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Statistical test of genotype-by-environment interaction patterns observed from a biplot

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This short article is to comment on the methodology and conclusions of Yang et al. (2009) concerning the use of biplots to reveal crossover genotype-by-environment interaction (GE) patterns, which was published in Crop Science. Yan et al. (2007) applied GGE (genotypic main effect plus genotype-by-environment interaction) biplot analysis to an Ontario winter wheat dataset and concluded that there were two mega-environments for winter wheat in Ontario, i.e., eastern Ontario represented by locations E5 and E7, and southwestern Ontario represented by seven other locations (Fig. 1), Yang et al. (2009) took this study as an example of “over-utilization or abuse of biplot analysis.” Yang et al. (2009) applied a bootstrap procedure to the same dataset and concluded that the crossover GE pattern revealed by the biplot analysis was false. They advocated that any future use of biplots should be supplemented with a statistical test, such as the bootstrap procedure they introduced. We believe their conclusion and recommendation are unfounded and misleading.

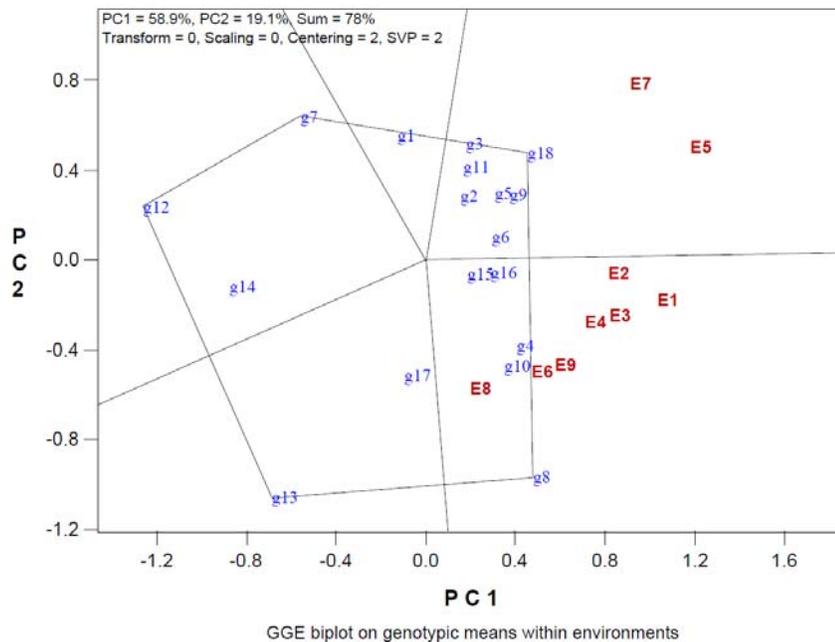


Figure 1 GGE biplot of genotype by environment table. The biplot was based on environment-centered data (Centering = 2) and was not scaled (standardized) (Scaling = 0). It was based on environment-focused singular value partitioning (SVP = 2). The genotypes are from g1 to g18 and the environments are from E1 to E9.

Biplots are a graphical display of a two-way dataset that has no measure of uncertainty. Patterns observed from a biplot, therefore, need to be tested using formal statistical methods, particularly when a critical decision is to be made (Yan and Hunt, 2003). The first step is to formulate a hypothesis based on the biplot pattern. For the current example, the hypothesis is: genotype G18 yielded more than genotype G8 in eastern Ontario *and* G8 yielded more than G18 in southwestern Ontario. The second step is to test these two contrasts against ‘within-group experimental errors’, for which replicated data are essential (in cases where replicated data are not available, each environment within a group may be treated as a replicate of the group). Table 1 represents the numerical contrast extracted from the original two-way table. Since g8 had higher yield than g18 at all seven locations in southwestern Ontario while the opposite was true in eastern Ontario, there is no need to resort to any statistical test to conclude that the crossover pattern revealed in Fig. 1 is true.

Table 1. Location-centered yield data (Mg ha⁻¹) extracted from the original genotype-by-location two-way table to test the crossover interaction revealed from the biplot in Fig. 1

Genotype	Eastern Ontario			Southwestern Ontario plus E1							
	E5	E7	Mean	E1	E2	E3	E4	E6	E8	E9	Mean
g8	-0.15	-0.07	-0.11	0.49	0.23	1.29	0.46	0.77	0.70	0.67	0.66
g18	0.96	0.78	0.87	-0.13	0.22	0.47	0.42	-0.23	0.00	0.21	0.14

Alternatively, a biplot of genotype-by-rep-within-environment can be used to visually verify the crossover GE pattern (Fig. 2). If the pattern is real, then all or most of the replicates within one environmental group should fall closely together and be separated from those in the other environmental group. This clearly is the case in Fig. 2, confirming the conclusion based on Fig. 1. For mega-environment delineation, which has long-term implications for plant breeding and cultivar recommendation, validation of crossover GE patterns using data from multiple years is more crucial than statistical tests using replicated data within years. Using the winter wheat-performance data from 1989 to 1998, Yan et al. (2000) showed that eastern Ontario and southwestern Ontario were distinct mega-environments, again confirming the conclusion based on Fig. 1.

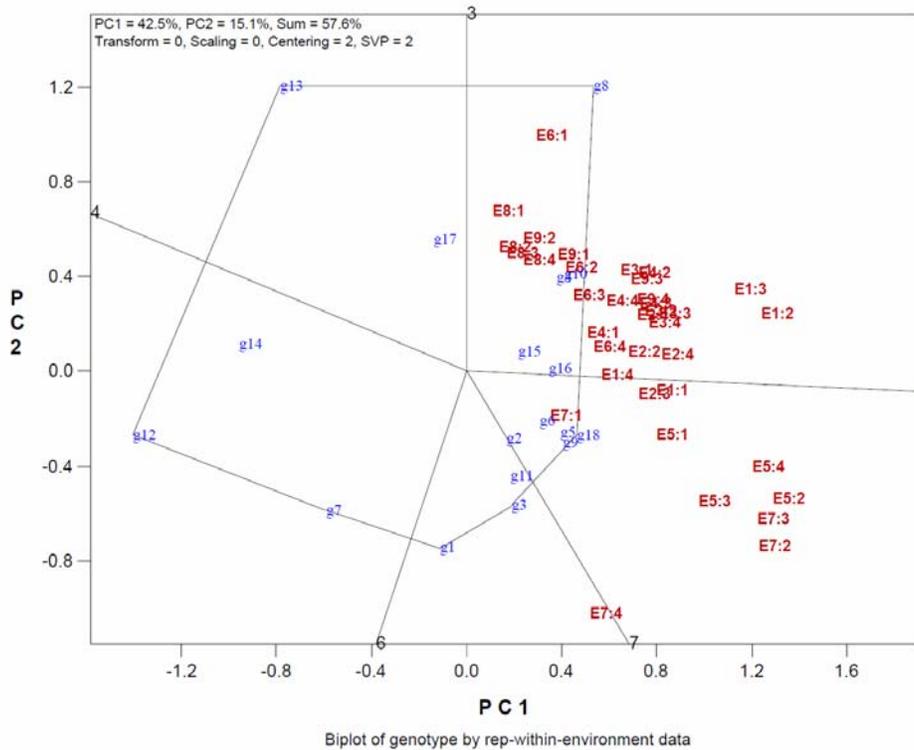


Figure 2 GGE biplot of genotype by replication-within-environment table. The biplot was based on rep-within-environment -centered data (Centering = 2) and was not scaled (standardized) (Scaling = 0). It was based on rep-within-environment-focused singular value partitioning (SVP = 2). The genotypes are from g1 to g18 and the environments are from E1 to E9. Each of the four replicates within an environment is represented by the environment code plus a replicate number.

The incorrect conclusion of Yang et al. (2009) about this particular dataset resulted from the use of a problematic bootstrap procedure. The crossover GE patterns of the full dataset can never be validated from its subsets. If there are no crossover GE patterns in the full data, no pattern can be expected from the subsets; if there are crossover patterns, they will be destroyed by the resampling process. Consequently, the only conclusion possible from such analysis would be to reject the existence of crossover GE. The bootstrap procedure cannot test whether the crossover GE or ‘which-won-where’ pattern observed in a biplot is real.

Another problem with the procedure of Yang et al. (2009) is that their focus was on principal component (PC) scores rather than on biplot patterns. The placements of genotypes and environments in a biplot are mutually defined, and it is the placement of the genotypes relative to the environments that is important. The sign and values of PC scores become meaningless when genotypic scores are detached from environmental scores; this is what happened in the procedure of Yang et al. (2009). It is common knowledge that a biplot can be flipped horizontally, vertically, or rotated at any angle; while this inevitably leads to different genotypic and environmental PC scores, the patterns remain unchanged. In other words, the PC scores of each genotype or environment can take an unlimited number of values during bootstrap permutations,

which will lead to unrealistic “confidence intervals” for each genotype and each environment, but such confidence intervals cannot be used to invalidate a biplot pattern. There have been discussions in the literature (e.g., Lebart, 2007) on the use of bootstrap in principal component analysis. The focus in such studies is on the robustness of the relationships among variables (or ‘environments’ in the context of genotype-by-environment data), treating observations (or ‘genotypes’ in the context of genotype-by-environment data) as random samples from a large population. Under such scenarios, meaningful confidence regions for each variable can be developed if, and only if, certain controls are implemented in the permutation to prevent possible change of sign for each PC, possible switch of axes between the first and the second PC, and possible rotation of the biplot (Lebart, 2007). The bootstrap procedure used in Yang et al. (2009) does not appear to have involved such controls. Further, while it is meaningful to resample observations to estimate confidence regions for the variables, it does not make sense to resample the variables to estimate confidence regions for individual observations.

In conclusion, when the significance of biplot patterns needs to be tested for making critical decisions, it should be done using established statistical methods, such as ANOVA, correlation analysis, etc. Unfortunately, the bootstrap procedure proposed by Yang et al. (2009) is not valid for this purpose.

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