

Biplot Analysis of Genotype by Environment for Cooking Quality in Hybrid Rice: A Tool for Line × Tester Data

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Abstract: A study of combining ability for improving rice cooking quality was carried out via genotype plus genotype × environment (GGE) biplot. Four restorer lines and three male sterile lines were used to obtain F₁ in a line × tester trial at the Rice Research Institute, Amol, Iran in 2009. GGE biplot analysis showed that Neda and IR56 were the best general combiners for amylose content (AC), whereas Nemat and IR28 had the highest general combining ability (GCA) effects for gelatinization temperature (GT), and IR58 and IR59 showed the highest GCA effects in terms of gel consistency (GC). Meanwhile, IR58 and IR59 showed large specific combining ability (SCA) effects for AC, while Neda and SA13 had high SCA effects for GT. Nemat and IR28 had large SCA effects for GC. Because intermediate levels of AC, GT and GC are ideal, Nemat × IR59 was considered as the best possible cross. Based on these results, the GGE biplot showed good potential for identifying suitable parents, heterotic crosses and the best hybrids in line × tester data.

Key words: line × tester trial; general combining ability; specific combining ability; hybrid rice; genotype plus genotype × environment biplot

Rice (*Oryza sativa* L.) has an important role in the nourishment of Asian societies. Data show that rice production in several Asian countries must double by the year 2025 to meet the requirements of the rising population. Hybrid rice technology is one of the feasible and readily available options for raising the yield (Yashitola et al, 2002).

In many countries, including Iran, the cooking quality of rice has a significant impact on the choice of varieties for cultivation and consumption. In view of this, a significant number of improved or introduced rice varieties are gradually being eliminated by farmers and consumers because of low grain quality. Thus, for rice breeding programs to be successful, quality improvement needs to be adequately considered along with quantity increase.

The cooking and eating qualities are the most important features of rice grains. Scientifically, cooked rice grain quality has been evaluated in terms of characteristics such as grain elongation, volume expansion and water absorption during cooking (Juliano, 1985). Studies spanning the past few decades have shown that the cooking quality is directly related to three physical and chemical characteristics of the starch in

the endosperm, namely amylose content (AC), gel consistency (GC) and gelatinization temperature (GT).

Improving the cooking quality of high yielding hybrid rice varieties requires comprehensive studies to understand the nature and action of the genes underlying cooked rice grain quality traits. Also, to transfer desirable cooked rice grain characteristics to new hybrid varieties, plant breeders need to find the best parental combinations.

Combining ability provides an important tool for selection of desirable parents and getting required information on gene action controlling desirable traits (Rastogi et al, 2011). Different mating systems have been used to study and evaluate combining ability in genetic research. Although diallel crosses have been applied in breeding programs, they are very labor-intensive, especially when the number of genotypes is increased. Therefore, Kempthorne (1957) suggested line × tester analysis as a faster and more reliable method to estimate combining ability and screen suitable lines for hybridization. Since then, many researchers have used line × tester analysis for hybrid rice genotypes (Kumar, 2007; Saidaiah et al, 2011; Tiwari et al, 2011; Patil et al, 2012; Raju et al, 2012). However, most of these studies have focused on yield and yield components using traditional methods.

Yan and Hunt (2002) developed a new genotype

plus genotype \times environment (GGE) biplot method to examine the combining ability and interrelationships of parents based on a graphical presentation using the first principal component (PC1) and the second principal component (PC2), which are derived from subjecting the tester-centered diallel data. They argue that although the GGE biplot method was developed for multi-environment trial (MET) data analysis, it should be applicable to all types of two-way data that assume an entry-by-tester data structure. They also note that conventional analysis is limited to obtain general combining ability (GCA) for each genotype and specific combining ability (SCA) for each cross while a biplot displays the most important entry by tester patterns of the data and allows the following information to be extracted visually: the GCA of each genotype, the SCA of each genotype, groups of parents with similar genetics, and superior hybrids (Yan and Hunt, 2002). Although there are some reports on the application of the GGE biplot in diallel data analysis (Yan, 2001; Rastogi et al, 2011; Sharifi and Safari Motlagh, 2011; Farshadfar et al, 2012; Sharifi, 2012, 2013), this method has not been used for line \times tester data analysis. There are some differences between the results obtained from diallel data and line \times tester data. In MET data, genotypes are entries and environments are testers while in diallel data, genotypes are both entries and testers. In this study (line \times tester data), it was assumed that restorer lines and male sterile lines were entries and testers, respectively.

The main objective of this study was to analyze line \times tester data using the GGE biplot method to gather information about the genetic interrelationships among parents and identify the best combinations for cooking quality in hybrid rice varieties.

MATERIALS AND METHODS

Experimental site

This study was conducted at the Rice Research Institute, Amol, Mazandaran Province, Iran (36°28' N and 52°23' E, at an altitude of 29.8 m) in 2009. The average annual temperature and rainfall were 16 °C and 800 mm, respectively.

Rice materials

Rice materials comprised 13 male sterile (MS) and 23 restorer (R) lines. In April, seeds were sown in the greenhouse. At the 3–4 leaf stage, single rice seedlings

were transferred to the main field and planted in a space of 20 cm \times 20 cm in a randomized complete block design with five replications. Plots were protected from unwanted pollen using nylon covers with a height of 2.5 m.

First, the flowering behavior and floral traits of the parental lines were evaluated according to the standard evaluation system for rice published by the International Rice Research Institute, the Philippines. Then, some parental lines with desirable flowering traits were selected to obtain F₁ hybrid seeds in a line \times tester fashion. These included four R lines *viz.* IR56, IR28, IR59606-119-3R (IR59) and SA13, and three MS lines *viz.* Neda A (Neda), Nemat A (Nemat) and IR58025 A (IR58). Seeds of the parental lines along with 12 derived F₁ hybrids were analyzed for AC, GT and GC according to the methods suggested by Juliano (1971), Little et al (1958) and Cagampang et al (1973), respectively.

Statistical analysis

Combining ability analysis was carried out using the GGE biplot method (Yan, 2001). The GGE biplot software version 6.3 was used to generate biplot figures (Yan, 2001). In the GGE biplot, the R and MS lines were considered as entries and testers, respectively.

The biplot model is defined as follows (Farshadfar et al, 2012):

$$\hat{Y}_{ij} - \mu - \beta_j = \lambda_1 \zeta_{i1} \eta_{j1} + \lambda_2 \zeta_{i2} \eta_{j2} + \varepsilon_{ij}$$

Where \hat{Y}_{ij} is the genotypic value of the combination between entry i and tester j ; μ is the grand mean; β_j is the mean of all combinations involving tester j ; λ_1 and λ_2 are the singular values for PC1 and PC2, respectively; ζ_{i1} and ζ_{i2} are the PC1 and PC2 eigenvectors for entry i , respectively; η_{j1} and η_{j2} are the PC1 and PC2 eigenvectors for tester j , respectively; and ε_{ij} is the residual of the model associated with the combination of entry i and tester j .

RESULTS

Amylose content

Fig. 1-A shows the GGE biplot software output obtained using the mean vs. stability option. This diagram has three main elements including: (i) the average tester coordinate (ATC), which is shown as a small circle, indicating the position of the average tester, (ii) the ATC abscissa, which is a thick arrowhead line that passes through the biplot origin and the ATC pointing to the average tester from the

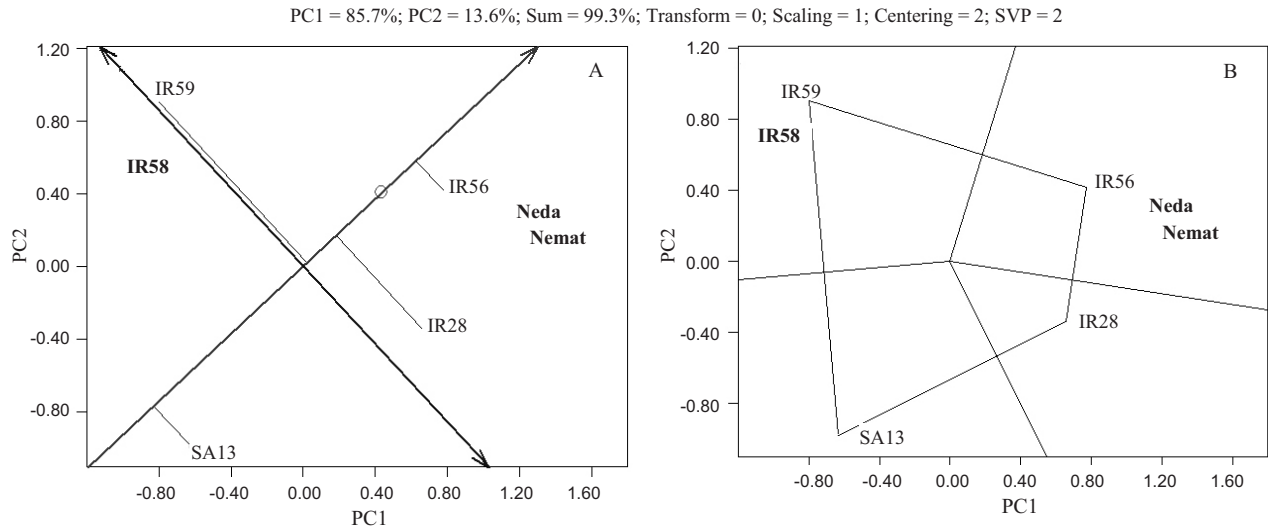


Fig. 1. Genotype plus genotype \times environment (GGE) biplot for amylose content showing average tester coordinate view (A) and polygon view (B) of three female and four male rice parents.

Parents showed with bold and regular letters are testers and entries, respectively.

biplot origin, and (iii) the ATC ordinate, which is a thick double-arrowhead line that is perpendicular to the ATC abscissa.

The GCA effect of an entry may be defined by the value of its hybrid with the average tester. Thus, the GCA effect increases by the direction of the arrow on the ATC abscissa. Therefore, Fig. 1-A indicates that IR56 had the highest GCA effect whereas SA13 had the lowest GCA effect for AC. The ACs of entries were ranked as IR56 > IR28 > IR59 > SA13. The ATC ordinate separated the entries with positive PCA scores from those with negative PCA scores. The positive end of the ATC abscissa was at the side of the biplot origin where the ATC was located. Thus, only SA13 had a negative GCA effect for AC.

The ATC is defined as a virtual tester whose PC1 and PC2 scores are equal to the average PC1 and PC2 scores, respectively, across all testers (Yan and Hunt, 2002). Hence, the GCA effects of testers are also approximated by their projection onto the ATC abscissa. Testers at the right side of the ATC abscissa should have a positive GCA effect, whereas those located at the left side of the ATC abscissa should have a negative GCA effect. Thus, Fig. 1-A shows that Neda followed by Nemat had the highest and positive GCA effect, whereas IR58 had the lowest and negative GCA effect for AC. As explained by Yan (2001), an ideal tester should be highly discriminating of the lines and be highly representative of all testers. It is therefore defined as the tester that has the longest

vector of all testers (i.e., the most discriminating) and zero projection onto the ATC ordinate (i.e., the most representative of the testers). Therefore, in this study, the female Neda was the best tester for AC.

The SCA effects are estimated based on the projection onto the ATC ordinate from the biplot origin (Yan, 2001). A larger projection onto the ATC ordinate indicates a higher SCA effect. Thus, the SCA effects for AC of the entries were ordered as IR59 > IR28 > SA13 > IR56. Also, among testers, the highest SCA effect for AC belonged to IR58, followed by Nemat and Neda (Fig. 1-A).

Fig. 1-B shows a polygon view of the biplot, which visualizes the interaction patterns between entries and testers. This polygon was formed by drawing straight lines that connected the entry markers positioned furthest from the plot origin. In this figure, the biplot was divided into four sectors for AC. A single tester, IR58, fell in sector 1 whereas genotype IR59 was the vertex of the polygon indicating that entry IR59 was the best mating partner with tester IR58 and the cross IR58 \times IR59 was heterotic. Also, testers Neda and Nemat fell in sector 2, whereas entry IR56 was the vertex of the polygon. Thus, the crosses Neda \times IR56 and Nemat \times IR56 should be heterotic. No tester fell in sectors 3 or 4, meaning that the genotypes IR28 and SA13 were not the best mating partners for any of the testers.

Gelatinization temperature

Based on the interpretation rules of the biplot

presented for AC and with regard to Fig. 2-A, entry IR28 had the highest GCA effect, whereas IR59 had the lowest GCA effect for GT. The GTs of entries were ranked as IR28 > IR56 > SA13 > IR59. Entry IR59 had a negative GCA effect for GT, as it was located at the left side of the ATC ordinate.

The testers were ordered as Nemat ≈ IR58 > Neda in terms of GCA effect for GT. The testers Nemat and IR58 had positive GCA effects for GT, as they were located at the right side of the ATC, whereas Neda, which was located at the left side of the ATC, had a negative GCA effect.

Based on the projection onto the ATC ordinate, the GTs of entries were ordered as SA13 > IR28 > IR56 > IR59, showing that SA13 and IR59 had the highest and lowest SCA effects for GT, respectively. Among the testers, Neda followed by IR58 and Nemat had larger SCA effects for GT as they had the highest projections onto the ATC ordinate (Fig. 2-A).

Fig. 2-B shows that the biplot was divided into three sectors for GT. Testers Nemat and IR58 fell in sector 1, whereas entry SA13 was the vertex of the polygon, indicating that SA13 was the best mating partner for Nemat and IR58, and that the crosses Nemat × SA13 and IR58 × SA13 were heterotic. Also, tester Neda fell in sector 2, whereas entry IR28 was the vertex of the polygon. Thus, the cross Neda × IR28 was heterotic. No tester fell in sector 3, meaning that IR59 was not the best mating partner for any of the testers.

Gel consistency

As demonstrated for AC and GT and with regard to

Fig. 3-A, entries IR59 and IR28 had the highest positive GCA effects, whereas SA13 had the lowest and negative GCA effect for GC. The GCs of entries were ordered as IR59 > IR28 > IR56 > SA13.

Also, the GCs of testers were ranked as IR58 > Nemat ≈ Neda in terms of GCA effect. The testers Nemat and IR58 had positive GCA effects for GC, as they were located at the right side of the ATC, whereas Neda, which was located at the left side of the ATC, had a negative GCA effect for GC (Fig. 3-A).

In addition, based on the projection onto the ATC ordinate from the biplot origin, entry IR59 had the highest SCA effect for GC, followed by entries IR28, IR56 and SA13. Also, based on the SCA effect, the GCs of testers were ordered as Neda ≈ Nemat > IR58 (Fig. 3-A).

Fig. 3-B shows that the biplot was divided into three sectors for GC. The tester Neda fell in sector 1, whereas restorer IR59 was the vertex of the polygon, indicating that IR59 was the best mating partner for Neda, and thus the cross Neda × IR59 was heterotic. Furthermore, testers Nemat and IR58 fell in sector 2, whereas genotype IR28 was the vertex of the polygon. Thus, the crosses Nemat × IR28 and IR58 × IR28 were heterotic. No tester fell in sector 3, thus, SA13 was not the best mating partner for any of the testers.

Best crosses

For the studied qualitative characteristics, high AC, GT and GC were not considered favorable factors in cooked rice quality. Therefore, genotypes with high GCA and SCA effects that formed heterotic crosses

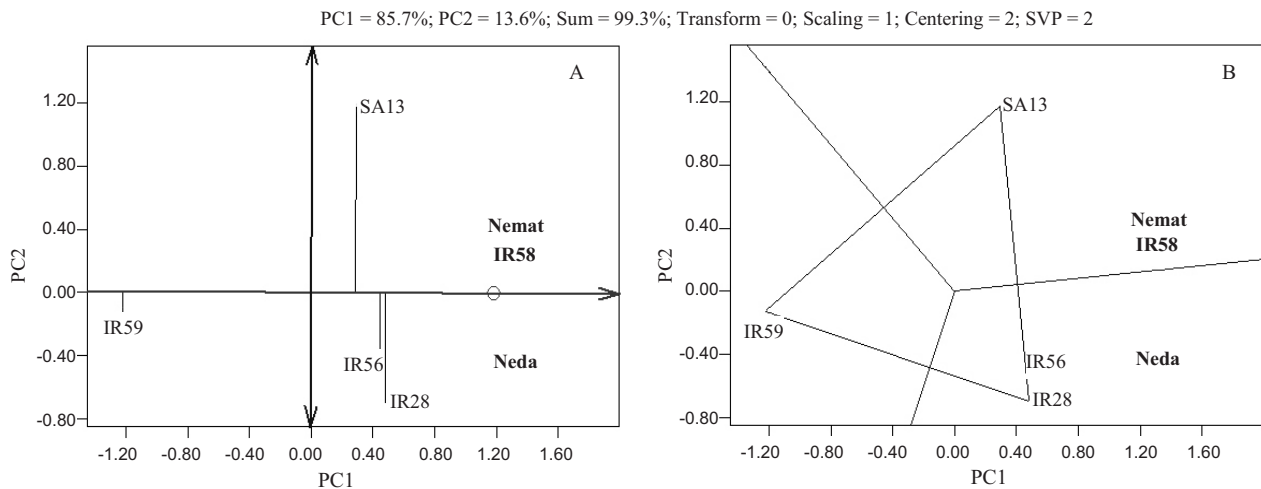


Fig. 2. Genotype plus genotype × environment (GGE) biplot for gelatinization temperature showing average tester coordinate view (A) and polygon view (B) of three female and four male rice parents.

Parents showed with bold and regular letters are testers and entries, respectively.

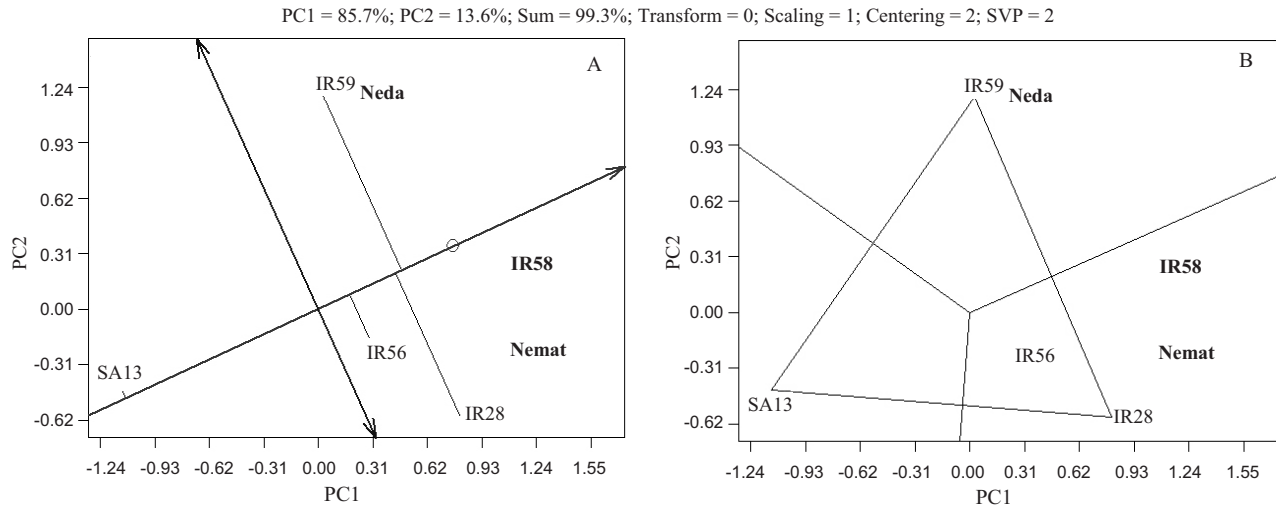


Fig. 3. Genotype plus genotype \times environment (GGE) biplot for gel consistency showing average tester coordinate view (A) and polygon view (B) of three female and four male rice parents.

Parents showed with bold and regular letters are testers and entries, respectively.

could not be recommended as superior genotypes for breeding programs. Instead, it was useful to choose genotypes that were in the second order regarding GCA or SCA values. In other words, considering Figs. 1-B, 2-B and 3-B, it was better to select genotypes located in different sectors as parents for crosses. For example, the crosses Neda \times IR28, Nemat \times IR59, and Nemat \times SA13 would be suitable for breeding AC, whereas the crosses Neda \times IR59, Nemat \times IR59, and IR58 \times IR59 would be suitable for breeding GT. Also, the crosses Neda \times IR59, Nemat \times IR28, Nemat \times IR56, Nemat \times IR59 and IR59 \times IR58 would be suitable for breeding GC. Considering the above results together, Nemat \times IR59 was selected as the best cross for breeding all the three assessed traits.

DISCUSSION

Enhancement of rice cooking quality is one of the major focused areas because it has a direct impact on consumer preference and thus sale and consumption of this product.

To achieve the desired enhancement in quality, genetical improvement of the crop is important, and thus various breeding techniques are used such as diallel, line \times tester. Improvement in crops can easily be achieved by using good combiners in hybridization programs followed by successive cycle of selection (Rastogi et al, 2011).

In this study, we used GGE biplot method for analysis of line \times tester data. So far, most of the

application of this method has been used for analysis of diallel data sets. However, there are differences between these two approaches. In diallel data, genotypes are both entries and testers in the entry \times tester classification and this is the main difference to line \times tester data (in line \times tester data, entries and testers are different). For the same reason, GGE biplot analysis is not appropriate for diallel data. The method essentially estimates the same GCA effects twice: The first time when subtracting tester and entry main effects from the data and the second time by suitable projections in the biplot (Möhring et al, 2011). Also, in a diallel analysis, the row and column main effects are identical, representing GCA effects, so it is wasteful to estimate row and column main effects separately. Therefore, the GGE biplot method is more compatible with line \times tester data which are entirely consistent with MET data.

To examine the validity of the biplot approach, our interpretations based on the biplots were compared with the results derived from the Kempthorne's line \times tester analysis method, which has been published before (Maleki et al, 2014). The biplot predictions are consistent with the conventional method. For example, the GCA values from biplot analysis and Kempthorne's method are entirely similar. However, the advantage of the biplot is its graphical presentation of the data, which enhances our ability to understand patterns in the data (Yan and Hunt, 2002). For example, the heterotic crosses as well as the best hybrids are easily distinguishable in a polygon view of the biplot.

Conversely, in the conventional method of line \times tester analysis, the parents are evaluated only on their GCA effects. The term SCA is associated with crosses and has little impact on the understanding of the parents (Yan and Hunt, 2002).

In this study, because the most appropriate crosses were not necessarily heterotic, the traditional method faces some difficulties in selecting suitable parental lines. In fact, the best parents cannot be distinguished regardless of progenies, and thus AC, GT and GC need to be measured in the hybrid lines as well as in the parental lines. Unlike the traditional method, the GGE biplot method does not need to measure characteristics in hybrid lines, and is better to identify suitable parents because of its graphical presentation.

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