at the low density of 1.15 plants m<sup>-2</sup> across two environments, while the rest statistics were obtained at the dense stand of 4.8 plants m<sup>-2</sup> across five environments (in parenthesis the genotype rank position for each parameter)

condition (about 9°C higher max and 4°C higher min), implying a heat stress in the greenhouse during that crucial for grain yield stage (Tokatlidis et al., 2010).

At the low density of 1.15 plants  $m^{-2}$ , the mean grain yield per plant ranged over the two environments from 148 g for genotype A3 to 309 g for genotype B17 (Tokatlidis et al., 2010). This top to bottom genotype gap of 109% indicated a set of diversified genotypes for yield potential. Analysis of these data resulted in *CYP* values varying from 2.95 for genotype A5 to 22.8 for genotype A8 (Table 1). The respective *CYP* values for landraces A and B were 2.98 and 4.74.

Analysis of variance on pooled data obtained from the five environments under the dense stand revealed high significance for environments, genotypes and the GxE interaction (P<0.001). Average yields in t ha<sup>-1</sup> for the five environments were, 2.35 (2005) and 1.72 (2006) for the greenhouse, 2.25 (2005) for the Field1, and 2.66 (2005) and 3.11 (2006) for the Field2. This means that across years the greenhouse averaged 24% lower yield than the typical open field conditions, probably due to heat stress at the grain filling stage. Genotypes B17, B13, A8 and A6 consistently exhibited higher yield than the average experimental yield (data not shown).

The statistic parameters for the 21 genotypes obtained at the dense stand are shown in Table 1. Regarding grain yield over the five environments, top was genotype B17, followed by B13, A8, A6, B11 and B14. These six genotypes were significantly superior over the respective mother landrace A or B, on the basis of an LSD value of 0.26 t ha<sup>-1</sup> (Tokatlidis et al., 2010). They also had of the highest regression coefficients ( $b_i$ ) and above one ( $b_i$  =1.23 for B11 up to 1.96

for B14) and consequently exhibited the best adaptability to favourable environments. This finding corroborated analogous results in dry bean (Mekbib, 2003) and durum wheat (*Triticum durum* Desf.) (Akcura et al., 2006; Mohammadi and Amri, 2008).

Rank correlation coefficients among all possible pairs of the statistic parameters are shown in Table 2, characterized strong, moderate and weak at the 0.001, 0.01 and 0.05 significance level, respectively. The CYP statistic was moderately correlated with yield,  $P_i$ , T/M/L,  $I_2$  and  $I_3$ , and weakly with  $I_1$ . Moreover, strong positive association among measures of yield,  $P_i$ , T/M/L,  $I_1$ ,  $I_2$  and  $I_3$  was observed. Strong positive relationship among yield,  $P_i$  and rank-sum index  $(I_i)$  was found by Kang and Pham (1991), and among yield,  $P_i$  and T/M/L was reported by Mohammadi and Amri (2008). Sabaghnia et al. (2006) as well as Solomon et al. (2007) found yield to correlate with T/M/L and  $I_i$ . As a sequel to these correlations, genotypes classified as top according to CYP,  $P_i$ , T/M/L,  $I_1$ ,  $I_2$  and  $I_3$  were those of the highest yielding. This was in agreement with, Kang and Pham (1991) for  $P_i$  and  $I_i$ , Mohammadi and Amri (2008) regarding  $P_i$  and T/M/L, and Sabaghnia et al. (2006) as well as Solomon et al. (2007) concerning T/M/Land  $I_i$ . The four top genotypes on the CYP model basis also occupied the four top positions according the  $P_i$  measure (i.e. B17, B13, A8, B11). Three of them were also among the four top genotypes while B11 was placed fifth for T/M/L. The rank-sum indices  $I_2$  and  $I_3$ , that place more emphasis on yield than on the stability variance ( $\sigma_i^2$ ), were in agreement with *CYP* measure regarding three of the top genotypes (i.e. genotypes B13, A8 and B17), whereas the  $I_1$  index ranked B13 at the first and A8 at the fourth position.

There was no association between *CYP* and the rest of the statistic measures (Table 2). Strong correlations (r=0.78-0.98) among variance in regression deviations ( $S_{di}^2$ ), stability

variance  $(\sigma_i^2)$  and ecovalence  $(W_i^2)$ , being consistent with results by Mekbib (2003), indicated that these three models measured similar aspects of stability. Mohammadi and Amri (2008) reached the same conclusion. Data by Akcura et al. (2006) also depicted a strong positive relationship between  $S_{di}^2$  and  $\sigma_i^2$ , but they were both negatively related to  $W_i^2$ . Regression coefficient  $(b_i)$  was correlated with  $\sigma_i^2$  and  $W_i^2$ but not with  $S_{di}^2$ , and these four statistics, as well as  $CV_i$  and  $S_i^{(2)}$ , were not related to yield. In turn, they did not support the highest yielding genotypes as more stable. Mohammadi and Amri (2008), and Solomon et al. (2007) also did not find yield to correlate with  $S_i^{(2)}$ . The coefficient of variation  $(CV_i)$  was not related to any of the parameters studied in this work. However, Mohammadi and Amri (2008) reported a negative association of  $CV_i$  with yield. Results of this work showed a marginally negative correlation of yield (and CYP as well) with  $S_i^{(3)}$  (r=-0.41, P<0.07), while Solomon et al. (2007) and Mohammadi and Amri (2008) reported highly negative connection between yield and  $S_i^{(3)}$ . Generally, genotype ranking based on  $b_i$  is reproducible but repeatability of  $S_{di}^2$  and  $\sigma_i^2$  is low (Leon and Becker, 1988; Jalaluddin and Harrison, 1993; Baxevanos et al., 2008).

The positive associations among  $P_i$ , T/M/L and  $I_i$ , as well as their strong correlation with yield, in fact mean that these measures represent criteria that could highlight grain yield and furthermore promote the agronomic concept of stability. It is worth mentioning the positive correlation of  $b_i$ with yield (r=0.79),  $P_i$  (r=0.84,), T/M/L (r=0.74,), and  $I_i$ (r=0.59-0.79) that was found when the genotypes of this work were ranked in descending order for  $b_i$  values (data not shown). Therefore, selection based on  $P_i$ , T/M/L and  $I_i$ would recommend genotypes adapted to favourable conditions. Mohammadi and Amri (2008) reached the same conclusion for  $P_i$  and T/M/L in 15 durum wheat genotypes. The statistics of  $P_i$ , T/M/L and  $I_i$ , were classified as criteria of stability in the agronomic sense by several other researchers (Kang and Pham, 1991; Sabaghnia et al., 2006; Mohammadi and Amri, 2008), while those of  $S_{di}^2$ ,  $\sigma_i^2$ ,  $W_i^2$ ,  $CV_i$ ,  $S_i^{(2)}$  and  $S_i^{(3)}$  were associated with the biological concept of stability (Nassar and Huehn, 1987; Sabaghnia et al., 2006; Mohammadi and Amri, 2008). Solomon et al. (2007) grouped together yield, T/M/L and  $I_1$ , and set  $S_i^{(2)}$ apart. Mohammadi and Amri (2008) speculated that  $S_{di}^2$ ,  $\sigma_i^2$ ,  $W_i^2$  and  $S_i^{(2)}$  allow the identification of genotypes adapted to environments with unfavourable growing conditions, while genotypes identified according to  $CV_i$  and  $S_i^{(3)}$  may not be

as good as the responsive ones under favourable conditions. Becker and Leon (1988) pointed out that the biological concept of stability is not acceptable by most agronomists and breeders, who prefer genotypes with high mean yields and the potential to respond to agronomic inputs or better environmental conditions. According to Sabaghnia et al. (2006) statistics that represent a biological concept of stability could be used as compromise methods that select genotypes with moderate yield and high stability.

The novel CYP statistic was correlated with yield, and with statistics associated with the agronomic concept of stability, i.e.  $P_i$ , T/M/L and  $I_i$ . Hence, selection through CYP may favour the highest yielding cultivars that respond more to enhanced environmental resources. This inference is bolstered by the fact the top genotypes on CYP basis were A8, B17, B11, B13 (Table 1) whose  $b_i$  values were above 1.297 (Table 2). Furthermore, one could gain useful insights if the CYP statistic is considered along with genotype and genotype by environment (GGE) biplot pattern of stability analysed in the previous work (Tokatlidis et al., 2010). Genotype B13 fell in within the point of an ideal genotype combining high yield and stable performance, while genotypes A8, B17 and B11 were found to produce higher grain yield and be more stable than other genotypes, thus closely representing an ideal genotype (Fig. 1). The GGE biplot model provides agronomists with a complete visual evaluation of all aspects of the GxE interaction by creating a biplot that simultaneously represents mean performance and stability; it removes the noise caused by the environment main effect (E) and puts emphasis on the two components of genotype effects and GxE interaction (González et al., 2006; Thapa et al., 2009; Cravero et al., 2010).

Hence, the outstanding genotypes according to the CYP  $_{C}^{P}$ model were the best on the basis of yield under dense stand,  $_{2}^{P}$ , T/M/L and  $I_{i}$ , and GGE biplot model as well, and

thus well adapted and stable. Cultivars with improved CYP are able to yield optimally at a wide range of densities, i.e. density-neutral cultivars (Fasoula and Fasoula, 2000, 2002; Fasoula, 2008; Fasoula and Tokatlidis, 2011). The hypothesis of density-neutral cultivars was verified in maize and bread wheat (Triticum aestivum L.) (Tokatlidis and Tsialtas, 2008; Tokatlidis et al., 2005; 2006; 2011). Density-neutral cultivars have a number of advantages such as independence of crop yield on seeding rate, elimination of the cultivar by density interaction and feasibility to exploit drought-prone environments (Fasoula and Fasoula, 2000, 2002; Duvick, 2005; Fasoula and Tokatlidis, 2011; Tokatlidis et al., 2005; 2011). If that is the case, CYP could be a useful and quick evaluating tool for existing cultivars to meet the requirements that arise from climate changes. Evaluation in the absence of competition in relation to conventional cultivar trials under a single dense stand, is devoid of the risk of biased results due to genotype by density interaction and interference between plots that may distort yield and result in misleading conclusions (Fasoula, 2008; Fasoula and Tokatlidis, 2011; Tokatlidis et al., 2005, 2006, 2011). The CYP measure may also be a promising selection tool for breeding programs at ultra-low densities that annihilates plant-to-plant interference for inputs. It is worth considering the magnitude of yield improvement obtained by a single-generation selection, i.e. up to 38% for landrace A (genotype A8) and up to 21% for landrace B (genotype B17) (Table 1), which is much higher than ever accomplished (Tokatlidis et al., 2010). Such an accomplishment highlights the potential of the honeycomb

breeding to become an essential contributor of stable, high yielding and well adapted cultivars that could meet the demands of the growing population in the future. Honeycomb procedure is capable of evaluating a large number of individual plants and reducing the time frame to release new and improved cultivars, while concurrent selection for plant vield potential and homeostasis in the absence of competition leads to high yielding, adaptable cultivars (Fasoula and Fasoula 2000, 2002; Fasoula, 2008; Fasoula and Tokatlidis, 2011). The previous work (Tokatlidis et al., 2010) along with the present study can be taken as a paradigm to substantially exploit the genetic variability of similar landraces in dry bean and other crops, and to meet future challenges, e.g. sustainable agriculture. Worldwide landraces offer an opportunity to transform their valuable agronomic traits into new cultivars for more sustainable production (Newton et al., 2010). Lastly, it should be noted that the commonly used technique of cultivar and landrace propagation under dense stands may accumulate undesirable mutations because of the negative relationship between yielding and competitive ability. On the other hand, the absence of competition presents the optimal situation to remove undesirable mutations and select for the desirable ones; indeed, successful breeding at ultra-low density has been achieved in several crops thanks to optimized environmental variance (Fasoula and Tokatlidis, 2011).



E1 and E4 is greenhouse in 2005 and 2006, E2 is Field1 in 2005, E3 and E5 is Field2 in 2005 and 2006

**Fig 1.** Genotype and Genotype by Environment (*GGE*) biplot for grain yield and stability of the 21 genotypes: Entry codes 1 to 9 correspond to lines derived from landrace A (code 21), and codes 10 to 19 correspond to lines derived from landrace B coded 20 (Adapted from Tokatlidis et al., 2010).

#### Materials and methods

Experimentation was performed across three growing seasons (2004-06) in two locations in northwest Greece, known as high-quality bean regions, both at the atypical for dry bean conditions of a greenhouse to induce heat stress, as well as the more appropriate open field conditions. One set of environment was located at the Technological Education

	СҮР	t ha <sup>-1</sup>	$b_i$	$S_{di}^2$	$\sigma_i^2$	$W_i^2$	$CV_i$	$P_i$	$S_{i}^{(2)}$	$S_{i}^{(3)}$	T/M/L	$I_1$	$I_2$
t ha <sup>-1</sup>	0.58**												
$b_i$	-0.15	-0.16											
$S_{di}^{2}$	0.03	0.39	0.34										
$\sigma_i^2$	0.13	0.27	0.60**	0.78***									
$W_i^2$	0.07	0.21	0.65**	0.80***	0.98***								
$CV_i$	0.13	-0.15	0.12	0.15	0.30	0.32							
$P_i$	0.55**	0.98***	-0.06	0.42	0.32	0.27	-0.20						
$S_{i}^{(2)}$	-0.11	0.15	0.11	0.19	0.26	0.30	0.08	0.19					
$S_{i}^{(3)}$	-0.41	-0.41	0.37	0.21	0.33	0.38	0.23	-0.34	0.76***				
T/M/L	0.57**	0.98***	-0.52*	0.29	0.17	0.12	-0.14	0.95***	0.11	-0.48*			
$I_1$	0.45*	0.77***	0.26	0.68***	0.77**	0.72***	0.10	0.78***	0.32	0.01	0.70***		
$I_2$	0.56**	0.93***	0.12	0.53**	0.53*	0.47*	-0.04	0.94***	0.25	-0.21	0.88***	0.93***	
$I_3$	0.55**	0.97***	-0.01	0.48*	0.44*	0.38	-0.11	0.96***	0.24	-0.28	0.93***	0.88***	0.98***

**Table 2**. Spearman's rank correlation coefficients among statistic parameters: Relative crop yield potential (*CYP*), mean grain yield (t ha<sup>-1</sup>), parametric measures ( $b_i$ ,  $S_{di}^2$ ,  $\sigma_i^2$ ,  $W_i^2$ ,  $CV_i$ ,  $P_i$ ), and non-parametric measures ( $S_i^{(2)}$ ,  $S_i^{(3)}$ , T/M/L,  $I_1$ ,  $I_2$ ,  $I_3$ )

\* P<0.05, \*\* P<0.01, \*\*\*P<0.001

Institute Farm of Florina (40° 46′ 7N, 21° 22′ 8E, 705m elevation), and a second set was located in the Prespa lakes region (40° 50' 1N, 21° 07' 2E, 856m elevation). The first location involved a greenhouse constructed of glass (sandy loam soil with a pH of 6.4, 1.6% organic matter, and a waterholding capacity of 0.35 cm<sup>3</sup> cm<sup>-3</sup>), and an adjacent field named Field1 (sandy loam soil with a pH of 5.8, 2.06% organic matter, and a water-holding capacity of 0.26 cm<sup>3</sup> cm<sup>-</sup> <sup>3</sup>). At the second location a field called Field2 was used (sandy loam soil with a pH of 6.8, 1.23% organic matter and a water-holding capacity of 0.39 cm<sup>3</sup> cm<sup>-3</sup>). Nineteen singleplant progeny lines and their two mother landraces (A and B) constituted the material of the study. The progeny lines derived from landraces A and B were coded A1-A9 and B10-B19, respectively. A detailed description of the breeding procedure established to isolate these lines has been given by Tokatlidis et al. (2010).

#### Entry evaluation under low density

During 2004, the 19 single-plant progeny lines and the two mother genotypes were tested in a replicated-21 honeycomb trial (R21) (Fasoulas and Fasoula, 1995) in the greenhouse and Field1, with single-plant hills spaced 100 x 100 cm (~1.15 plants m<sup>-2</sup>). A total of 532 plants (~25 plants per entry) were established in the greenhouse (sowing date 15 March) and 994 plants (~47 plants per entry) were similarly established in the Field1 (sowing date 6 May). Each plant was harvested individually and the mean yield per plant ( $\overline{x}_i$ ) as well as the respective standard deviation  $(s_i)$  for the  $i_{th}$ genotype were calculated. To comparably estimate the relative crop yield potential (CYP) of the genotypes, relative plant yield potential  $(\bar{x}_i/\bar{x}_t)^2$  and relative homeostasis  $(\bar{x}_i/s_i)^2$  were computed from each environment separately, where  $\bar{x}_t$  is the average over genotypes experimental mean yield per plant. Means of  $(\bar{x}_i/\bar{x}_t)^2$  and  $(\bar{x}_i/s_i)^2$  across environments were used to compute CYP:  $CYP_i = (\overline{x}_i / \overline{x}_t)^2 \cdot (\overline{x}_i / s_i)^2$ .

#### Entry evaluation under dense stand

Entry evaluation under the dense stand of 4.8 plants m<sup>-2</sup> was performed across five environments at trials according to the randomized complete block design including four blocks. In the greenhouse, sowing dates were 12 March 2005 and 13 March 2006. The spacing was consistent with the farming practice. Each plot consisted of two rows 4.8 m in length, 70 cm apart and 60 cm between double-plant hills on row, and yield per plot was adjusted to yield per hectare (ha). Similar trials were established in the Field1 on 28 April 2005 and in the Field2 on 26 April 2005 and on 15 May 2006, where each plot included four rows of 6.6 m in length from which the two central rows were harvested. Mean entry yields were compared via the LSD test (P<0.05) after analysis of variance, where environment (location and/or year) effects were considered random and the genotype effect was fixed. To determine stability across environments, from the numerous stability models available, the following parametric and non-parametric measures were obtained: The regression approach of GxE interaction (Finlay and Wilkinson, 1963), estimated on the basis of the slope  $(b_i)$  of the linear regression of the mean yield  $\overline{y}_{ij}$  of the genotype *i* in the

environment *j* with the mean yield  $\overline{y}_{.j}$  of all genotypes in the environment *j*. Genotypes with  $b_i = 1$  are assumed more stable and expected to have general adaptation, whereas genotypes with  $b_i > 1$  are presumably more able to exploit beneficially favourable growing conditions. In this work, the less  $b_i$  deviated from the value 1, the more stable the genotype was regarded. The variance in regression deviations ( $S_{di}^2$ ) by Eberhart and Russell (1966) was calculated by the formula:

$$\frac{1}{e-2} \left[ \sum_{i} (\overline{y}_{ij} - \overline{y}_{i.} - \overline{y}_{.j} + \overline{y}_{..}) - (b_i - 1)^2 \sum_{i} (\overline{y}_{.j} - \overline{y}_{..})^2 \right]$$

where *e* is the number of environments,  $\overline{y}_{i.}$  is the mean of the genotype *i* over environments, and  $\overline{y}_{..}$  is the grand mean of all observations. The rest of the notations are consistent with those previously explained. Genotypes with variances equal to zero would be more stable, whereas increasing variance indicates low stability due to environmental stimulus. The stability variance ( $\sigma_i^2$ ) by Shukla (1972), describing the contribution of a genotype to the total *GxE* interaction, was measured by the formula:

$$\frac{g(g-1)\sum_{j}(\overline{y}_{ij}-\overline{y}_{i.}-\overline{y}_{.j}+\overline{y}_{..})^{2}-\sum_{i}\sum_{j}(\overline{y}_{ij}-\overline{y}_{i.}-\overline{y}_{.j}+\overline{y}_{..})^{2}}{(e-1)(g-1)(g-2)}$$

where g is the number of genotypes. The lowest the  $\sigma_i^2$  value of a genotype the greater its stability is. The ecovalence  $(W_i^2)$  by Wricke (1962) was computed, with  $W_i^2 = 0$  denoting greatest stability:

$$\sum \left(\overline{y}_{ij} - \overline{y}_{i.} - \overline{y}_{.j} + \overline{y}_{..}\right)^2$$

The coefficient of variation ( $CV_i$ ) by Francis and Kanenberg (1978) was measured:

$$\sqrt{\sum_{i} \left(\overline{y}_{ij} - \overline{y}_{i.}\right)^2 / (e-1)} / \overline{y}_{i.}$$

The superiority measure  $(P_i)$  by Lin and Binns (1988) was computed as the distance mean square between the genotype and the maximum response:

$$\sum_{j=1}^{n} (\bar{y}_{ij} - \max_j)^2 / 2e$$

where max<sub>j</sub> is the maximum response among all genotypes in the j environment. The smaller the  $P_i$  value, the better the genotype is. The  $S_i^{(2)}$  and  $S_i^{(3)}$  parameters (Nassar and Huehn, 1987) were obtained, the former giving equal weight to each environment, and the latter measuring stability in units of the mean rank of each genotype:

$$S_{i}^{(2)} = \sum_{j=1}^{e} \left( r_{ij} - \overline{r_{i.}} \right)^{2} / (e-1) , \qquad S_{i}^{(3)} = \left( \sum_{j=1}^{e} \left( r_{ij} - \overline{r_{i.}} \right)^{2} \right) / \overline{r_{i.}}$$

where  $r_{ij}$  is the rank of the *i* genotype in the *j* environment and  $\bar{r}_{i.}$  its mean rank across all environments. The stratified ranking procedure by Fox et al. (1990) was applied in each environment. The number of environments in which each genotype ranked in the top, middle, and bottom thirds of trial entries were scored multiplied by 3, 2 and 1, respectively. The sum of the scores each genotype achieved resulted in the integrated T/M/L measure. High T/M/L value indicated a widely adapted genotype. The rank-sum index  $(I_i)$  by Kang and Pham (1991) is based on yield and  $\sigma_i^2$  ranks. In more detail, for ranks assigned according to mean yield, the genotype with the highest yield represents the rank one; for ranks assigned according to  $\sigma_i^2$ , rank one is represented by the lowest estimated. Accordingly, the  $I_1$  index was the sum of yield rank and  $\sigma_i^2$  rank. The  $I_2$  index was the sum of 2(yield rank) and  $\sigma_i^2$  rank, whereas the  $I_3$  index was the sum of 3(yield rank) and  $\sigma_i^2$  rank. The lowest rank-sum was

assumed to be the most desirable one.

## Conclusions

The results of this study highlight the possibility of the CYP statistic to be a useful tool for cultivar comparisons and selection purposes. The data presented provide evidence that genotype ranking using the CYP matches the respective based on  $P_i$ , T/M/L and  $I_i$ , and GGE biplot, particularly for the outstanding genotypes. It was found that the CYP constitutes a criterion for stability in an agronomic sense, identifying genotypes that are able to utilize resources more efficiently. Cultivar evaluation on the CYP basis in the absence of competition is devoid of the risk of biased results due to genotype by density interaction and interference between plots. Honeycomb breeding via the CYP appears to be a promising procedure to exploit the within commonly cultivated landraces variability and develop highly yielding and stable new cultivars that could meet the future challenges regarding the demand for higher quantity and quality of food.

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