

Comparison of Biplot Analysis and Kang's YSi Statistic in Estimating Maize Hybrids Stability

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Summary: Integration of mean yield and stability evaluation into a single analysis is practical for maize hybrids recommendation and approval in multi-environment trials. The objective of this study was (i) to investigate grain yield performance and stability of 21 experimental maize hybrids *via* the use of the Kang's yield-stability statistics (YSi) and *via* the sites regression biplot (GGL) from multi-location trial conducted in Serbia; and (ii) to determine if hybrids yield stability was influenced by the linear effect of an environmental index. Hybrid main effect was highly significant and captured 9% of total sum of squares. Hybrid × location interaction was highly significant and accounted for 8.1% of the total sum of squares. Heterogeneity caused by environmental index was statistically non-significant. The same genotypes were recognized as stable by YSi and GGL biplot, but we recommend the sites regression biplot as more informative and precise tool for simultaneous hybrids mean performance and stability estimation.

Keywords: GGL biplot, grain yield, hybrids, maize, yield stability, YSi statistics

Introduction

The phenomenon of genotype × environment interaction (GEI) is characteristic of multi-environment trials (MET) and represents permanent interest for breeders and biometricians along with practical and theoretical aspects (Kempton & Fox 1997). Genotype stability estimation and GEI are specifically interrelated. While it would be nearly impossible to package into one cultivar all high-yield genes and genes conditioning all possible stresses that might be encountered from location to location and year to year, identification of cultivars that are both high yielding and stable in performance across multiple environments (locations or years or locations-years combination), or that have specific adaptation, is an appropriate strategy (Kang et al. 2005). Grain yield is a quantitative trait and routinely exhibits GEI, and as it represents agronomically and economically most important trait in maize, different genotypes should be evaluated across multiple environments in the advanced stages of selection (Annicchiarico 2002).

The effects of genotypes and environments are statistically non-additive, which means that

differences between genotypes depend on the environment or in other words differences between environments are not the same for different genotypes (Comstock & Moll 1963). GEI indicates importance of environments and their variable factors on adaptability and stability and is favourable phenomenon only if it is correlated with above-average yield (Yan & Hunt 2003). The environmental index is believed to represent a majority of the differences among locations, which includes differentials in fertility, cultural practices, insect or disease incidence, humidity, insolation, etc. (Haynes et al. 1995). The differences could relate to the growing season and/or to the period just before the growing season. Information on individual environmental covariates, including environmental index is needed to understand the nature and causes of genotype × environment interaction as well as to exploit it (Kang et al. 2005).

The estimation of performance and stability becomes important to identify consistent-performing and high-yielding genotypes. Various concepts of stability are described (Becker & Leon 1988). In varying environments, genotypes that provide high average yields with minimum GEI have been gaining importance over increased yields (Gauch & Zobel 1997).

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The sites regression model (SREG) (Crossa & Cornelius 1997) expresses the empirical mean of the i_{th} genotype in the j_{th} environment, as a sum of linear (additive) components (i.e. main effects of locations), a sum of bilinear (multiplicative) components combining the effects of genotypes (G) and genotype \times environment (GE), and a residual error. Yan et al. (2000) referred to biplots based on singular value decomposition (SVD) of environment-centered or within-environment standardized GE data as "GGE biplot" and this term emphasizes that the understanding of both G and GE is relevant to genotype evaluation and must be considered simultaneously for appropriate genotype and test environment evaluation.

The yield-stability statistic (YS_i) proposed by Kang (1993) represents modified rank-sum method (KRM) (Kang 1991) but is less likely to commit Type II errors (choosing a genotype for cultivation when it is unstable in its performance) relative to selection for both yield and stability. Stability component in YS_i is based on Shukla's (1972) stability-variance statistic (σ^2), which provides the contribution of a genotype to the total GE interaction, attributable to all genotypes in the test (Kang 1993). The YS_i was first applied in maize (Magari & Kang 1993) and soybean trials (Pazdernik et al. 1997). Madry (2003) used YS_i in a series of yield trials of spring wheat and

oat to investigate its possible use as a sufficient selection index for selecting generally adapted genotypes. Investigation of yield stability of cool-season pasture grasses by YS_i was conducted by Waldron et al. (2002); of rice (*Oryza sativa* L.) cultivars by Gravois & Bernhardt (2000); of potato (*Solanum tuberosum* L.) seed families by Upadhyya & Cabello (2000a,b); of common bean (*Phaseolus vulgaris* L.) genotypes by Mekbib (2003).

The objective of this study was to (i) investigate grain yield performance and stability of experimental maize hybrids *via* the use of the Kang's YS_i statistic and *via* the SREG biplot analysis from MET conducted in Serbia; and (ii) determine if hybrids stability was influenced by the linear effect of environmental index.

Material and Methods

Genetic material used in this research was represented by 21 experimental maize hybrids tested in official variety trials organized by the Department for Protection and Recognition of Varieties of Ministry of Agriculture, Water Management and Forestry of Republic Serbia during 2004. Experimental data were mean grain yields (t ha⁻¹) of tested hybrids over eight locations. The names of the test locations used are shown in Table 1 along with their codes, latitudes and longitudes.

Table 1. Test locations and climatic variables involved in maize multi-location yield trial during 2004. Average values for the period April to October 2004 meteorological series.

Location	Code	Latitude	Longitude	T _{max} [†] (°C)	T _{min} [‡] (°C)	T _{mean} [§] (°C)	pp [#] (mm)	RH [¶] (%)	SH [*] (h)
Bečej	BC	45°37'05"N	20° 02' 06"E	23.4	11.8	17.5	48.2	68.5	7.7
Kikinda	KI	45°49'28"N	20° 27' 33"E	23.2	11.6	17.3	43.6	68.9	8.1
Pančevo	PA	44°51'49"N	20° 39' 33"E	23.7	11.7	17.3	54.9	70.6	7.7
Rimski Šančevi	RS	45°19'51"N	19° 50' 59"E	23.1	11.5	17.2	60.9	73.2	7.8
Sremska Mitrovica	SM	44° 59'N	19°37' E	23.5	11.4	17.0	62.9	77.5	7.3
Sombor	SO	45°46'27"N	19° 06'44"E	23.6	10.9	16.8	62.7	68.0	7.4
Zemun Polje	ZP	44°52' N	20° 19'E	23.1	12.4	17.7	60.7	74.0	7.1
Zaječar	ZA	43°54'15"N	22° 17' 05"E	23.7	10.1	16.6	43.3	72.5	6.8

[†]T_{max}-maximum temperature; [‡]T_{min}-minimum temperature; [§]T_{mean}-average temperature; [#]pp- precipitation; [¶]RH-relative humidity; ^{*}SH-insolation

The experimental design was a randomized complete block design with four replications and elementary plot size was 10 m². Standard cultural and agronomic practices generally used were applied at all locations. Nitrogen, phosphorus, and potassium, were added according to fertilizer recommendations for each location. Density of tested hybrids was 57.142 plants ha⁻¹. Grain yield evaluation was performed by measurement of ears mass for each elementary plot using average

sample from each replication and grain yield with 14% moisture ha⁻¹ was calculated.

Additional information on climatic variables during vegetation period from April 15 to October 15 was provided by the Republic Hydro-meteorological Service of Serbia. Maximum temperature, minimum temperature, mean temperature, precipitation, relative humidity and insolation duration average values for test locations are given (Table 2).

For the data analysis a two-way fixed effect ANOVA was used based on randomized complete block design (RCBD) and the model is as follows:

$$Y_{ijk} = \mu + \gamma_{jk} + g_i + e_j + (ge)_{ij} + \varepsilon_{ijk} \quad (1)$$

where Y_{ijk} is the grain yield response of i_{th} hybrid in j_{th} location for k_{th} block; μ is the grand mean, g_i is the effect of the i_{th} hybrid, e_j is the effect of the j_{th} location, $(ge)_{ij}$ is the interaction effect of the i_{th} hybrid with j_{th} location and ε_{ijk} is the error term associated with response variable. All effects in model were considered as fixed.

In order to evaluate hybrids for their performance and stability both hybrid (G) and hybrid by location interaction (GL) effects must be considered simultaneously, and we used the sites regression (SREG) model (Crossa & Cornelius 1997) to obtain GGL biplots. Data analysis was done within an R computing environment (R Development Core Team 2010). The average-site coordination (ASC) view of the GGL biplot was used to estimate mean performance and stability of tested hybrids. The GGL biplot for this purpose originated using genotype-metric preserving singular value procedure (SVP = 1) and is, therefore, more appropriate for hybrids evaluation.

A yield-stability statistic (YS_i) developed for simultaneous selection of yield and stability was calculated according to Kang (1993). Location means of hybrids were subjected to stability analysis using Kang & Magari's (1995) program 'STABLE' to obtain estimates of YS_i (Kang 1993) and σ_i^2 (Shukla 1972). The STABLE program also provided a partition of the total G×L interaction into heterogeneity (non-additivity or the linear effect of environmental index

(EI); $EI = \bar{X}_{.j} - \bar{X}_{..}$, where $\bar{X}_{.j}$ is mean of all hybrids in the location j and $\bar{X}_{..}$ is mean of all hybrids across all locations) and residual G × L interaction.

Results and Discussion

In consideration to the fact that the most stable genotype(s) may not be the highest yielding, the use of methods that integrate yield performance and stability to select superior genotypes becomes more important and advantageous, especially when target environment encompasses great range of environmental conditions (Kang & Magari 1995). If high yielding maize hybrids with stable performance across environments are identified, the need for regional breeding strategy in order to exploit the positive GE interaction, would be reduced and lead to the preservation of breeders resources (Ceccarelli et al. 1994). In this study integration of mean yield performance and stability of experimental maize hybrids is achieved by SREG biplot methodology and Kang's yield-stability statistic YS_i.

ANOVA analysis showed highly significant ($P < 0.01$) location effect that accounted for 82.9%, of the total sum of squares for grain yield variation in the set of tested locations, (Table 2). The variance due to location effect is regarded as irrelevant for genotype evaluation in MET and is removed from the observed phenotypic data in order to focus on genotype (G) and genotype × environment (GE) effects, which are relevant for genotype evaluation (Yan & Kang 2003). Both YS_i and GGE methodology are based on the G + GE (Setimela et al. 2007). Hybrid (G) and hybrid × location (GL) captured 9% and 8.1% (Table 2), respectively, in the set of tested locations.

Table 2. ANOVA for yield data obtained from maize multi-location trial conducted in Serbia

Source of variation	df	SS	SS (%)†	MS	F value
Total	167	6405.95			
Hybrids (G)	20	576.50	9.0	28.82	7.78**
Locations (L)	7	5311.00	82.9	758.72	983.44**
G × L	140	518.44	8.1	3.70	4.80**
Heterogeneity	20	106.10	1.7	5.30	1.54
Residual G × L	120	412.35	6.4	3.44	4.45**
Pooled error	480			0.77	

** $P < 0.01$; † tested with respective mean square error term

The partitioning of GL interaction into heterogeneity caused by environmental index and residual GL interaction revealed that the linear effect of EI accounted for 1.7%, (Table 2) but it was statistically non-significant. Residual G × L obtained after heterogeneity caused by EI is removed was highly significant ($P < 0.01$) and accounted for 6.4% of the total sum of squares (Table 2). The use of covariates, which can be any environmental factor such as humidity,

precipitation, temperature, or environmental index, allows researchers to investigate possible causes of GEI (Magari & Kang 1993). In our study the partitioning of GL interaction into heterogeneity caused by EI and residual GL interaction revealed that the linear effect of EI was statistically non-significant. Obtained results suggested that the cause of these interactions must be factors other than those captured by the linear effect of the environmental index. This is in accordance with

the results of Fan et al. (2007) who conducted maize MET trials in China and also observed no significant heterogeneity caused by environmental index. Kang & Gorman (1989) found heterogeneity caused by environmental index to be significant for the maize MET in a Louisiana, but individual weather factors (e.g., rainfall, humidity, temperature) did not cause significant heterogeneity. Magari & Kang (1993) estimated contribution of environmental index, minimum temperature, maximum temperature, pre-season rainfall, rainfall during the growing season, and relative humidity to GEI by determining heterogeneity attributable to each of these covariates. They found that in five of eight trials, heterogeneity due to environmental index was significant. Heterogeneity due to the other environmental factors was not significant in any trial. When heterogeneity is significant statistically, it means that the linear effect of EI influenced stability of cultivars. In such cases, genotypes-wise comparisons of σ^2 (Shukla 1972), stability-variance statistic derived from the total genotype \times location (GL) interaction, and S^2 (Kang & Miller 1984), stability-variance statistic derived from residual GL after removal of heterogeneity, caused by EI or by another covariate from total GL, is desirable (Kang et al. 2005).

The average-site coordination view of the site regression biplot was used to estimate mean performance and stability of tested hybrids. The ASC abscissa is the average location axis, and the projection of the hybrids markers onto this line represents the main

effects of the hybrids (G) (Yan & Tinker 2006). The ASC ordinate expresses the hybrids' contributions to GL effect, and thus is convenient for estimation of hybrids stability of performance (Yan & Tinker 2006). According to Yan et al. (2001) ideal genotypes should have large projections of their markers towards the ASC abscissa to show high performance and small, preferably zero projection onto the ASC ordinate to show high stability of performance. Plant breeders and agronomists have found SREG biplot analysis to be useful for genotype evaluation (Butron et al. 2004, Malvar et al. 2005, Preciado-Ortiz et al. 2006, Fan et al. 2007).

GGL biplot explained 77.8% of the total G+GL variation for the set of all tested locations (Fig. 1). The length of the average-location vector was sufficient to select hybrids based on mean yield performance. Hybrids with above-average mean yield performance were G14, G5, G12, G3, G17, G8, G2, G18, G11, G1, G9 and G13, whereas hybrids with below-average mean yield were: G4, G6, G15, G10, G20, G16, G19, G7, G21. Hybrid G13 was with the highest mean yield performance and also the most stable hybrid. Hybrids G9, G2, G17 and G3 also showed good above-average mean yield performance and good stability. Hybrids with below-average mean yield performance showed greater instability in comparison to the hybrids with above-average mean yield performance for the set of all tested locations. The least stable hybrid of all tested was G6 which also showed second worst mean yield performance.

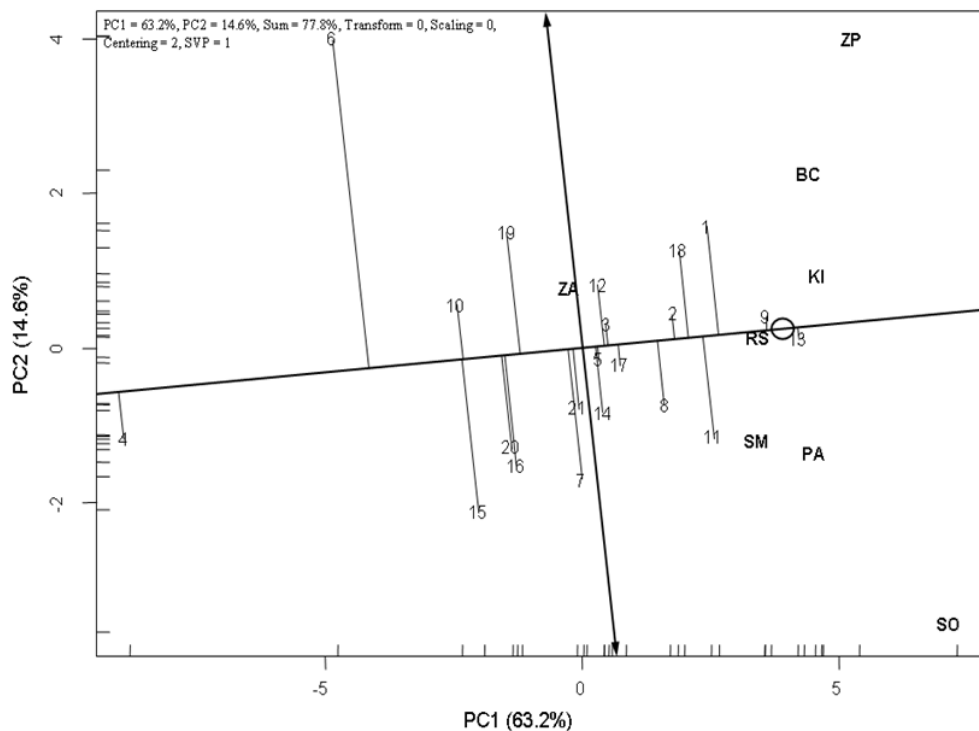


Figure 1. The average-environment coordination (AEC) view to show the mean performance and stability of hybrids. The details for test locations and hybrids are given in Tables 1 and 2.

According to Kang's analysis, a genotype with YS_i statistics more than mean YS_i and non-significant stability variance (Shukla 1972) is ideal for selecting genotypes for both high yield and stability. The mean YS_i value was 4.71 and 12 hybrids with $YS_i > 4.71$ had potential for further testing (Table 3). The mean grain yield of these hybrids was 14.02 t ha⁻¹, which is statistically equal to the mean yield (13.98 t ha⁻¹) of the 13 hybrids that could be selected on the basis of their grain yields alone (grain yield of hybrid > mean trial yield) (Table 3). Of the 21 tested hybrids G3 and G17 showed non-significant stability variance (σ_i^2) and YS_i values higher than the mean YS_i (Table 3). The mean grain yield of these two hybrids was 13.61 t ha⁻¹. On this basis, only two hybrids with good stability and high grain yields can be recommended. Bertoia et al. (2002) identified maize inbred lines with improved ear and stover yield and quality of silage using YS_i . The integration of yield and stability using YS_i statistics helps to identify genotypes that perform well for both

grain yield and stability, and also reduces the probability of committing Type II errors. A consequence of committing a Type I error would be that growers could miss using a stable genotype, but the consequence of committing a Type II error could be disastrous for growers as they might grow an unstable genotype and suffer economically (Kang 1993). It also helps to reduce the number of genotypes recommended for Advanced Trials and Official Variety Trials selected solely on mean grain yield, thus saving the total testing costs of METs. Kang (1993) showed that selecting for yield and stability will not automatically result in lower yield but if high yielding, unstable genotypes are used under poor environment conditions it can cause crop failures. Although YS_i -based selection would continue to identify unstable genotypes, the number of unstable genotypes selected is usually fewer than that identified by selection based on yield alone and when significant interaction is present, selection process becomes more precise (Kang & Magari 1995).

Table 3. Yield stability statistics (YS_i) for simultaneous selection for mean grain yield (t ha⁻¹) and stability of 21 experimental maize hybrids across the set of all the tested locations in 2004

Hybrid	Mean grain yield (t ha ⁻¹)	Yield rank (Y#)	Adjustment to Y# ^a	Adjusted Y (Y)	Stability variance (σ_i^2)	Stability rating (S)	$YS_i = (Y + S)$
G1	14.38	19	3	22	1.41*	-2	20+
G2	13.96	16	2	18	3.22***	-8	10+
G3	13.69	13	1	14	0.96	0	14+
G4	10.49	1	-3	-2	8.02***	-8	-10
G5	13.64	12	1	13	3.79***	-8	5+
G6	12.08	2	-3	-1	15.71***	-8	-9
G7	13.50	9	1	10	3.44***	-8	2
G8	13.82	15	2	17	2.62***	-8	9+
G9	14.45	20	3	23	4.72***	-8	15+
G10	12.72	4	-2	2	2.75***	-8	-6
G11	14.30	17	3	20	2.35***	-8	12+
G12	13.78	14	1	15	2.78***	-8	7+
G13	14.74	21	3	24	4.68***	-8	16+
G14	13.60	11	1	12	1.36*	-2	10+
G15	12.66	3	-3	0	2.66***	-8	-8
G16	12.97	6	-2	4	4.55***	-8	-4
G17	13.53	10	1	11	1.24	0	11+
G18	14.31	18	3	21	2.65***	-8	13+
G19	12.93	5	-2	3	3.08***	-8	-5
G20	13.18	7	-1	6	3.42***	-8	-2
G21	13.33	8	-1	7	2.32***	-8	-1
Mean	13.43						4.71

LSD (0.05): 0.36188

^a Adjustment to Y# (+ 1 for mean yield > overall mean yield (OMY), + 2 for mean yield > 1 LSD above OMY, + 3 for mean yield > 2 LSD above OMY, -1 for mean yield < OMY, -2 for mean yield < 1 LSD below OMY, -3 for mean yield < 2 LSD below OMY); *** P < 0.01; ** P < 0.05; * P < 0.1; + Genotype selected on the basis of YS_i

Out of 21 hybrids tested in the set of all tested locations, two hybrids showed non-significant stability variance (σ_i^2) and YSi values higher than the mean YSi (Table 3). On this basis, only two hybrids with good stability and high grain yields can be recommended. If the decisions were based only on mean hybrids performance, then also the rest of 11 hybrids (grain yield of hybrid > mean trial yield) would have been recommended, leading to a Type II error or to a biological or static concept of stability (Becker & Leon 1988). This concept is not acceptable to most breeders and agronomists who would prefer an agronomic or dynamic concept of stability favouring the high yielding genotypes which has a potential to respond positively to agronomic inputs or better environmental conditions (Becker 1981, Becker & Leon 1988).

GGL biplot for the set of all tested locations also confirmed good stability and above-average mean yield performance (Fig. 1) of these two selected hybrids, although also showed seven more hybrids with higher mean yield performance, and two of these seven hybrids were with better stability. Yan & Kang (2003) and Fan et al. (2007) reported a highly significant association between rankings of genotypes based on mean performance and stability in the SREG biplot and genotype ranking based on the YSi statistic.

Conclusions

The average-site coordination (ASC) view of the GGL biplot and Kang's yield stability statistic-YSi were confirmed in our study as appropriate tools to integration and estimation of maize hybrid's mean performance and stability. On the basis of the YSi statistic two hybrids with good stability and high grain yields can be recommended, and regarding GGL biplot two same hybrids and two more, not confirmed as stable by the YSi statistic.

Although both used models allows interpretation of agronomic or dynamic concept of stability, we recommend the sites regression biplot as more informative and precise tool for simultaneous hybrids mean performance and stability estimation.

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Poređenje biplot analize i Kangove statistike stabilnosti (YS_i) u proceni stabilnosti hibrida kukuruza

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Sažetak: Istovremena procena prinosa i stabilnosti prinosa na osnovu rezultata iz multi-lokacijskih ogleda ima veliki značaj u preporuci hibrida kukuruza. Cilj ovog istraživanja bio je da se ispita (i) prinos i stabilnost prinosa eksperimentalnih hibrida kukuruza pomoću Kangove statistike stabilnosti (YS_i) i metodom SREG GGL biplot analize, na osnovu podataka iz multi-lokacijskih ogleda u Srbiji, i (ii) da li su linearni efekti indeksa spoljašnjih sredina uticali na stabilnost hibrida. Glavni efekti genotipa (hibrida) bili su visoko značajni i obuhvatali su 9% od ukupne sume kvadrata. Interakcija hibrid \times lokalitet je takođe bila visoko značajna i obuhvatala je 8,1% od ukupne sume kvadrata. Heterogenost uslovljena razlikom između spoljašnjih sredina bila je statistički neznačajna. Na osnovu rezultata obe analize isti genotipovi su izdvojeni kao stabilni ali je prednost na strani SREG modela kao preciznijeg i informativnijeg.

Ključne reči: GGL biplot, hibridi, kukuruz, prinos zrna, stabilnost prinosa, YS_i statistika