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Testing the Validity of CV for Single-Plant Yield in the Absence of Competition as a Homeostasis Index

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Abstract: The coefficient of variation (*CV*) of yield may functionally be related to the mean. The expected exponential *CV* decline with increasing mean, i.e., the Taylor's power law (TPL), is not always valid. Removal of this scale dependency allows for a scale-independent assessment of stability. The objective of this study was to interpret the validity of the homeostasis index (*HI*), i.e., the inverse *CV* value, suggested in breeding under nil competition as a selection criterion for progeny lines that oppose the acquired interplant variation. Data concerning the single-plant yield of various crops under a nil-competition regime were studies against the theoretical background of the above hypothesis. Simple correlations between logarithms of variances and respective means were used to assess the reliability of *CV* as a stability statistic in breeding trials under nil competition. A total of 8 of the 24 case analyses revealed a systematic variance dependence on the mean. The impact was more prevalent in experiments with extensive spatial heterogeneity and high *CV* scores. Conversion of variance to remove systematic dependence did not validate the *CV*~mean negative relationship. Because of variance dependence, caution is needed when interpreting the *HI* as a stability index. Misuse of the *HI* may entail the risk of bias, upgrading or downgrading a progeny line in its ability to withstand acquired dissimilarity between plants. Testing the validity of the variance seems necessary, and the calculation of *HI* on a converted variance may enhance the accuracy of identifying the most promising progeny lines.

Keywords: acquired dissimilarity; coefficient of variation; intragenotype competition; interplant variation

1. Introduction

The quantitative nature of yield and stability constitutes a considerable difficulty that breeders face in their attempt to increase selection efficiency in breeding [1]. The necessity for the resilience of grain-producing crops, serving sustainability in the enormously fluctuating environment, brings to the fore the 'productive' instead of the 'competitive' ideotype [2]. The productive ideotype combines low interspecific competitive ability and high plant yield efficiency. These two attributes are closely related due to the inverse connection of productivity with competitive ability [3–10]. Low interspecific competitive ability is valuable for varieties to evade acquired interplant dissimilarities and promote equality and resource use efficiency, whereas high plant yield efficiency allows for crop spacing without compromising grain yield per unit of area [2,9].

Owing to the confounding effects of intergenotypic competition on genotype expression and differentiation, the productive ideotype is recognizable under ultra-low densities, i.e., the '*nil*-competition' regime. Fasoulas [5,7,11] defined the nil-competition regime as widely spaced individual plants to preclude interference with each other for any input,

such as space, light, water, and nutrients. Each plant grows seamlessly and unhindered to exploit the available inputs and express its genetic background. The space share is decisive and should be large enough to allow for underground and above-ground autonomous plant development. Fasoulas [5,7,11] developed the 'honeycomb breeding method', in which nil competition is the first and inviolable principle [1,2,12]. Fasoulas also constructed the 'honeycomb selection design' (HSD), in which each plant lies in the centre of a circle surrounded by six equidistant plants [5,13,14]. Several documents have explained this method in detail [1,5,7,8,12,14–16].

The honeycomb experimental configuration has been deployed to deal with spatial heterogeneity [5,13,14,17,18]. A standardized, even, and systematic entry layout instead of a randomized configuration and implementation of the main principles of other models, such as blocking, replication, and nearest-neighbour adjustment on the same baseline (Figure 1), make this experimental model advantageous over other popular models in reducing experimental error [17,18]. Single-plant selection results in the respective progeny line (PL), and each PL is evaluated in terms of its mean yield and homeostasis index. Among *n* PLs, for a particular PL_i (with index *i* from 1 to *n*) comprising *k* plants, with the yield of each plant symbolized by X_{ij} (with index *j* from 1 to *k*), mean yield (μ_i) is computed across all plants grown in a uniform dispersion across the entire experimental area (e.g., code 15 in Figure 1), i.e., $\mu_i = \sum_1^k X_{ij}/k$. The inverse value of the single-plant coefficient of variation (*CV*), known as the 'homeostasis index' (*HI*) and computed on the basis of the respective variance, i.e., (σ^2), $\sigma_i^2 = \sum_1^k (X_{ij} - \mu_i)^2/(k-1)$, qualifies the ability of the PL to withstand environmentally induced acquired plant-to-plant dissimilarity and inequality: $HI_i = 1/CV_i$ and $CV_i = \sigma_i/\mu_i$. Therefore, PLs that are distinguished for high μ and high *HI* (in essence, low *CV*) are considered for further single-plant selection.

Homeostasis Index of entry 15:

$$HI_{15} = \mu_{15}/\sigma_{15}$$
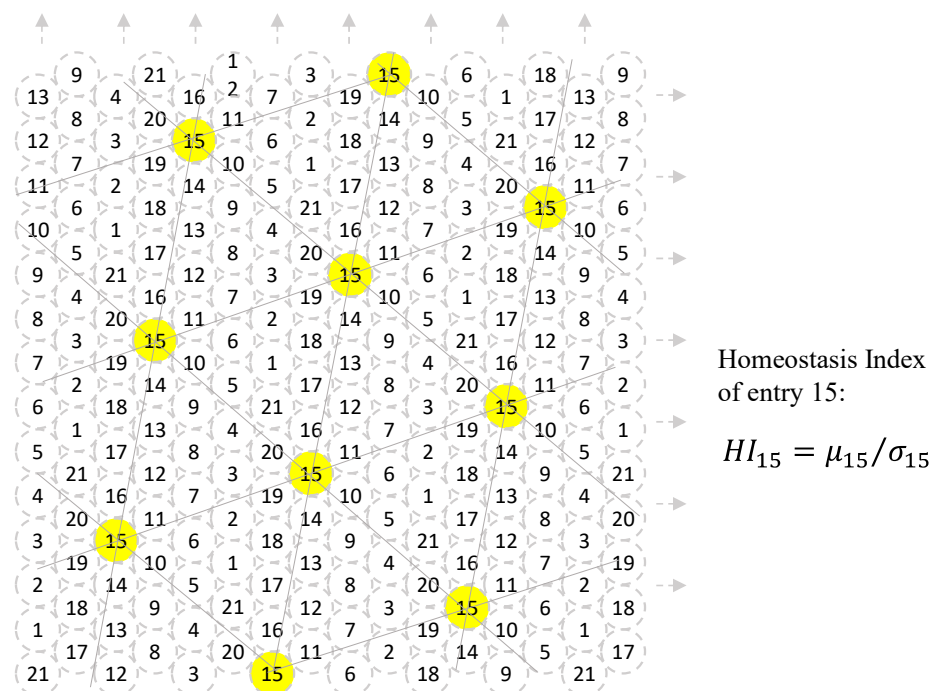
Figure 1. The honeycomb selection design-21 (HSD-21), where 21 entries (e.g., progeny lines) are evenly distributed across the experimental area to sample the spatial heterogeneity, e.g., entry 15. In order to evaluate an entry, the homeostasis index (HI_i) is calculated based on mean yield (μ_i) and standard deviation (σ_i), as shown for entry 15 (Figure constructed according to Fasoulas and Fasoula [14]).

The *CV* usually decreases with an increase in the respective mean following a power-law pattern, i.e., the Taylor's power law (TPL) relationship [19]. The TPL model has been set

as a theoretical basis to interpret the association of yield variation with mean yield; however, the reliability of *CV* for yield as a stability index has been questioned [20,21]. Because standard deviation (σ) is divided by the mean (μ), large means may be automatically accompanied by a low *CV*, implying a systematic (scale) dependence of σ, variance (σ^2), and *CV* on the mean. In such a case, a low *CV* is a mathematical artefact rather than an agronomically meaningful mechanism and needs to be interpreted with caution. In order to investigate the potential dependence of the *CV* on the mean, it was proposed to test the hypothesis using the slope (*b*) of the linear correlation between logarithms of variance ($\log_{10}(\sigma^2)$) and mean ($\log_{10}(\mu)$), that, is $\log_{10}(\sigma_i^2) = b * \log_{10}(\mu_i) + \alpha$ [20,21]. Theoretically, the slope (*b*) is either negative or (most likely) falls within the $0 < b < 2$ range. If *b* is negative, the standard deviation decreases with increasing mean, and the hypothesis of scale dependence of the *CV* on mean is not valid; thus, the negative *CV*~mean correlation is true. When *b* falls within the $0 < b < 2$ range, the *CV* systematically decreases with increasing μ in a non-linear manner, resulting in a negative *CV*~mean association of mathematical rather than biological essence. Standard deviation rises at a similar rate with mean when the slope (*b*) equals 2. Therefore, variance and *CV* do not exhibit a systematic dependence on the mean when a significant *b* approaches the value of 2; otherwise, a variance conversion is needed to set *b* equal to 2 and produce a scale-adjusted *CV* [21].

The validity of the theoretical framework of TPL has been studied regarding inter-crop yield variation, i.e., the over-location and/or over-season variation for yield per unit of area [20,22–24]. Regarding the intracrop variation, i.e., the plant-to-plant yield variation, relevant reports were made in [25,26]. The above studies occasionally discovered a systematic dependence of yield variance on mean yield, implying that the *CV*~yield correlation might not always be biologically meaningful; conversion of variance to remove this kind of dependence on mean did not validate the negative *CV*~yield relationship. This study is the first to test the validity of the theoretical framework of TPL in the *CV*~mean relationship concerning the single-plant yield recorded in honeycomb breeding experiments under a nil-competition regime and investigate the soundness of the *HI*. The analyses reported herein were based on relevant data provided in previously published studies.

2. Materials and Methods

2.1. Source Studies

Datasets including mean grain yield per plant and the respective *CV* value stemmed from honeycomb experiments in different crops and comprised 24 case analyses (c.a.) (Table 1). Most of the studies concerned breeding within registered cultivars or landraces of self-pollinated crops. In those studies, PLs were genetically homogeneous, interplant variation for grain yield included only the environmental variability, and the *CV* value of a PL represented its vulnerability to acquired inequality. Exceptions were studies of partial interspecific cotton lines [27] and open-pollinated maize lines [28,29], in which part of the single-plant yield variation was due to PL heterogeneity. More specifically, data were drawn from the following studies.

Bean (*Phaseolus vulgaris* L.)

In a study by Papathanasiou et al. [30], single-plant selection within three cultivars resulted in 18 1st generation PLs; the PLs, along with the original cultivars, were tested in two HSD-21 trials: one with normal irrigation and the other with deficit irrigation. Tokat-lidis et al. [31] derived 19 single-plant PLs from two landraces; progeny testing, including the original landraces, was performed in two HSD-21 trials: the first in a greenhouse to simulate heat stress and the second in an open field.

Cotton (*Gossypium hirsutum* L.)

Selection of six plants for high-seed cotton yield within each of three commercial cultivars resulted in 18 1st generation PLs [32]. The PLs and the maternal cultivars were evaluated across three locations according to the HSD-21 configuration. At one location, ten

selfed (i.e., hand-pollinated), high-yielding plants were selected from each 1st generation PL, and mixed ginned seed of the selected plants formed the respective 2nd generation PLs. The 2nd generation PLs were tested in a similar way to their ancestors. Another source study is a review paper by Fasoula [12], who presented data from an HSD-31 trial including 30 PLs and the source cultivar 'Celia.'

Cotton (partial interspecific lines)

In a doctoral thesis, Pankou [27] evaluated partial interspecific lines in two honeycomb trials: 21 (HSD-21 design) and 16 (HSD-16 design). Those lines had been formed from F_1 plants of *G. barbadense* × *G. hirsutum* crosses pollinated with pollen from *Hibiscus cannabinus* [33].

Lentil (*Lens culinaris* Medik.)

Two breeding studies emphasized tolerance to seedborne viruses, dealing with huge spatial heterogeneity.

A landrace well adapted to and commercially cultivated in northeast Greece was the source material in the first study [34,35]. Among 1088 landrace plants, single-plant selection led to 15 1st generation PLs; in parallel, mass selection at five different selection pressures led to the respective 1st generation populations. The 1st generation PLs and populations, along with the maternal landrace, were tested in an HSD-21 experiment. The experimentation was repeated for the same number of 2nd generation PLs and populations originating from six of the best 1st generation PLs.

The lentil landrace 'Lefkada' was the source material to derive a set of 30 1st generation PLs [36]. The set of PLs, along with their ancestor, was tested in three locations in Greece, i.e., Thessaloniki (site 1), Orestiada (site 2), and Larissa (site 3), according to the HSD-31 honeycomb configuration. In this study, analysis depicted an unexpected positive correlation of *CV* with mean yield at site 1, and results of analysis were presented either for site 1 separately or pooled across the three sites.

Wheat (*Triticum aestivum* L.)

From the bread wheat cultivar 'Nestos', single-plant selection led to 20 1st generation PLs tested along with the mother cultivar in an HSD-21 trial [37]. The experimentation was repeated for 20 2nd generation PLs formed via selection within five of the 1st generation PLs.

Maize (*Zea mays* L.)

Tokatlidis et al. [38] evaluated two sets, each comprising 40 S_6 lines (A and B) originated from single-plant selection within the F_2 of the commercial hybrid 'PR3183', as well as 40 random AxB ($S_5 \times S_5$) crosses. Additionally, two doctoral theses investigated the possibility of obtaining highly productive open-pollinated (half-sib, HS) lines. In the first thesis, sets of 41 HS_1 and 21 HS_2 lines, along with the original hybrid, 'Costanza', were tested in two successive seasons (2008 and 2009) in the region of Florina, Greece [28,39]. In the second thesis [29], 31 HS_2 and 21 HS_3 lines were tested in successive seasons allocated according to HSD-31 and HSD-21 honeycomb configurations, respectively. In addition, unpublished data from an ongoing research program include 13 hybrids evaluated during 2020 in two adjacent HSD-13 experiments in the region of Florina, Greece, in one of which the hybrids were inoculated with mycorrhizae.

Soybean [*Glycine max* (L) Merr.]

For soybean, analysis was applied the data reported by Fasoula [12] concerning 20 PLs derived via single-plant selection within the cultivar 'Haskell'; the HSD-21 entry arrangement was employed to evaluate PLs and the mother cultivar.

Bean (*Phaseolus vulagis* L.)

2.2. Data Analysis

The theoretical framework of the TPL model was investigated on a per plant basis in the absence of competition. Hereafter, the terms 'usual variance' and 'adjusted variance'

are used before and after conversion, respectively (see below), and the same terminology is used for the *CV*.

Table 1. Case analyses (c.a.) of genotype evaluation under a nil-competition regime concerning the mean (μ)~CV relation (df = degrees of freedom). (I) *r* is the coefficient correlation between μ and the usual *CV*. (II) *r* is the coefficient correlation between $log_{10}(\mu)$ and $log_{10}(\sigma_i^2)$, and *b* is the respective slope. (III) *r* is the coefficient correlation between μ and the adjusted *CV*.

c.a.	Source Study	df	μ Range (g Plant⁻¹)	Usual CV Range (%)	I r	II r	II b	III r
				Bean (*Phaseolus vulagis* L.)				
1	Papathanasiou et al. [30] (deficit irrigation)	19	83–121	44–83	−0.44 *	+0.39 ns		
2	Papathanasiou et al. [30] (normal irrigation)	19	115–178	35–57	−0.53 *	+0.41 ns		
3 ‡,§	Tokatlidis et al. [31] (open field)	19	122–341	32–73	−0.83 ***	+0.66 **	+0.75 **	−0.03 ns
4	Tokatlidis et al. [31] (greenhouse)	19	149–277	22–41	+0.02 ns			
				Cotton (*Gossypium hirsutum* L.)				
5	Tokatlidis et al. [32] (1st generation)	19	415–522	27–37	−0.09 ns			
6 §	Tokatlidis et al. [32] (2nd generation)	19	281–408	29–45	−0.73 ***	+0.25 ns		
7 §	Fasoula [12]	29	286–427	21–56	−0.42 *	−0.14 ns		
				Cotton (partial interspecific *G. barbadense x G. hirsutum* lines)				
8	Pankou [27]	19	209–514	17–71	−0.61 **	+0.29 ns		
9	Pankou [27]	14	323–430	22–45	−0.67 **	−0.42 ns		
				Lentil (*Lens culinaris* L.)				
10	Kargiotidou et al. [35] (1st generation)	19	5.75–10.4	93–151	−0.04 ns			
11 ‡	Kargiotidou et al. [35] (2nd generation)	19	10.2–27.9	84–175	−0.88 ***	+0.67 ***	+0.62 ***	−0.07 ns
12	Vlachostergios et al. [36] (Site 1)	29	1.2–6.4	30–56	+0.66 ***			
13 ‡,§	Vlachostergios et al. [36] (across 3 sites)	29	2.2–13.7	88–163	−0.81 ***	+0.93 ***	+1.32 ***	−0.10 ns
				Wheat (*Triticum aestivum* L.)				
14 ‡	Tokatlidis et al. [37] (1st generation)	19	20–34	47–75	−0.82 ***	+0.54 *	+0.64 *	+0.05 ns
15 ‡,§	Tokatlidis et al. [37] (2nd generation)	19	18–29	43–61	−0.67 ***	+0.68 ***	+1.02 ***	+0.01 ns
				Maize (*Zea mays* L.)				
16	Tokatlidis et al. [38] (lines A)	38	190–657	19–46	−0.13 ns			
17	Tokatlidis et al. [38] (lines B)	38	227–507	25–50	−0.01 ns			
18 ‡	Tokatlidis et al. [38] (crosses AxB)	38	673–1438	19–35	−0.62 ***	+0.41 **	+0.75 **	−0.02 ns
19 §	Greveniotis [28] (HS₁)	39	266–699	17–69	−0.77 ***	−0.13 ns		
20 ‡,§	Greveniotis [28] (HS₂)	19	354–664	31–60	−0.45 *	+0.53 *	+1.13 *	−0.02 ns
21	Tzantarmas [29] (HS₂)	29	418–796	30–61	+0.15 ns			
22 ‡	Tzantarmas [29] (HS₃)	19	574–1080	28–56	−0.81 ***	+0.49 *	+0.62 *	−0.01 ns
23 §	Tokatlidis et al. (unpublished) (hybrids)	24	625–1169	22–41	−0.76 ***	−0.08 ns		
				Soybean (*Glycine max* (L) Merr.)				
24	Fasoula [12]	19	150–212	12–23	−0.24 ns			

‡, variance dependence on mean; §, exponential rather than linear decline in CV with increasing mean, simulating TPL; * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), ns (non-significant).

To investigate the validity of TPL, the within-PL plant-to-plant variability was assessed for grain yield. Mean yield (μ) across plants was computed, and the usual coefficient of variation (CV) was computed based on the respective usual variance (σ^2). More specifically, data from each experiment were analysed separately in the following successive stages (I–III).

2.2.1. Linear Regression of Usual CV against Mean (Stage I)

For each PL_i, mean (μ_i), usual variance (σ^2) and usual (CV_i) were computed according to following equations:

$$\mu_i = \sum_1^k X_{ij}/k, \tag{1}$$

$$\sigma_i{}^2 = \sum_1^k (X_{ij} - \mu_i)^2/(k-1), \tag{2}$$

and

$$CV_i\% = 100\sqrt{\sigma_i{}^2}/\mu_i. \tag{3}$$

The mean values (independent variable) were plotted against the respective CV values (dependent variable) to perform the linear regression of CV against the mean and compute the correlation coefficient (r). In case of a significant negative correlation, a variance-dependence test was performed; otherwise, it was assumed that the usual variance was not scale-dependent on the mean and that the usual CV could be used as a stability index.

2.2.2. Testing Variance Dependence on Mean (Stage II)

To qualify the systematic dependence of the usual variance on the mean, the slope (b) was obtained according to the following linear regression [20]:

$$log_{10}\left(\sigma_i{}^2\right) = b * log_{10}(\mu_i) + \alpha \tag{4}$$

If the r value was nonsignificant, the process was interrupted, assuming the usual variance is not scale-dependent on the mean. Otherwise, the value and significance of b were examined. If the b value was negative, the usual variance was assumed not to be scale-dependent on the mean. If the b value was below 2, the analysis of variance of the regression was used to test the significance of b. A nonsignificant b implied that the usual variance was not scale-dependent on the mean. For all three cases of absence of systematic variance dependence mentioned above, values of the usual CV obtained in stage I were assumed to be valid, and there was no need for further analysis. The analysis proceeded into the next stage if the b value was within the $0 < b < 2$ range and significant.

2.2.3. Conversion of Usual Variance into Adjusted Variance (Stage III)

In case of a significant b, the usual variance (σ^2) was converted into adjusted variance ($\widetilde{\sigma^2}$) following the steps suggested in [21,23]. The residual u from the above regression was calculated as:

$$u_i = log_{10}\left(\sigma_i{}^2\right) - [a + b * log_{10}(\mu_i)] \tag{5}$$

Through the average $log_{10}(\mu_i)$ value, $\overline{log_{10}}(\mu)$, the logarithm of the adjusted variance ($log_{10}(\widetilde{\sigma^2})$) resulted from the following equation:

$$log_{10}\left(\widetilde{\sigma_i{}^2}\right) = 2 * log_{10}(\mu_i) + (b-2) * \overline{log_{10}}(\mu) + \alpha + u_i \tag{6}$$

The adjusted CV ($\widetilde{CV}$) was computed as

$$\widetilde{CV_i}\% = 100\sqrt{\widetilde{\sigma_i{}^2}}/\mu_i \tag{7}$$

which was substituted for the usual CV in the linear regression of stage I. To evaluate the homeostasis index (HI), it was computed according to the usual CV

$$HI_i = 1/CV_i \qquad (8)$$

and the adjusted *CV*

$$\widetilde{HI_i} = 1/\widetilde{CV_i} \qquad (9)$$

3. Results

3.1. Linear Regression of Usual CV against Mean (Stage I)

Table 1 summarises the results of 24 case analyses (c.a.). The c.a. numbered 12 was limited to stage I because means and *CVs* exhibited a positive correlation (Figure 2) instead of a negative correlation, indicating the absence of possible systematic dependence of variance on the mean. An additional seven c.a. (4, 5, 10, 16, 17, 21, and 24) were also interrupted at this stage because the correlation coefficient was not significant (e.g., Figure 2 for c.a. 16), and there was no systematic increase in variance with increasing mean. For 8 of the 16 remaining c.a. exhibiting a significant negative *CV* according to the mean relationship, an exponential rather than the linear decline in *CV* with increasing mean fit better, following the TPL model, i.e., c.a. 3, 6, 7, 13, 15, 19, 20, and 23.
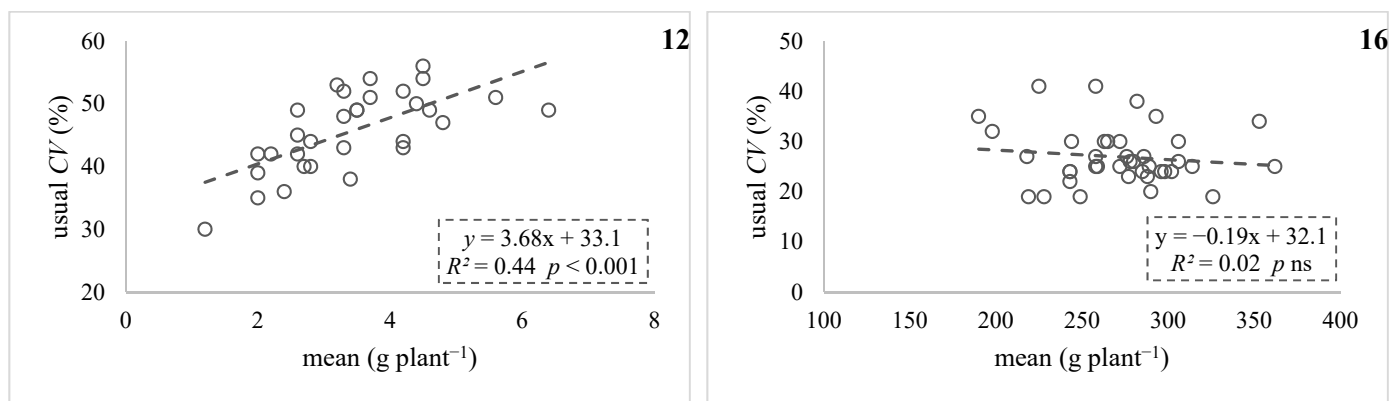
Figure 2. The correlation of the usual *CV* with mean was positive in c.a. 12 and negative but non-significant in c.a. 16. The absence of a negative correlation implies that the usual variance is not scale-dependent on the mean and that the usual *CV* is valid as a stability statistic and selection criterion.

3.2. Testing Variance Dependence on Mean (Stage II)

Eight of the sixteen c.a. performed at stage II (i.e., 1, 2, 6, 7, 8, 9, 19, and 23) indicated the absence of correlation in the linear regression of $log_{10}(\sigma_i{}^2)$ with $log_{10}(\mu_i)$; hence, the usual variance was not scale-dependent on the mean, and the negative relationship of usual *CV* with mean was valid (e.g., Figure 3 for c.a. 7 and 19). For the remaining eight c.a. (3, 11, 13, 14, 15, 18, 20, and 22), the linear regression of $log_{10}(\sigma_i{}^2)$ with $log_{10}(\mu_i)$ resulted in significant *b* values falling within the range of $0 < b < 2$ (shown in Figure 4 for c.a. 13 and 18), implying a systematic dependence of usual variance on the mean and therefore the need to proceed to variance conversion (stage III).

3.3. Conversion of Usual Variance into Adjusted Variance (Stage III)

Conversion of the usual variance resulted in the adjusted variance. Substitution of the logarithm of adjusted variance for the logarithm of usual variance in the linear regression rendered slope (*b*) values equal to 2 (left side of Figure 4 for c.a. 13 and 18) and removed the systematic dependence of the variance on the mean. The simple correlation coefficient between the usual *CV* and the adjusted *CV* was positive and significant, except c.a. 15, 20, and 22 (not shown). However, when the adjusted *CV* replaced the usual *CV* in the linear regression analysis, the *CV* correlation with the mean ceased to be significant (Table 1, right side of Figure 4 for c.a. 13 and 18).

Table 2 shows data concerning the mean yield per plant (μ) and the usual and adjusted *CV*, along with the respective *HI* values for the A × B (S$_5$ × S$_5$) crosses in the maize study of Tokatlidis et al. [38]. Two crosses (A29 × B3 and A16 × B31) consistently achieved top rankings for μ and *HI* before and after conversion. However, conversion differentiated the rank position of other crosses. For example, the conversion downgraded the A10 × B18 cross on account of adjusted *HI*. On the other extreme, the conversion of *HI* upgraded the A34 × B19 and A12 × B9 crosses. The results presented in Supplementary Table S1 for lentil PLs from a study by Vlachostergios et al. are similar [36].
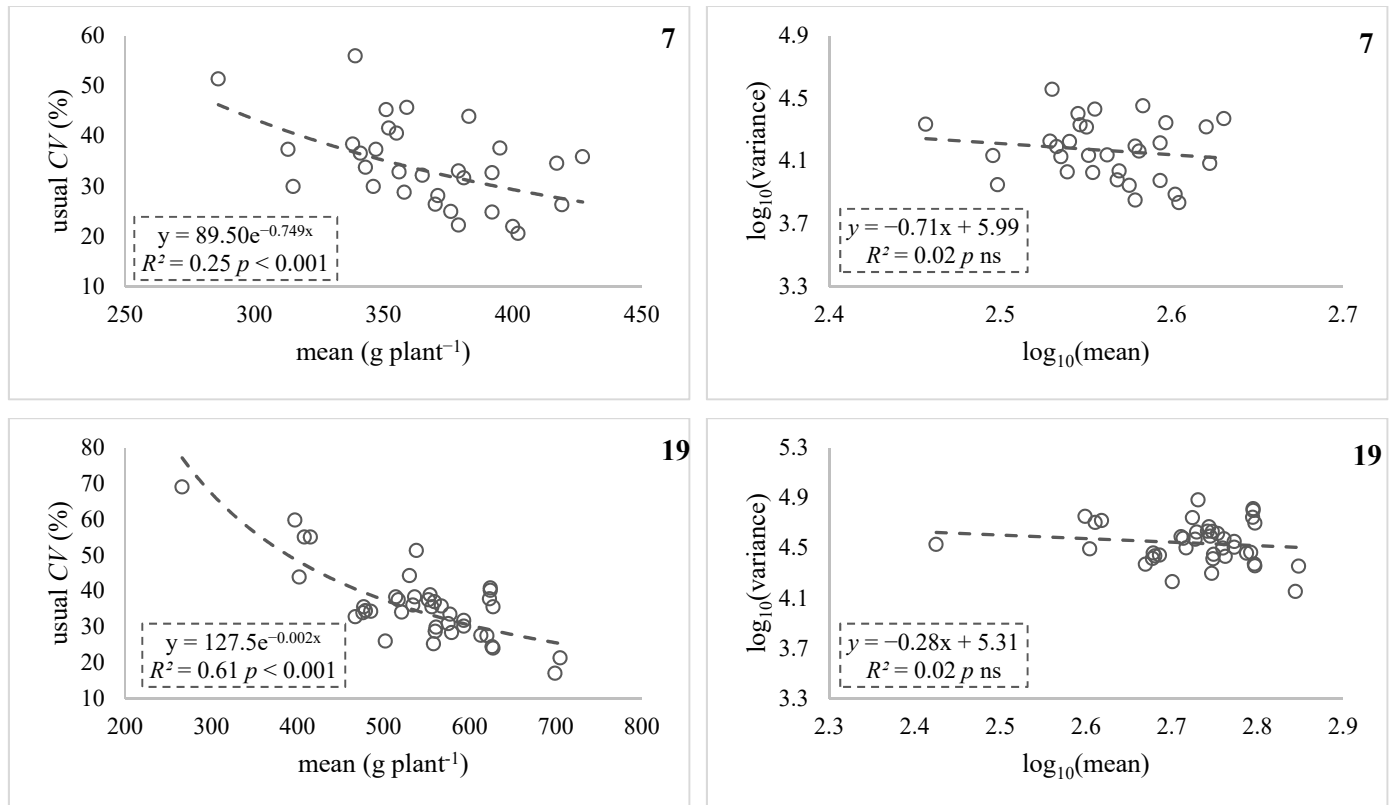

Figure 3. The correlation between the usual *CV* and mean was negative and significant in c.a. 7 and 19, simulating the exponential TPL model (**left** side). The absence of significance in the linear regression of the logarithm of usual variance with the logarithm of the mean (**right** side) implies that the usual variance is not scale-dependent on the mean and that the *CV* is valid to be used as a stability index and selection criterion.
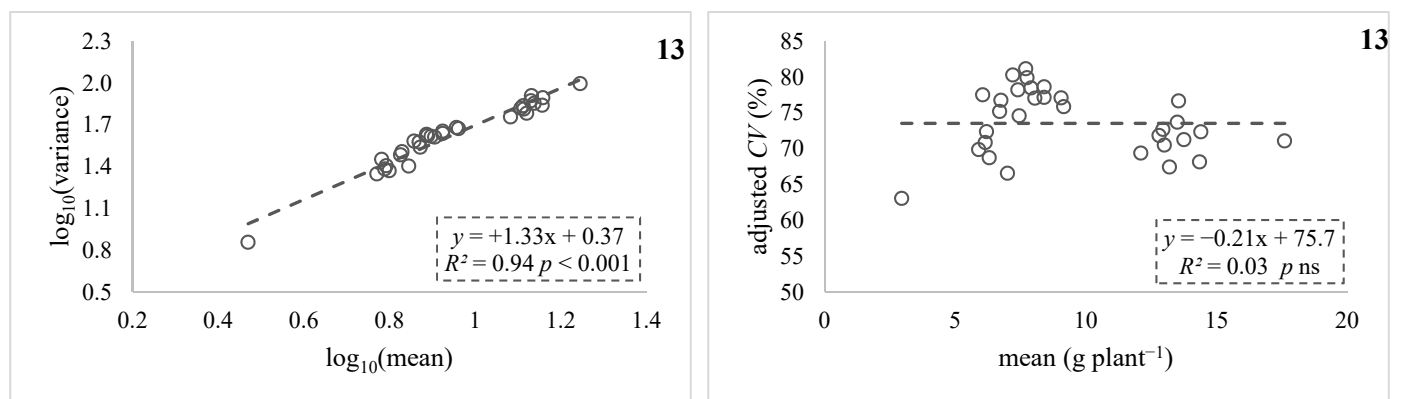
Figure 4. *Cont.*
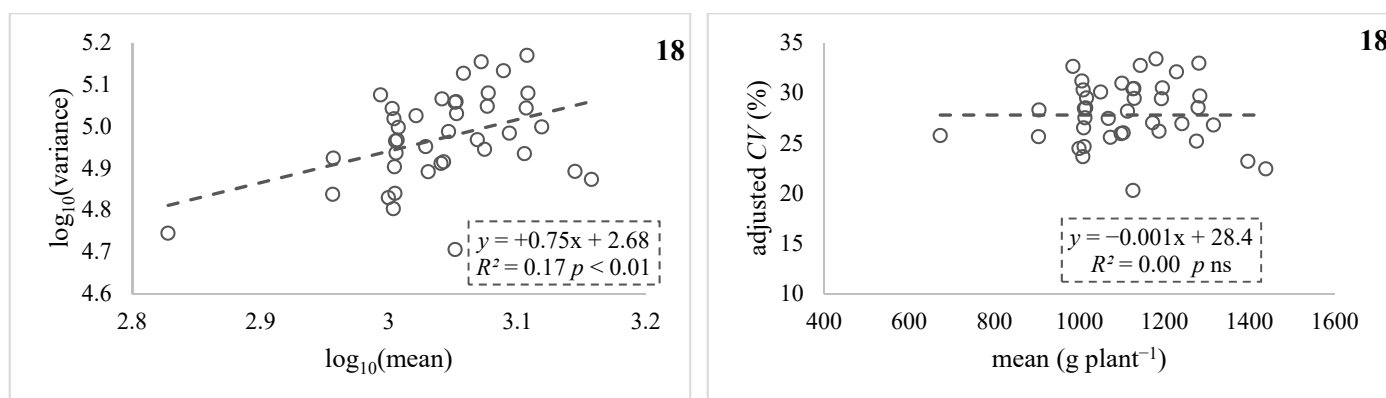
Figure 4. The linear regression of the logarithm of usual variance with the logarithm of mean in c.a. 13 and 18 was positive and significant, accompanied by a slope (*b*) falling within the $0 < b < 2$ range (**left** side); there is a mathematical dependence of usual variance, and the negative *CV*~mean association (Table 1) is not of biological essence to apply usual *CV* as a stability statistic. The adjusted CV obtained after variance conversion does not correlate with the mean (**right** side).

Table 2. Descending rank of $S \times S_5$ crosses for mean yield (μ), homeostasis index (*HI*) derived from usual *CV*, and homeostasis index ($\widetilde{HI}$) derived from adjusted *CV* ($\widetilde{CV}$). Occasional crosses are coloured to highlight their rank position. (Data from Tokatlidis et al. [38]).

$S_5 \times S_5$	μ (g Plant^{-1})	$S_5 \times S_5$	*CV*	**HI**	$S_5 \times S_5$	$\widetilde{CV}$	$\widetilde{HI}$
A29 × B3	1438	A29 × B3	0.19	5.26	A2 × B26	0.20	4.94
A16 × B31	1396	A16 × B31	0.20	5.00	A29 × B3	0.22	4.46
A22 × B23	1315	A2 × B26	0.20	5.00	A16 × B31	0.23	4.32
A32 × B4	1283	A36 × B11	0.23	4.35	A9 × B2	0.24	4.23
A14 × B39	1281	A22 × B23	0.24	4.17	A37 × B33	0.24	4.09
A10 × B18	1279	A40 × B24	0.25	4.00	A1 × B6	0.25	4.06
A36 × B11	1275	A28 × B14	0.25	4.00	A36 × B11	0.25	3.97
A40 × B24	1241	A9 × B2	0.25	4.00	A26 × B27	0.26	3.91
A30 × B30	1228	A10 × B18	0.26	3.85	A38 × B36	0.26	3.90
A15 × B35	1195	A25 × B12	0.26	3.85	A12 × B9	0.26	3.89
A8 × B16	1193	A31 × B17	0.26	3.85	A18 × B40	0.26	3.86
A28 × B14	1187	A18 × B40	0.26	3.85	A31 × B17	0.26	3.85
A17 × B5	1180	A26 × B27	0.26	3.85	A28 × B14	0.26	3.82
A25 × B12	1172	A1 × B6	0.26	3.85	A3 × B29	0.27	3.77
A39 × B1	1143	A37 × B33	0.26	3.85	A22 × B23	0.27	3.73
A5 × B22	1129	A32 × B4	0.27	3.70	A40 × B24	0.27	3.72
A7 × B34	1128	A8 × B16	0.28	3.57	A25 × B12	0.27	3.70
A2 × B26	1126	A21 × B20	0.28	3.57	A6 × B10	0.27	3.64
A24 × B8	1126	A6 × B10	0.28	3.57	A11 × B28	0.28	3.64
A21 × B20	1113	A3 × B29	0.28	3.57	A21 × B20	0.28	3.55
A31 × B17	1103	A15 × B35	0.29	3.45	A34 × B19	0.28	3.53
A35 × B15	1100	A5 × B22	0.29	3.45	A19 × B38	0.28	3.52
A18 × B40	1098	A11 × B28	0.29	3.45	A23 × B37	0.28	3.51
A26 × B27	1073	A38 × B36	0.29	3.45	A10 × B18	0.29	3.51
A6 × B10	1068	A14 × B39	0.30	3.33	A8 × B16	0.29	3.40
A33 × B13	1050	A30 × B30	0.30	3.33	A5 × B22	0.29	3.40
A4 × B21	1017	A7 × B34	0.30	3.33	A4 × B21	0.29	3.39
A23 × B37	1015	A24 × B8	0.30	3.33	A32 × B4	0.30	3.37
A11 × B28	1013	A23 × B37	0.30	3.33	A33 × B13	0.30	3.33
A19 × B38	1012	A19 × B38	0.30	3.33	A13 × B32	0.30	3.30
A1 × B6	1011	A35 × B15	0.31	3.23	A24 × B8	0.30	3.29
A3 × B29	1010	A33 × B13	0.31	3.23	A7 × B34	0.30	3.29
A13 × B32	1009	A4 × B21	0.31	3.23	A15 × B35	0.30	3.28
A9 × B2	1008	A17 × B5	0.32	3.13	A35 × B15	0.31	3.23

Table 2. *Cont.*

$S_5 \times S_5$	μ (g Plant^{-1})	$S_5 \times S_5$	CV	HI	$S_5 \times S_5$	$\widetilde{CV}$	$\widetilde{HI}$
A27 × B7	1006	A39 × B1	0.32	3.13	A27 × B7	0.31	3.21
A37 × B33	999	A13 × B32	0.32	3.13	A30 × B30	0.32	3.12
A20 × B25	985	A34 × B19	0.32	3.13	A20 × B25	0.33	3.07
A34 × B19	905	A27 × B7	0.33	3.03	A39 × B1	0.33	3.06
A38 × B36	904	A20 × B25	0.35	2.86	A14 × B39	0.33	3.04
A12 × B9	673	A12 × B9	0.35	2.86	A17 × B5	0.33	3.00

4. Discussion

The simple statistical tool of *CV* (standard deviation divided by the respective mean) is a relative measure of variation and stability [22,23,40]. Regarding the variation for grain yield per area (intercrop variation), a widespread consensus exists about an inverse connection between *CV* and the respective mean, usually following the exponential TPL model [20,24]. This correlation was found to be true concerning plant-to-plant variation in dense stands (intracrop variation) in studies analysing the implication of inter-plant variation on genotype stability [25,26]. In genetically homogeneous crops, the assessment of the usual *CV* for single-plant yield has been suggested as a stability measure against environmental forces that induce acquired interplant differences [9,41,42]. Interplant variation intensifies competition and inequality among plants, resulting in inefficient use of resources, decreased profit, and substantial yield loss [2]. That is why under Fasoulas' honeycomb breeding method, the inverse value of *CV* for single-plant yield was employed as a measure of PL stability known as the homeostasis index (*HI*) [1,12].

4.1. Soundness of HI

The present study is the first investigation of the theoretical framework of TPL in the *CV* ~ mean relationship for single-plant yield with widely spaced plants to prevent plant-to-plant interference for any input. Such a study could add value to the credibility of *CV* (and *HI*) in the honeycomb breeding method as a predictive measure of a genotype's ability to withstand spatial heterogeneity and evade acquired interplant differences. In the endeavour to study PL stability, one critical issue is the reliability of *CV* assessed by the correlation between logarithms of variances and means [20,21,23].

A total of 16 of the 24 c.a. verified the negative correlation of *CV* with mean, half of which the exponential rather than the linear decline in usual *CV* with increasing mean fit better, following the TPL model (Table 1), as demonstrated for c.a. 7 and 19 on the left side of Figure 3. A possible reason for the absence of the TPL model in the remaining cases is the relatively limited number of *CV*–mean pairs accompanied by a narrow range of means and/or *CVs* [24,26]. The range of means in the TPL regression should be as extensive as possible [43], which is why TPL tends to become more visible [20]. Nevertheless, the log_{10}(variance)~log_{10}(mean) regression and the subsequent variance conversion highlighted the potential unreliability of using the usual *CV* as a criterion for stability.

Regardless of the *CV* amount, there were cases in which the analysis from the first stage showed an absence of scale dependence of the variance on the mean, e.g., c.a. 24 (low *CVs*) and 10 (high *CVs*). On the other hand, c.a. showing an absence of systematic dependence of variance averaged lower min and max *CV* values compared to those showing variance to be scale-dependent on the mean (i.e., 30 vs. 47% for min and 58 vs. 87% for max; Figure 5). Therefore, there is a tendency for systematic dependence of variance on the mean to occur when high spatial heterogeneity increases plant-to-plant variability—a hypothesis that needs further investigation.

4.1.1. Cases Analyses Confirming the Validity of HI

The absence of systematic dependence of variance on the mean was due either to a positive correlation or a lack of significance in the *CV* ~ mean regression (stage I), as well as to a lack of significance in the $log_{10}(\sigma_i^2)$~$log_{10}(\mu_i)$ regression. An additional possible

reason (not observed in this study) is a negative value of *b*, implying that variance declines with increasing mean [20,22,24–26]. The absence of such a variance dependence renders the reverse association of the usual *CV* with yield biologically relevant [20,21], and for c.a. falling into this category, the *CV~* mean regression is valid, regardless of whether the pattern is linear (Figure 2) or simulates the TPL (Figure 3). This outcome implies that a low *CV* highlights a genotype's ability to withstand acquired interplant inequality and that *HI* can be used as a stability criterion [9].
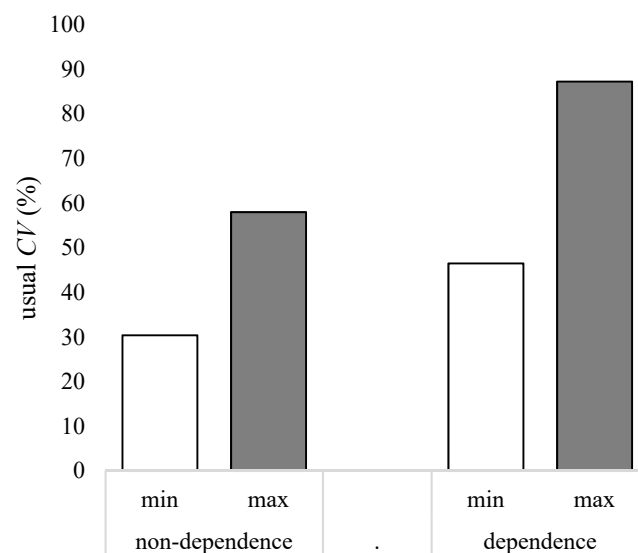
Figure 5. Both average min and max *CV* values derived from data exhibiting the dependence of usual variance on the mean are higher than the respective values when variance is independent of the mean.

4.1.2. Case Analyses Not Confirming the Validity of HI

Parameters for assessing yield variation need to show the absence of scale dependence on the mean. A significant slope (*b*) falling within the range of $0 < b < 2$ indicates a systematic dependence of *CV* on the mean (Figure 4). Such results were observed in case studies of intercrop variation [20,23,24], as well as intracrop variation [26]. Therefore, the negative relationship between *CV* and the mean might be a mathematical artefact rather than biologically meaningful. To remove the systematic dependence of *CV* on the mean and validate the *CV* value, a slope (*b*) value of 2 was set as a 'benchmark' [20,21]. However, after adjusting the slope (*b*) to a value of 2, there was no significant correlation between the adjusted *CV* and the mean (Figure 4), despite the correlation of the adjusted *CV* with the usual *CV*, in agreement with other studies [21,24,26]. When yield is log-transformed to remove scale dependence of variance, the negative correlation between the usual *CV* and the mean is likely to disappear; the usual *CV* value needs to be interpreted with caution when scale dependence of variance is detected [20]. Consequently, in honeycomb breeding studies, a scale dependence of the variance on the mean is detected, and applying the *CV* (or *HI*) could influence the results and their interpretation.

4.2. General Implications

The plant population density of a crop is a resource-limited regime used to satisfy each plant's input needs, e.g., space, water, nutrients, and light. Developmental dissimilarities of individual plants and intracrop inequality cause an unequal sharing of limited growth resources. The within-field-acquired interplant (intra-crop) variation is crucial because the plant-to-plant interference, in connection with an unbalanced use of inputs and decreased harvest index, constitutes a root source of yield loss [25,26,44]. Increased crop density (crowding) and intense competitive ability of a variety's genotype component are the primary sources of acquired interplant differences [1,41,42]. To address intracrop

variation, Tokatlidis [2] highlighted the 'productive' ideotype, which combines improved plant yield efficiency and low competitive ability. High plant yield efficiency enables individual plants to respond to additional inputs and increase their grain yield, allowing for crop spacing (lower than currently used densities) to mitigate interplant dissimilarities. Low interspecific competitive ability reduces interplant competition and plant-to-plant acquired differences. Nil competition, the inviolable principle of the honeycomb breeding method, focuses on the productive ideotype. Thus, the *HI* is a reasonable criterion for low interspecific competition. However, the use of *HI* presupposes several conditions. Because *HI* exclusively concerns the variability from acquired (not genetic) differences, it should be used with great caution when progeny lines are still genetically heterogeneous, particularly in the first segregating generations [2,9]. The present study highlights that great caution is also required if experimental data show a scale dependence of variance on the mean. Analyses showed that such a risk might be greater when the experiment has extensive spatial heterogeneity and large interplant variability is recorded (Figure 5). The generalization of such an impact remains to be confirmed by further investigations. Moreover, a PL may show high interplant variation and a low *HI*, but this does not preclude it from including a genotype that is, for example, resistant to the disease that causes the acquired dissimilarity. Extensive spatial heterogeneity in breeding experiments is often desirable, e.g., when screening for tolerance to viruses or fungal diseases [35,36,45,46]. Either way, scales and factors structuring data affect the reliability of the usual *CV* [20]. Hence, caution is needed in interpreting the effect of stability on the usual *HI*. Testing the validity of the usual variance seems necessary. Converting the usual variance to an adjusted variance may enhance the accuracy of the *HI* to identify the most promising progeny lines—an assumption that requires further investigation.

5. Conclusions

Experiments involving extensive spatial heterogeneity and progeny lines with high *CV* scores were more prone to exhibit a scale dependence of variance on the mean. A total of 8 of the 24 total analyses concerning single-plant yield in the absence of competition indicated a scale dependence of variance on the mean. A scale dependence of variance suggests that the *CV* value is a mathematical artefact rather than an agronomically meaningful statistic. Because *HI* is the inverse of the *CV* value, it may be incorrectly interpreted if there is a systematic variance dependence on the mean. Conversion of variance to remove its dependency eliminates the *CV*~ yield correlation. Therefore, caution is needed in interpreting genotype stability according to the *HI*. Misuse of the *HI* as a selection criterion against environmentally induced acquired variation may result in overestimation or underestimation of a progeny line in terms of its ability to withstand acquired interplant dissimilarity. Testing the validity of the variance seems necessary, and the calculation of *HI* based on a converted variance may enhance the accuracy of identifying the most promising progeny lines. However, confirmation of the assumption is a matter for further investigation. This study is the first attempt to assess the reliability of the *HI*; however, it relies on a wide range of experimental data to support the validity of the above outcomes.

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Abbreviations

CV, coefficient of variation; *HI*, homeostasis index; HSD, honeycomb selection design; PL, progeny line; TPL, Taylor's power law.

References

1. Fasoula, V.A.; Tokatlidis, I.S. Development of crop cultivars by honeycomb breeding. *Agron. Sustainable Dev.* **2012**, *32*, 161–180. [CrossRef]
2. Tokatlidis, I. Crop resilience via inter-plant spacing brings to the fore the productive ideotype. *Front. Plant Sci.* **2022**, *13*, 934359. [CrossRef] [PubMed]
3. Papadakis, J. The relation of the number of tillers per unit area to the yield of wheat and its bearing on fertilizing and breeding this plant-the space factor. *Soil Sci.* **1940**, *50*, 369–388. [CrossRef]
4. Donald, C.M. Competition among crop and pasture plants. *Adv. Agron.* **1963**, *15*, 1–118. [CrossRef]
5. Fasoulas, A.C. (Ed.) *The Honeycomb Methodology of Plant Breeding*; GR 54006: Thessaloniki, Greece, 1988.
6. Sedgley, R.H. An appraisal of the Donald ideotype after 21 years. *Field Crops Res.* **1991**, *26*, 93–112. [CrossRef]
7. Fasoulas, A.C. (Ed.) *Principles of Crop Breeding*; GR 54006: Thessaloniki, Greece, 1993.
8. Fasoula, D.A.; Fasoula, V.A. Competitive ability and plant breeding. *Plant Breed. Rev.* **1997**, *14*, 89–138. [CrossRef]
9. Tokatlidis, I.S. Crop adaptation to density to optimise grain yield: Breeding implications. *Euphytica* **2017**, *213*, 92. [CrossRef]
10. Fischer, R.A.; Rebetzke, G.J. Indirect selection for potential yield in early-generation, spaced plantings of wheat and other small-grained cereals: A review. *Crop Pasture Sci.* **2018**, *69*, 439–459. [CrossRef]
11. Fasoulas, A. *Principles and Methods of Plant Breeding*; Department of Genetics and Plant Breeding, Aristolelian University of Thessaloniki: Thessaloniki, Greece, 1981; Volume 11.
12. Fasoula, V.A. Prognostic breeding: A new paradigm for crop improvement. *Plant Breed. Rev.* **2013**, *37*, 297–347.
13. Fasoulas, A.C.; Zaragotas, D.A. *New Developments in the Honeycomb Selection Designs*; Pub. 12; Department of Genetics and Plant Breeding, Aristotle University of Thessaloniki: Thessaloniki, Greece, 1990.
14. Fasoulas, A.C.; Fasoula, V.A. Honeycomb selection designs. *Plant Breed. Rev.* **1995**, *13*, 87–139. [CrossRef]
15. Fasoula, V.A.; Fasoula, D.A. Honeycomb breeding: Principles and applications. *Plant Breed. Rev.* **2000**, *18*, 177–250.
16. Fasoula, V.A.; Fasoula, D.A. Principles underlying genetic improvement for high and stable crop yield potential. *Field Crops Res.* **2002**, *75*, 191–209. [CrossRef]
17. Kargiotidou, A.; Vlachostergios, D.; Tzantarmas, C.; Mylonas, I.; Foti, C.; Menexes, G.; Polidoros, A.; Tokatlidis, I.S. Addressing huge spatial heterogeneity induced by virus infections in lentil breeding trials. *J. Biol. Res. Thessalon.* **2016**, *23*, 2. [CrossRef] [PubMed]
18. Tokatlidis, I.S. Sampling the spatial heterogeneity of the honeycomb model in maize and wheat breeding trials: Analysis of secondary data compared to popular classical designs. *Exp. Agric.* **2016**, *52*, 371–390. [CrossRef]
19. Taylor, S.L.; Payton, M.E.; Raun, W.R. Relationships between mean yield, coefficient of variation, mean square error, and plot size in wheat field experiments. *Commun. Soil Sci. Plant Anal.* **1999**, *30*, 1439–1447. [CrossRef]
20. Döring, T.F.; Knapp, S.; Cohen, J.E. Taylor's power law and the stability of crop yields. *Field Crops Res.* **2015**, *183*, 294–302. [CrossRef]
21. Döring, T.F.; Reckling, M. Detecting global trends of cereal yield stability by adjusting the coefficient of variation. *Eur. J. Agron.* **2018**, *99*, 30–36. [CrossRef]
22. Knapp, S.; van der Heijden, M.G.A. A global meta-analysis of yield stability in organic and conventional agriculture. *Nat. Commun.* **2018**, *9*, 3632. [CrossRef]
23. Reckling, M.; Döring, T.F.; Bergkvist, G.; Stoddard, F.L.; Watson, C.A.; Seddi, S.; Chmielewski, F.-M.; Bachinger, J. Grain legume yields are as stable as other spring crops in long-term experiments across northern Europe. *Agron. Sustainable Dev.* **2018**, *38*, 63. [CrossRef]
24. Smutná, P.; Tokatlidis, I.S. Testing Taylor's Power Law association of winter wheat variation with mean yield at two contrasting soils. *Eur. J. Agron.* **2021**, *126*, 126268. [CrossRef]
25. Tokatlidis, I.S.; Remountakis, E. The Impacts of interplant variation on aboveground biomass, grain yield, and harvest index in maize. *Int. J. Plant Prod.* **2020**, *14*, 57–65. [CrossRef]
26. Pankou, C.; Koulymboudi, L.; Papathanasiou, F.; Gekas, F.; Papadopoulos, I.; Sinapidou, E.; Tokatlidis, I.S. Testing Taylor's Power Law association of maize interplant variation with mean grain yield. *J. Integr. Agric.* **2022**, *21*, 3569–3577. [CrossRef]

27. Pankou, C. Effect of Reciprocal crosses on the Stability of Partial Interspecific Lines and Improvement of Cotton Varieties. Ph.D. Thesis, Aristotle University of Thessaloniki/School of Agriculture, Thessaloniki, Greece, 2015. (In Greek with Abstract in English)

28. Greveniotis, V. Investigating the Possibility of Replacing Maize Hybrids with Open Pollinated Lines. Ph.D. Thesis, Democritus University of Thrace/Department of Agricultural Development, Orestiada, Greece, 2012. (In Greek with Abstract in English)

29. Tzantarmas, C. Investigating the Possibility of Recombination of Favorable Genes from Different Maize Hybrids to Develop Improved Open Pollinated Lines. Ph.D. Thesis, Democritus University of Thrace/Department of Agricultural Development, Orestiada, Greece, 2016. (In Greek with Abstract in English)

30. Papathanasiou, F.; Papadopoulou, F.; Mylonas, I.; Ninou, E.; Papadopoulos, I. Single-plant selection at ultra-low density of first generation lines of three been cultivars under water stress. *Agric. For.* **2019**, *65*, 27–34. [CrossRef]

31. Tokatlidis, I.S.; Papadopoulos, I.I.; Baxevanos, D.; Koutita, O. Genotype × Environment effects on single-plant selection at low density for yield and stability in climbing dry bean. *Crop Sci.* **2010**, *50*, 775–783. [CrossRef]

32. Tokatlidis, I.S.; Tsikrikoni, C.; Tsialtas, J.T.; Lithourgidis, A.S.; Bebeli, P. Variability within cotton cultivars for yield, fibre quality and physiological traits. *J. Agric. Sci.* **2008**, *146*, 483–490. [CrossRef]

33. Mavromatis, A.G.; Kadarzi, S.K.; Vlachostergios, D.N.; Xynias, I.N.; Skaracis, G.N.; Roupakias, D.G. Induction of embryo development and fixation of partial interspecific lines after pollination of F$_1$ cotton interspecific hybrids (*Gossypium barbadense* x *Gossypium hirsutum*) with pollen from *Hibiscus cannabinus*. *Aust. J. Agric. Res.* **2005**, *56*, 1101–1109. [CrossRef]

34. Kargiotidou, A. Pedigree and Mass Selection for Yield in a Lentil Population under Low-Density, Low-Input Conditions, and the Influence of Selection Intensity on Grain Productivity and Tolerance to Viruses. Ph.D. Thesis, Democritus University of Thrace/Department of Agricultural Development, Orestiada, Greece, 2012. (In Greek with abstract in English).

35. Kargiotidou, A.; Chatzivassiliou, E.; Sinapidou, E.; Papageorgiou, A.; Skaracis, G.; Tokatlidis, I.S. Selection at ultra-low density highlights plants escaping virus infection and leads towards high-performing pure-line cultivars in lentil. *J. Agric. Sci.* **2014**, *152*, 749–758. [CrossRef]

36. Vlachostergios, D.N.; Tzantarmas, C.; Kargiotidou, A.; Ninou, E.; Pankou, C.; Gaintatzi, C.; Mylonas, I.; Papadopoulos, I.; Foti, C.; Chatzivassiliou, E.; et al. Single-plant selection within lentil landraces at ultra-low density: A short-time tool to breed high yielding and stable varieties across divergent environments. *Euphytica* **2018**, *214*, 58. [CrossRef]

37. Tokatlidis, I.S.; Xynias, I.N.; Tsialtas, J.T.; Papadopoulos, I.I. Single-plant selection at ultra-low density to improve stability of a bread wheat cultivar. *Crop Sci.* **2006**, *46*, 90–97. [CrossRef]

38. Tokatlidis, I.S.; Koutsika-Sotiriou, M.; Fasoulas, A.C.; Tsaftaris, A.S. Improving maize hybrids for potential yield per plant. *Maydica* **1998**, *43*, 123–129.

39. Greveniotis, V.; Fasoula, V.A.; Papadopoulos, I.I.; Sinapidou, E.; Tokatlidis, I.S. Bridging the productivity gap between maize inbreds and hybrids via selection of plants excelling in crop yield genetic potential. *Aust. J. Crop Sci.* **2012**, *6*, 1448–1454.

40. Nielsen, D.C.; Vigil, M.F. Wheat yield and yield stability of eight dryland crop rotations. *Agron. J.* **2018**, *110*, 594–601. [CrossRef]

41. Andrade, F.H.; Abbate, P.E. Response of maize and soybean to variability in stand uniformity. *Agron. J.* **2005**, *97*, 1263–1269. [CrossRef]

42. Fasoula, V.A.; Tollenaar, M. The impact of plant population density on crop yield and response to selection in maize. *Maydica* **2005**, *50*, 9–48.

43. Taylor, R.A.J.; Lindquist, R.K.; Shipp, J.L. Variation and consistency in spatial distribution as measured by Taylor's power law. *Environ. Entomol.* **1998**, *27*, 191–201. [CrossRef]

44. Pan, X.Y.; Wang, G.X.; Yang, H.M.; Wei, X.P. Effect of water deficits on within-plot variability in growth and grain yield of spring wheat in northwest China. *Field Crops Res.* **2003**, *80*, 195–205. [CrossRef]

45. Kargiotidou, A.; Tzantarmas, C.; Chatzivassiliou, E.; Tokatlidis, I.S. Seed propagation at low density facilitates the selection of healthy plants to produce seeds with a reduced virus load in a lentil landrace. *Seed Sci. Technol.* **2015**, *43*, 31–39. [CrossRef]

46. Vlachostergios, D.N.; Roupakias, D.G. Screening under low-plant density reinforces the identification of lentil plants with resistance to fusarium wilt. *Crop Sci.* **2017**, *57*, 1285–1294. [CrossRef]