

Yield Stability of Contrasting Orchardgrass (*Dactylis Glomerata* L.) Genotypes Over the Years and Water Regimes

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2 **genotypes over the years and water regimes**

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1 **Abstract**

2 Stability of combining ability and the nature and extent of genetic and genotypexenvironment
3 interaction is poorly understood in orchardgrass especially under climate change conditions. In the
4 present study, first-generation half-sib families of orchardgrass derived from the polycross of 25
5 parental genotypes were evaluated in the field during five years under two irrigation regimes of
6 normal and water deficit. Considerable genotypic variation was observed among half-sib families for
7 all of the evaluated traits, demonstrating high potential for improving these traits through half-sib
8 mating. The effects of water deficit on dry forage yield increased from the first to fifth year and
9 consequently declined the persistence of half-sib families. Results showed that both genetic and non-
10 genetic gene actions played a role in the control of dry forage yield; indicating that selection based on
11 an index would be more useful to attain genetic progress. Moreover, the estimates of narrow-sense
12 heritability for most of the traits were higher under deficit irrigation, which is advantageous for
13 successful selection. The stability of general combining ability, plant productivity, and drought
14 tolerance clearly identified G4, G5, G6, and G14 as the superior and stable parental genotypes able to
15 transmit both stability and forage productivity to their progenies.

16 **Keywords:** Combining ability- GGE biplot- orchardgrass- polycross mating- synthetic variety.

17

18 **Introduction**

19 Growth and productivity of plants are affected by a wide range of biotic and abiotic stresses such as
20 severe temperatures, excessive light, nutrient deficiencies, salinity, drought, and cold (Chaves et al.
21 2009; Crisp et al. 2016). Among them, drought is the most significant and acute problem which
22 adversely affects growth, survival, persistence, and productivity of plants in arid and semi-arid

1 regions of the world (Cai et al. 2011; Ciais et al. 2005) and is expected to become increasingly
2 important due to desertification and climate change (Cullen et al. 2009; IPCC 2014).
3 Effective adoption of perennial forage grasses as an alternative to annuals depends on their ability to
4 survive successive summer droughts and retain forage productivity, which is defined as persistence
5 (Annicchiarico et al. 2011). Under drought stress conditions, perennial forage grasses such as
6 orchardgrass can be valuable alternative to annuals because they offer higher optimization of
7 available water due to higher and faster growth at the beginning of autumn rains and more efficient
8 exploitation of residual soil water in late spring. The ecophysiological bases of persistence in
9 orchardgrass are relatively well known; but the genetic determinism of this persistence is not so well
10 known.

11 In most forage species, half-sib (HS) matings, including polycross and open-pollination are
12 widely used for estimation of general combining ability of parental clones for the development of
13 synthetic cultivars, the nature and relative extent of genetic variability and heritability in quantitative
14 genetic studies (Araujo et al. 2002; Aastiveit and Aastiveit 1990). Estimates of variance components
15 are used to determine the proportion of total genetic variance due to additive genetic effects
16 (heritability) and predict genetic gain from selection. Since the genetic variance among HS families is
17 equal to the covariance between HS individuals and to one-fourth of the additive genetic variance
18 (assuming there is no additive x additive types of epistatic variance), estimation of heritability based
19 on analysis of HS family gives a good prediction of narrow-sense heritability (h^2_n) (Nguyen and
20 Sleper 1983; Amini et al. 2013).

21 As a multi-layered factor, drought stress causes genotype by environment (GE) interactions.
22 Therefore, understanding genotype \times environment interaction (GEI) is an essential and challenging
23 issue among plant breeders and agronomists. GEI usually hinders the accuracy of yield estimation,

1 reduces the association between genotypic and phenotypic values, leads to bias in the estimation of
2 heritability and in the prediction of genetic advance, and complicates the process of selecting
3 genotypes with superior performance (Gauch 2006; Oral et al. 2018). Different methods have been
4 used to study and interpret G×E interaction. These methods include univariate linear regression
5 models (Finlay and Wilkinson 1963; Eberhart and Russell 1966) and multivariate models such as
6 Additive Main effects and Multiplicative Interactions (AMMI) (Zobel et al. 1988) and
7 Genotype×Genotype-Environment interaction (GGE) biplot (Yan 2001). Among these, GGE biplot is
8 more interpretative and has been recognized as preferred tool to analyze and visualize the pattern of
9 GEI in multi environment studies (Brar et al. 2010; Jandong et al. 2011). GGE biplots have been
10 identified as an effective tool in mega-environment analysis, genotype and environment evaluation,
11 trait-association and trait-profile analyses, and heterotic pattern analysis (Amira et al. 2013; Zhang et
12 al. 2016; Teodoro et al. 2015). They have thus been increasingly used in GE interaction studies in
13 plant breeding (Samonte et al. 2005; Dehghani et al. 2006; Kaya et al. 2006; Blanche et al. 2007).
14 However, there has been no report of its application to orchardgrass so far.

15 Little information is available about the nature and extent of GE interactions effects using HS
16 families in cool season grasses. Such information could simplify selection of parents able to transmit
17 high persistence, forage yield, and stability under drought to develop new synthetic cultivars.
18 Accordingly, this study attempted to i) assess the genetic basis of agro-morphological traits,
19 persistence, and drought tolerance of Iranian and foreign HS families of orchardgrass derived from
20 polycross, ii) estimate general combining ability for dry forage yield of parental genotypes of
21 preceding HS families and evaluate its stability in order to select superior genotypes, iii) visually
22 assess the adaptability and forage yield stability of orchardgrass families across 10 environments
23 based on the GGE biplot in order to facilitate comparison among HS families and environments and

1 iv) identify suitable families in terms of drought tolerance, persistence, and stability for future
2 breeding studies.

3

4 **Materials and Methods**

5 **Experimental site**

6 This research was conducted during five years (2010–2014) on a Typic Haplargid, silty clay loam soil
7 at the research farm of the Isfahan University of Technology, located in Lavark, Najaf-Abad, Isfahan,
8 Iran (32° 30' N, 51° 20' E; 1630 m amsl). According to the classification of Koppen, this region had
9 a semi-arid and cold climate with dry summers and had a mean annual rainfall and temperature of
10 140 mm and 14.5 °C, respectively. In this region, there is no rain during the summer (from late May
11 to mid-October) and supplemental irrigation is needed for growing crops during this period.

12

13 **Plant materials**

14 In this study 25 HS families derived from the polycross of 25 parental genotypes of orchardgrass
15 were used as genetic material (Table 1). The parental genotypes of the polycross were selected based
16 on agronomical and morphological data from previous studies. To produce half-sib (HS) progeny
17 families of these parental plants, they were polycrossed in an isolated nursery with eight replications.
18 In June 2009, seeds from each genotype were harvested per replication and mixed together in equal
19 proportions. A random sample was taken out in the bulked seed lot of each parental genotype to grow
20 25 HS families. Polycross seeds were grown in plastic boxes in a greenhouse during the winter of
21 2010. In March of 2010, the established and uniform seedlings were space planted in the field
22 according to a randomized complete block design with four replications. In each plot, 20 seedlings of

1 a given family were planted in two rows with 40 cm apart and 40 cm between plants within row. The
2 distance between neighboring plots was 60 cm.

3

4 **Evaluation of HS families**

5 After establishment of HS families, one half of the replications were assigned to the normal irrigation
6 and the other half were allocated to deficit irrigation for five years (during the 2010-2014 growing
7 seasons). Under the normal and deficit irrigations, water was supplied when 50% and 85% of the total
8 available soil moisture (field capacity) was depleted from the root zone respectively, according to the
9 methods of determination of evapotranspiration proposed by Allen et al. (1998). Water deficit
10 treatment was continuously applied during the growing season (from the first of May to the first of
11 October) of each year. To determine the amount of irrigation water needed to restore the soil moisture
12 to the field capacity and determine the irrigation times, soil samples were taken from different sites of
13 each irrigation regime before irrigation at depths of 0–20, 20–40, and 40–60 cm, using a hand auger
14 and the gravimetric soil–water content was measured (Clarke Topp et al. 2008). A volumetric counter
15 was used for measurement of the supplied water for each irrigation regime.

16 The following plant measurements were undertaken during five years. Days to panicle
17 emergence (DPE) and days to anthesis (DA) were recorded as the time from March 1st until
18 appearance of three panicles in each plant and onset of pollen shedding, respectively. At full anthesis,
19 the distance from the plant base to the top of the three tallest panicles was measured and considered
20 as plant height (PHT). Number of fertile stems per plant (NS) was recorded at the pollination stage.
21 Crown diameter (CD) was measured as the width of plant basal cover remaining after the first cut.
22 After the complete flowering (about early summer), the produced forage of each HS family was
23 harvested by cutting the grass from 5 cm above the ground and the harvested materials were then

1 dried at 75°C for 48 h. The dry forage yield per family (DFY1) was then computed. To assess
2 complete growth, a second cut was undertaken in late summer and the dry forage yield was recorded
3 (DFY2) following the same protocol as for the first cut. Percentage of dry matter in forage yield was
4 calculated at each cut (PDFY1 and PDFY2). The drought tolerance index (STI) (Fernandez 1992)
5 was calculated based on the DFY (g plot⁻¹) under normal and deficit irrigation regimes using the
6 following formulae:

$$7 \text{ STI} = (Y_{si} \times Y_{pi}) / (Y_{mp})^2$$

8 where Y_{si} is the yield of the i th family in the deficit irrigation, Y_{pi} is the yield of the i th family in the
9 normal irrigation, and Y_{mp} is the yield mean over all families in the normal irrigation. The
10 persistence (PER) of HS families was calculated on the combined data of two irrigation regimes by
11 subtracting the DFY of first cut in the second year (2014) from the DFY of first cut in the fifth year
12 (2011).

13

14 **Statistical analyses**

15 In the stability analysis, a combination of a single year and irrigation regime was considered as the
16 test environment; thus, 10 test environments were created (Online Resource 1). The Bartlett's test and
17 Kolmogorov–Smirnov method were used to examine the homogeneity of residual variance and
18 normality distribution of data, respectively. Combined analysis of variance was performed to examine
19 the effect of the irrigation regimes, years, families, and their interactions, using Proc GLM of SAS
20 release 9.4 (SAS Institute, Cary, NC, USA). As the experiment was conducted for 5 years in two
21 irrigation regimes, a split-plot in time (year) model was used for this analysis as proposed by Steel
22 and Torrie (1980) and Nguyen and Sleper (1983). The effects of irrigation regime were considered as

1 fixed, and year was considered as random effect. Mean comparison was conducted using the LSD test
2 at $P < 0.05$ (Steel and Torrie 1980).

3 Data were also subjected to ANOVA separately for normal and deficit irrigation across five years
4 using a split-plot in time model with HS families as the main plots and years as subplots. Variance
5 components were estimated for individual irrigation regime (normal and water deficit conditions)
6 from mean squares of the ANOVA after being equated to their expected variance components
7 (Nguyen and Sleper 1983). The estimation of narrow-sense heritability was calculated on the basis of
8 family means for normal and deficit irrigation regimes, using the following equation (Nguyen and
9 Sleper 1983):

$$10 \quad h_{nHS}^2 = \frac{\sigma_f^2}{\sigma_f^2 + \frac{\sigma_{fy}^2}{y} + \frac{\sigma_{fr}^2}{r} + \frac{\sigma_e^2}{yr}}$$

11 where h_n^2 is the narrow-sense heritability, σ_f^2 is the family variance, σ_{fy}^2 is the family \times year variance,
12 and σ_e^2 is the residual variance, while y and r represent the number of years and replications,
13 respectively. The level of genetic variation was estimated with the calculation of genotypic
14 coefficient of variation (GCV) as follow:

$$15 \quad GCV = (\sigma_g / \mu) 100$$

16 where σ_g is the square root of the genotypic variance and μ is the phenotypic mean. General
17 combining ability (GCA) was calculated on the combined data of two irrigation regimes as the
18 deviation of each HS family from the population mean as defined by Wricke and Weber (1986).

19

20 **Stability analysis**

1 In the present study, analysis of adaptability and phenotypic stability was conducted by the GGE
2 biplot, using the model outlined by Yan (2002). The data were graphically analyzed for interpreting
3 the GE interaction to identify stable and adaptive HS families by the GGE biplot, as described by Yan
4 and Tinker (2006). This methodology uses a biplot to show the factors (G and GE) that are important
5 in genotype evaluation and that are also the sources of variation in GE interaction analysis of MET
6 (multi-environmental trial) data (Yan et al. 2000, 2001). For this purpose, the first two components
7 that resulted from singular value decomposition (SVD) using MATLAB programming language were
8 used to draw the desired biplots with Microsoft Office Excel 2016. The remaining PCs were regarded
9 as residuals (Yan and Tinker 2006).

10

11 **Results**

12 **Analysis of variance and genetic analysis**

13 Results from the ANOVA showed that there were significant differences between the normal and
14 deficit irrigation regimes for all of the measured traits except for DPE and DA. The effect of family
15 was significant for all traits, indicating significant variation among the selected families. Family \times
16 irrigation regime effects were also significant for most of the traits (Tables 2 and Online Resource 2).
17 Moreover, significant differences were observed among HS families from Iranian parental genotypes
18 on one hand and from foreign parental genotypes on the other hand for most of the studied traits.
19 Meanwhile, the Iranian vs. foreign effect was only significant for PDMY1 and DFY (Tables 2 and
20 Online Resource 2). The environment effect was a predominant source of variation which explained
21 58.6% of total variation, whereas G and GE captured 4.2% and 21.5%, respectively (Table 2). The
22 partitioning of GGE through GGE biplot analysis showed that the first two PC, obtained by singular

1 value decomposition, accounted for 65% and 11% of the GGE sum of squares respectively,
2 explaining a total of 76% variation (Figs. 2-6).

3 Mean comparisons showed that under the drought stress condition, the mean performance was
4 decreased for some of the evaluated traits (PH, NS, CD, DFY1, and DFY2), while it was significantly
5 increased for the remaining traits (DPE, DA, PDFY1, and PDFY2) during 2010-2014 (Table 3). As
6 expected, drought stress strongly reduced dry forage yield of cut 1 (DFY1), dry forage yield of cut 2
7 (DFY2), and mean dry forage yield (MDFY) during five consecutive years of experiment. Compared
8 with normal condition, drought stress approximately reduced DFY1 by 22, 30, 65, 69, and 30%
9 during 2010-2014. For DFY2, these reductions were approximately 17, 45, 62, 65, and 42%, in the
10 same five years relative to normal condition (Table 3). To assess drought tolerance of the
11 orchardgrass families, the STI index was calculated. The highest values of STI were obtained for F4
12 (from Hungary), F18 (from Hungary), and F7 (from Isfahan, Iran), and the lowest values were
13 detected for F3 (from Najafabad, Iran), F12, and F16 (both from Isfahan, Iran) (Table 1; Fig. 1a).
14 Moreover, prolonged water deficit was applied in five consecutive years and reduced the persistence
15 of HS families (data not shown). Based on the biplot depicted in Figure 1a, the higher values of
16 persistence were observed for HS families F23 (from Isfahan, Iran), F11 (from Hungary), F12 (from
17 Isfahan, Iran), F21, and F22 (both from Hungary), respectively. The families of F6, F18 (both from
18 Hungary), F7 (from Isfahan, Iran), and F15 (from Semnan, Iran) had the lower persistence,
19 respectively (Fig. 1a).

20 For most of the evaluated traits, the genetic variation was higher under deficit irrigation than
21 under normal irrigation. Based on GCV, highest range of genetic variation was observed for NS and
22 relatively lower one was detected for PDFY2 and DA (Table 4). The estimates of narrow-sense
23 heritability and variance components for each irrigation regime are displayed in Table 3. According

1 to the results, heritability estimates ranged from 14.33% for DFY2 to 57.36% for DA in normal
2 irrigation and from 25.66% for DFY2 to 75.35% for DPE in deficit irrigation. Higher heritability
3 estimates were obtained under deficit irrigation for most of the studied traits. Moreover, in both the
4 normal and deficit irrigation regimes, the heritability of yield components (such as PH, NS, and CD)
5 was higher than that of forage yield (Table 4).

6 In the present study, HS families showed a wide range of general combining ability (GCA) for
7 DFY (Table 1). The highest GCA was observed for the parental genotypes F3 (from Najafabad, Iran),
8 and F7 (from Isfahan, Iran) (Table 1). Therefore, these genotypes are good combiners for forage yield
9 and may be suggested for developing synthetic varieties. Parental genotypes F18 (from Hungary),
10 F16 (from Isfahan, Iran) and F15 (from Semnan, Iran) had relatively low GCA for DFY and, hence,
11 are not good combiners. GCA had a significant and positive correlation with STI (Fig. 1a); therefore
12 it is possible to identify genotypes having high values of combining ability and drought tolerance at
13 the same time. In this respect, some families including F4 (from Hungary) and F7 (from Isfahan,
14 Iran), were identified as superior (Fig. 1b).

15

16 **Stability analysis based on GGE biplot**

17 In this study, GE interaction effect was highly significant and explained 21.55% of total variation of
18 DFY (Table 2). As significant GEI complicates the selection process through minimizing the
19 association between genotypic and phenotypic values; therefore, there is a need for the stability
20 analysis. The GGE biplot analysis showed that the first two PC accounted for 65% and 11% of the
21 G+GE sum of squares respectively, explaining a total of 76% variation (Figs. 2-6).

22 To effectively visualize the “which-won-where” pattern of the interaction between HS families
23 and environments and also to interpret their biplot for DFY, the polygon view of the GGE biplot is

1 shown in Figure 2. In the polygon biplot analysis, the families located on the vertices of the polygon,
2 i.e. F3, F5, F11, F13, and F18, are the best or worst ones in one or more environments, because they
3 have the largest distances from the biplot origin in their direction and therefore, are considered
4 specifically adapted (Yan et al. 2007). Moreover, the family F3 was the highest yielding family at the
5 environments E4 and E10; family F5 produced the highest yield at environments E6 and E8; F11
6 showed high yield at E5 and E7; and family F18 was identified as the winner family at E1, E2, E3,
7 and E9, because these environments were located in their related sections, respectively. The vertex
8 family F13 was not the highest yielding family at any environment, because it fell in the section
9 where there was not any environment at all (Fig. 2).

10 The mean forage yield and stability performance of HS families are graphically visualized
11 through the average environment coordination (AEC) method (Fig. 3). In this method, the abscissa
12 and the ordinate axes of the AEC are defined by the average PC1 and PC2 of all environments to
13 approximate the mean forage yield and stability performance of HS families. Projections of HS
14 families onto the AEC abscissa approximate the mean forage yield of the families. The families
15 farthest from the origin would have higher (on the positive side of the AEC abscissa) or lower (on the
16 negative side of the AEC abscissa) mean forage yield. According to Fig. 3, the families were divided
17 into two groups. The first group, with above-average performance, included F15, F10, F1, F9, F13,
18 F2, F4, F6, F7, F18, F8, F11, F14, and F5, respectively. The second group that included the
19 remaining families had below average performance. An ideal genotype should have the highest mean
20 performance of all genotypes and be absolutely stable to show wide adaptability in the wide range of
21 environments (Sharma et al. 2010; Akcura et al. 2011). Therefore, considering both forage yield and
22 stability performance, F6 and F14 were more stable as well as relatively high yielding in terms of
23 forage and could be regarded as the most favorable HS families.

1 The performance of the family in the respective environment can be determined based on the
2 angle between the vector of each HS family and the vector of each environment (Yan and Tinker
3 2006). The angle of less than 90° shows the better performance than the average; the angle of more
4 than 90° indicates poorer performance than the average; and the angle of about 90° demonstrates that
5 the family performs near the average. For example, in E3, E6, E8, and E9 the families F2, F4, F5, F6,
6 F7, F8, and F14 showed performance better than the average, the families F1, F9, F10, F11, F13, F22,
7 and F25 performed near the average, and the families F3, F12, F15, F19, F20, F21, and F24 had
8 poorer performance (Fig. 4).

9 The GGE biplot analysis allows comparison among genotypes in respect to a reference genotype
10 named “ideal genotype”. An ideal genotype is defined as one that is the highest yielding across all
11 environments and is absolutely stable in performance (Yan and Tinker 2006). In this context, the
12 desirable genotypes are those located close to the ideal genotype on the biplot. According to the ideal-
13 genotype view (Fig. 5), families F14, F5, F6, and F4 are positioned closest to the ideal genotype on
14 the biplot and could be considered as desirable HS families. These families seem to be widely
15 adapted across environments. On the other hand, the lower yielding families i.e. F3, F21, F12, F24,
16 and F19, are unfavorable ones; because they are located far away from the ideal genotype,
17 respectively (Fig. 5).

18 The discriminating power and representativeness view of the GGE biplot is indicated in Figure 6.
19 In the biplot, the environments having longer vectors have more discriminating power of the
20 genotypes and vice versa (Yan et al. 2007). Another important feature of an environment is its
21 representativeness of the other environments, which is measured based on the angle between the
22 environment vector and the “average environment coordinate” (AEC) axis. The environments which
23 have small angle with the AEC are more representative of other environments. In this study, E3, E7,

1 and E5 had the long vectors and therefore were the most discriminating environments (Fig. 6).
2 Among them, two environments of E5 and E7, which had wide angles with the AEC, were not
3 representative of the other environments and therefore, not useful for selecting the superior HS
4 families. On the other hand, E10 with the shortest vector length was the least discriminating
5 environment; and the environments E9, E6, and E8 were in the next ranks, respectively; and therefore
6 provide little or no information about the families' variability (Jalata 2011; Yan and Tinker 2006).
7 Environments E8, E6, E9, and E3 with the lower angles from the AEC axis were the most
8 representative environments, respectively. Among these environments E6, E8, and E9 were low
9 discriminative environments, due to the short vector length. However, the E4 environment was the
10 least representative environment due to the wider angle with AEC axis, followed by E10 and E1.
11 Environment E8 had relatively high discriminating power and representativeness. Therefore, this
12 environment can be used to effectively select superior HS families which can perform consistently
13 best across all of the environments.

14

15 **Discussion**

16 Combined analysis of variance indicated that all the main effects (families, irrigation regimes, and
17 years) and all of the interactions significantly influenced DFY and all of the other traits evaluated in
18 the present study. Significant variation among HS families revealed that there is considerable
19 genotypic variation for forage yield and its components. The significant GE interaction demonstrated
20 the different response of genotypes to environmental variations. Therefore, it was possible to proceed
21 and calculate phenotypic stability. The GE effect was about five times greater than the G effect of the
22 total variation, suggesting the possible existence of different mega environments with different top-
23 yielding genotypes (Yan and Kang 2003). This along with a highly significant GEI indicated the need

1 for the stability analysis. Moreover, the large yield variation due to environment, which is irrelevant
2 to cultivar evaluation and mega environmental investigation, justifies selection of SREG procedures
3 for analyzing the MET data (Segherloo et al. 2010). This is the reason that E is removed from the
4 observed phenotypic data, which helps to concentrate on genotype and GE, which are relevant for
5 genotype evaluation (Yan and Kang 2003; Fan et al. 2007).

6 Water deficit caused a significant reduction in all of the measured traits except for DPE and DA
7 in five years of study. As water deficit was applied each year from 1 May onwards, which coincided
8 exactly with the start of flowering and just before pollination, these results were expected and are
9 consistent with the findings of previous studies in orchardgrass (Saeidnia et al. 2017; Majidi et al.
10 2016) and smooth brome grass (Abtahi et al. 2019; Saeidnia et al. 2020). The region in which the
11 study was conducted (Najafabad, Isfahan, Iran) is a warm and dry area where the summer
12 temperature reaches as high as 45°C and precipitation is null. Therefore, reductions are expected in
13 most of the evaluated traits.

14 Genotypic coefficients of variation (GCV) for most of the measured traits under the deficit
15 irrigation regime were higher than their estimates under the normal one, which suggests that selection
16 under deficit irrigation may be more effective. The findings in this context are contradictory. For
17 example, some researchers stated that GCV and genetic gain through selection is higher under normal
18 irrigation than deficit irrigation (Blum 2011; Majidi et al. 2016). While others reported higher GCV
19 and genetic advance through selection under deficit irrigation (Abtahi et al. 2018; Saeidnia et al.
20 2019).

21 Estimation of heritability can be used for determining the influence of environmental and genetic
22 factors on the traits of interest for assessing the efficiency of selection. Estimates of h^2_n were higher
23 under deficit irrigation than at normal one for all of the traits. This may be due to the fact that

1 environmental changes can induce changes in gene expression and increase genetic variation of these
2 traits. Lower amounts of heritability for forage yield as the most economically important trait, results
3 in smaller expected gain by direct selection on this trait. In these cases, indirect selection of the traits
4 having high heritability and high correlation with forage yield can be promising (Blum 2011). This
5 was generally consistent with previous reports in smooth brome grass (*Bromus inermis* L.) (Araghi et
6 al. 2014) and orchardgrass (Jafari and Naseri 2007).

7 In grasses such as orchardgrass, due to self-incompatibility which results to cross-pollination,
8 developing synthetic varieties is the most common breeding method (Nguyen and Sleper 1983). In
9 order to select parental genotypes for a synthetic variety, information about GCA is desirable and can
10 be obtained from the analysis of HS progenies (Araujo et al. 2002). In the present study, HS families
11 showed a wide range of GCA for DFY. Araujo (2001) stated that large differences in GCA enable
12 effective selection of superior parents based on a polycross progeny test. As parental genotypes F3
13 and F7 had higher GCA values for DFY; the parental genotypes of these families could be considered
14 as superior parents in breeding programs.

15 The GE interaction has been an important and challenging issue among plant breeders,
16 geneticists, and agronomists engaged in performance testing. The presence of GEI complicates the
17 selection process as GEI reduces the usefulness of genotypes through minimizing the association
18 between genotypic and phenotypic values (Crossa et al. 1990). Therefore, breeding researchers are
19 always looking for high yield potential genotypes with low GE interactions.

20 According to “which-won-where” pattern of GGE biplot, the test environments could be
21 separated into four groups. Environments E1, E4, and E10 formed one group; which had least
22 discriminating power and was also least representative of other environments and therefore provide
23 little or no information about the families’ variability (Jalata 2011; Yan and tinker 2006). The second

1 group included environments E5 and E7 with more discriminating power and relatively low
2 representative of other environments. Environments E6, E8, and E9 marked the third group. These
3 environments had low discriminating power and hence provide little information about studied
4 families. However, these environments showed most representative of other environments. The two
5 remaining environments, i.e. E2 and E3, fell into the fourth group with more discriminating power and
6 relatively high representative of other environments. In total, environment E3 with relatively high
7 discriminating power and representative of other environments may be used to effectively select
8 superior HS families which can perform consistently best across all of the environments.

9 Results of present study indicated that GGE biplot could not separate the normal and water
10 deficit environments. The reasons for this observation could be ascribed to the amount of
11 environmental variation. In this study environment contributed to 58.6% of the total variation in the
12 data. Meanwhile Gauch and Zobel (1997) stated that in normal multi-location yield experiments,
13 location accounted for about 80% of the total variation. In barley (*Hordeum vulgare*) yield trials,
14 Dehghani et al. (2006) reported that more than 80% of variation was explained by environment.

15 In terms of mean performance, HS families were divided into two groups. In the first group,
16 which had above-average performance, two families of F6 and F14 were highly stable, whereas three
17 HS families of F11, F13, and F18 showed more variability (lower stability). Other families of this
18 group had moderate to low stability. In the second group with below-average performance, two HS
19 families of F20 and F24 had high stability and the other families showed moderate stability. From ten
20 foreign HS families used in this study, seven of them were placed in the first group and therefore,
21 most of the foreign families performed better than average. Two families of F6 and F14 with high
22 yield and highest stability can be considered as most desirable ones for different environments.

1 However, families of F11, F13, and F18 which had high yield and low stability were desirable for
2 specific environments.

3 An ideal genotype should have the highest mean performance of all genotypes and be absolutely
4 stable to show wide adaptability in the wide range of environments (Sharma et al. 2010; Akcura et al.
5 2011). In this respect, family F14 was identified as an ideal family, and families F5, F6, and F4 were
6 considered as most desirable families respectively, because they were located at the closest position
7 to the ideal family. Among them two HS families of F14 and F6 which had above-average
8 performances and also were located almost on the AEC abscissa and had near-zero projections onto
9 AEC ordinate, could be considered as the most stable and desirable families. These families have
10 broad adaptation to various environments. In contrast, two high-yielding families of F4 and F5 had
11 lower stability and therefore tended to be specifically adapted to certain environments.

12 In conclusion, high genotypic variation among HS families indicated the possibility to find and
13 select desirable parental genotypes for synthetic cultivar development with high stability and forage
14 productivity using GGE biplot analysis. Water deficit had negative effects on forage yield and its
15 components, and increased the genotypic variation of evaluated traits. Its effects on DFY were
16 increased from the first to fifth year of study; and its consequence manifested as decline in
17 persistence of HS families. However, some HS families with more drought tolerance and persistence
18 were identified in these populations. Since relatively low narrow-sense heritability was obtained for
19 DFY; both genetic and non-genetic effects play a role in the control of this trait. Therefore, selection
20 based on an index, which is a weighted linear combination of several traits, would be more effective
21 to achieve genetic progress in recurrent selection programs. Results of this study verified the
22 efficiency of the GGE biplot method for selecting stable, high yielding, and responsive families.
23 From analysis of HS families through GGE biplot method some families (F4, F5, F6, and F14) were

1 identified as stable ones. Among them F4 was more drought tolerant than other families. The parental
2 genotypes of these families can be suggested as superior parents for developing of synthetic varieties.
3 Moreover, crosses between contrasting genotypes would be a suitable option when planning for the
4 development of mapping population for genome studies of stability and drought tolerance in this
5 species.

6

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10

11 **Conflicts of interests**

12 The authors declare that they have no conflict of interest.

13

14 **Ethics approval**

15 Not applicable.

16

17 **Consent to participate**

18 Not applicable.

19

20 **Consent for publication**

21 Not applicable.

22

23 **Availability of data and materials**

24 The datasets analyzed during the current study are available from the corresponding author on
25 reasonable request.

26

27 **Code availability**

28 Not applicable.

1 **Authors' contributions**

2 FS, MMM and AM conceived and designed the experiments; FS performed the experiments,
3 analyzed the data and wrote the manuscript with the supervision of MMM and AM; MRD analyzed
4 the data; BH performed the experiments; all authors discussed the results and reviewed the
5 manuscript.

6

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Table 1- Codes, origin, general combining ability (GCA), stress tolerance index (STI), and persistence (PER) of 25 half-sib families of orchardgrass evaluated in five years at two irrigation regimes

Codes of families	Origin	GCA of parents (g per plant)	STI	PER (g per plant)
F1	Hungary	-2.86	0.605	-45.56
F2	Iran, Semnan	4.18	0.697	-56.92
F3	Iran, Najafabad	-15.20	0.467	-32.61
F4	Hungary	8.68	1.486	-47.89
F5	Hungary	8.53	0.921	-46.39
F6	Hungary	4.23	0.812	-70.65
F7	Iran, Isfahan	9.87	1.288	-52.99
F8	Iran, Kuhrang	7.12	0.685	-39.44
F9	Iran, Isfahan	-3.66	0.551	-32.38
F10	Netherland	6.13	0.683	-41.53
F11	Hungary	8.15	0.561	-21.10
F12	Iran, Isfahan	-9.92	0.497	-26.26
F13	Iran, Najafabad	8.81	0.950	-44.37
F14	Iran, Isfahan	5.37	0.647	-41.93
F15	Iran, Semnan	1.88	0.645	-51.59
F16	Iran, Isfahan	-1.14	0.505	-30.41
F17	Iran, Isfahan	-6.97	0.602	-31.50
F18	Hungary	0.58	1.318	-64.57
F19	Iran, Isfahan	-4.58	0.666	-42.58
F20	Iran, Semnan	-5.85	0.535	-37.91
F21	Hungary	-7.99	0.575	-27.82
F22	Hungary	-2.77	0.541	-28.89
F23	Iran, Isfahan	-4.39	0.577	-21.05
F24	Iran, Najafabad	-2.83	0.648	-40.20
F25	Hungary	-5.38	0.644	-48.31

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Table 2- Results of analysis of variance for forage yield of 25 half-sib families of orchardgrass evaluated in 10 environments (five years and two irrigation regimes).

Source of variation	df	Sum of squares	Mean squares	Total variation
Environment (E)	9	307181.94	34131.33 **	58.60%
Replication /E	10	8191.71	819.17	
Genotype (G)	24	29834.30	1243.10 **	4.22%
Iranian (I)	15	20150.98	1439.36**	
Foreign (F)	8	8290.91	921.21**	
I × F	1	1392.42	1392.42*	
G × E	216	112959.93	522.96 **	21.55%
Error	229	73740.53	307.25	
Total	488	524204.10		

* and ** show significance at the 0.05 and 0.01 probability levels, respectively.

n.s: not significant

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Table 3- Means of phenological and agronomic traits of 25 half-sib families of orchardgrass under normal and deficit irrigation during 2010-2014.

Traits	2010			2011			2012			2013			2014		
	Normal	Deficit	Change† (%)	Normal	Deficit	Change (%)	Normal	Deficit	Change (%)	Normal	Deficit	Change (%)	Normal	Deficit	Change (%)
DPE (day)	73.40	76.98	4.88**	40.61	35.17	-13.39**	44.25	32.85	-25.76**	46.60	35.20	-24.46**	73.06	66.68	-8.73*
DA (day)	80.73	84.30	4.42*	55.14	49.72	-9.83*	63.85	52.28	-18.12*	66.20	54.63	-17.48*	87.66	78.06	-10.95**
PH (cm)	85.15	79.32	-6.85 ^{ns}	85.06	75.18	-11.62*	82.69	68.13	-17.61*	83.19	64.23	-22.79**	-	-	-
NS	5.79	3.78	-34.72*	41.44	37.21	-10.21 ^{ns}	122.07	63.01	-48.38*	129.96	65.99	-49.22*	-	-	-
CD (cm)	13.78	11.33	-17.78**	25.33	21.41	-15.47**	23.77	19.29	-18.85**	24.75	20.27	-18.10**	24.28	21.54	-11.28**
DFY1 (g/plant)	82.91	64.58	-22.11*	106.67	74.68	-29.99*	98.38	34.92	-64.50**	102.87	31.85	-69.04**	58.33	41.11	-29.52*
PDFY1 (%)	36.26	46.57	28.43*	35.53	45.30	27.50*	42.69	50.11	17.38*	43.79	50.52	15.37*	37.30	47.16	26.43*
DFY2 (g/plant)	107.04	88.85	-16.99*	63.86	35.17	-44.93*	28.03	10.66	-61.97**	32.61	11.32	-65.29**	30.27	17.58	-41.92*
PDFY2 (%)	33.11	42.05	27.00*	35.23	40.33	14.48*	39.84	45.85	15.08*	42.46	50.02	17.80*	30.11	37.52	24.61*

DPE, days to panicle emergence; DA, days to anthesis; PH, plant height; NS, number of stems per plant; CD, crown diameter; DFY1 and 2, dry forage yield of cuts 1 and 2; PDFY1 and 2, percentage dry forage yield of cuts 1 and 2.

† The test of null hypothesis for 'Change' is computed from comparison means based on an ANOVA per year.

Table 4- Estimates of variance components, narrow-sense heritability (h^2_n), and genetic coefficient of variation (GCV) of measured traits in 25 half-sib families of orchardgrass under normal and deficit irrigations during 2010-2014. These results come from the combined ANOVA per irrigation regime.

Traits	Normal irrigation					Deficit irrigation					GCV	
	σ^2_f	σ^2_{fy}	σ^2_e	σ^2_p	h^2_n	σ^2_f	σ^2_{fy}	σ^2_e	σ^2_p	h^2_n	Normal	Stress
DPE	3.45	3.20	16.07	7.62	45.27	9.44	2.43	18.55	12.53	75.35	3.34	6.22
DA	6.54	2.73	15.42	11.41	57.36	6.37	1.17	15.30	11.22	56.77	3.62	3.96
PH	6.88	0.46	35.00	20.28	33.93	7.71	-0.70	32.39	20.13	38.32	3.12	3.87
NS	55.59	-2.54	664.70	202.49	27.45	96.30	40.26	267.35	185.32	51.97	9.97	23.09
CD	0.07	0.10	1.04	0.22	32.33	0.14	0.12	0.66	0.22	62.88	1.18	1.99
DFY1	76.35	458.88	1201.62	414.70	18.41	14.23	41.34	165.47	53.10	26.80	9.73	7.63
PDFY1	3.62	4.72	56.34	14.74	24.53	5.54	7.07	25.50	11.45	48.38	4.86	4.91
DFY2	7.40	44.46	119.11	51.66	14.33	9.43	24.65	96.66	36.74	25.66	5.20	9.39
PDFY2	0.66	6.35	8.21	3.66	18.09	5.52	-1.30	67.01	18.74	29.46	2.25	5.44

DPE, days to panicle emergence; DA, days to anthesis; PH, plant height; NS, number of stems per plant; CD, crown diameter; DFY1 and 2, dry forage yield of cuts 1 and 2; PDFY1 and 2, percentage dry forage yield of cuts 1 and 2.

σ^2_f , family variance; σ^2_{fy} , family \times year variance; σ^2_e , error variance; σ^2_p , phenotypic variance; h^2_n , narrow-sense heritability; GCV, genetic coefficient of variation. Negative values of variance components assumed to be zero for estimation of heritability.

1 **Figure captions**

2 **Fig. 1-** Biplots of stress tolerance index (STI) vs (a) general combining ability (GCA) and (b)
3 persistence (PER) for 25 families of orchardgrass. The red codes refer to foreign families and the
4 black codes to Iranian families

5 **Fig. 2-** ‘Polygon’ view of the GGE biplot to show which family performed better in which
6 environment for forage yield of orchardgrass

7 **Fig. 3-** GGE biplot showing the ranking of HS families of orchardgrass based on yield performance
8 and stability

9 **Fig. 4-** GGE biplot showing the performance of each HS families of orchardgrass in each
10 environment

11 **Fig. 5-** Comparison of HS families of orchardgrass against the position of an ‘ideal’ genotype for
12 forage yield and stability of performance across the environments

13 **Fig. 6-** GGE biplot showing the ranking of HS families of orchardgrass based on yield performance
14 and stability

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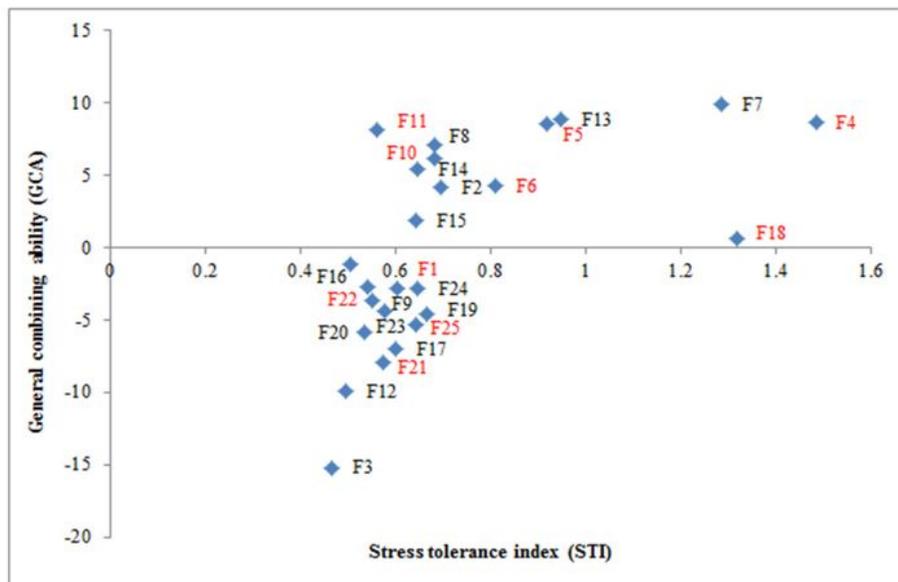
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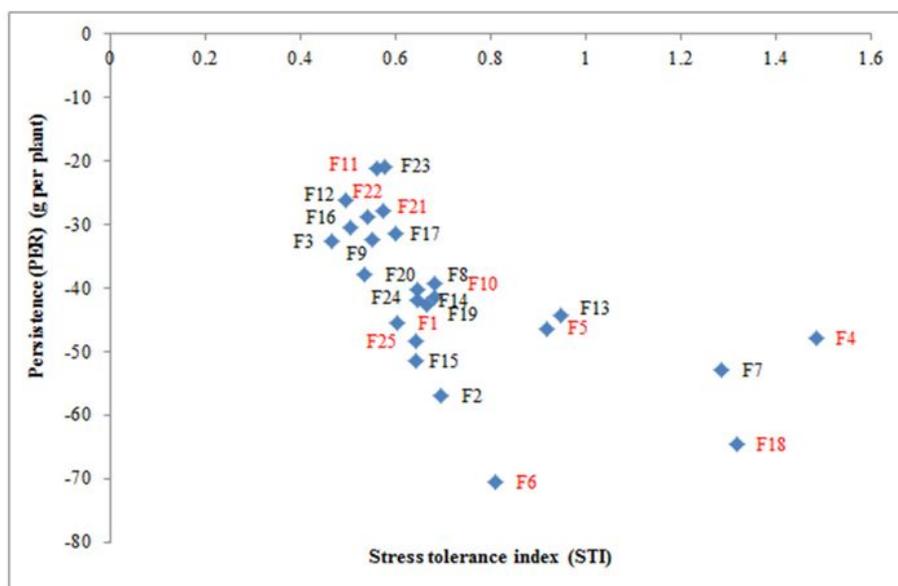
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Figures



(a)



(b)

Figure 1

Biplot of stress tolerance index (STI) vs (a) general combining ability (GCA) and (b) persistence (PER) for 25 families of orchardgrass. The red codes refer to foreign families and the black codes to Iranian families

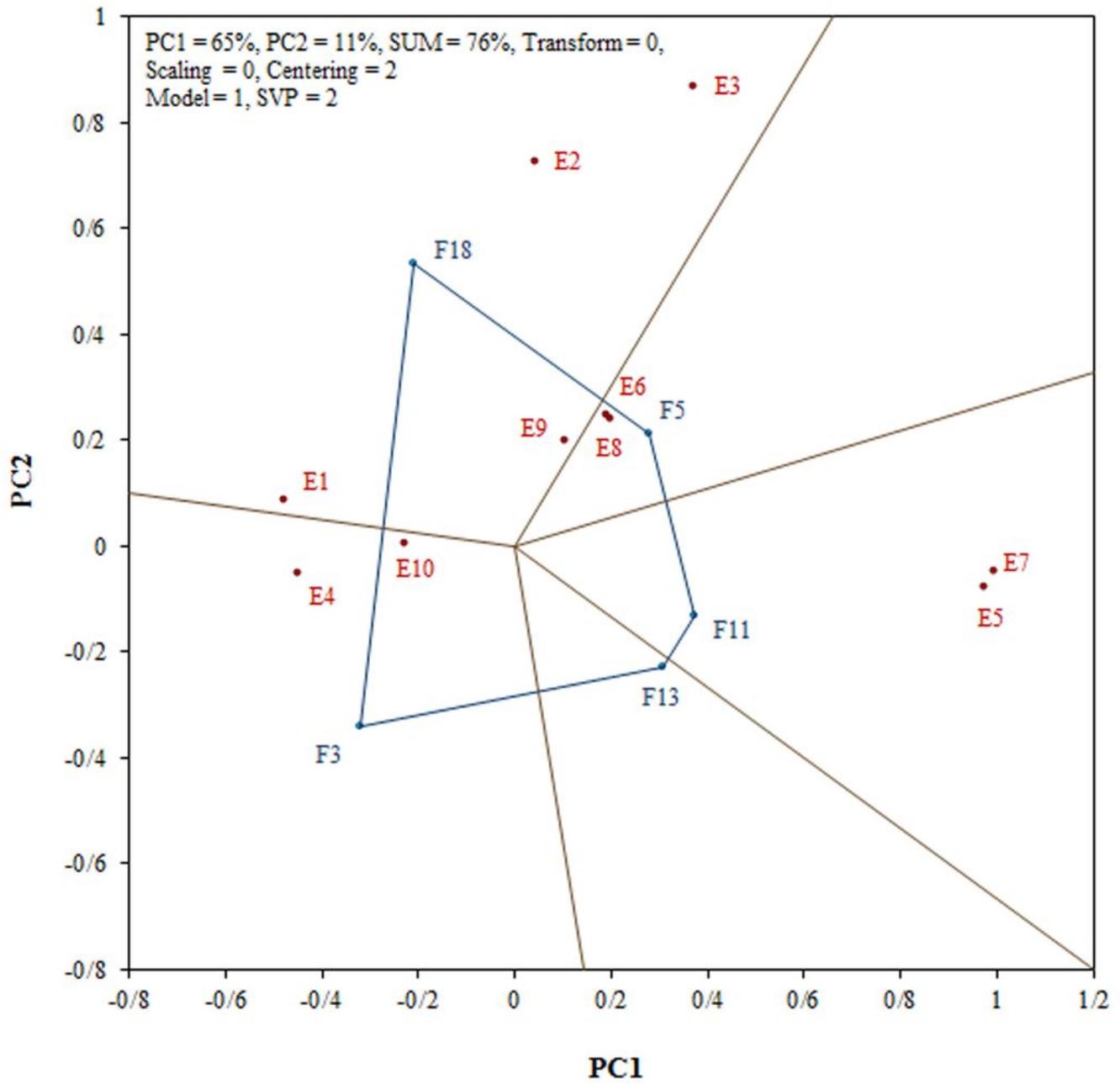


Figure 2

'Polygon' view of the GGE biplot to show which family performed better in which environment for forage yield of orchardgrass

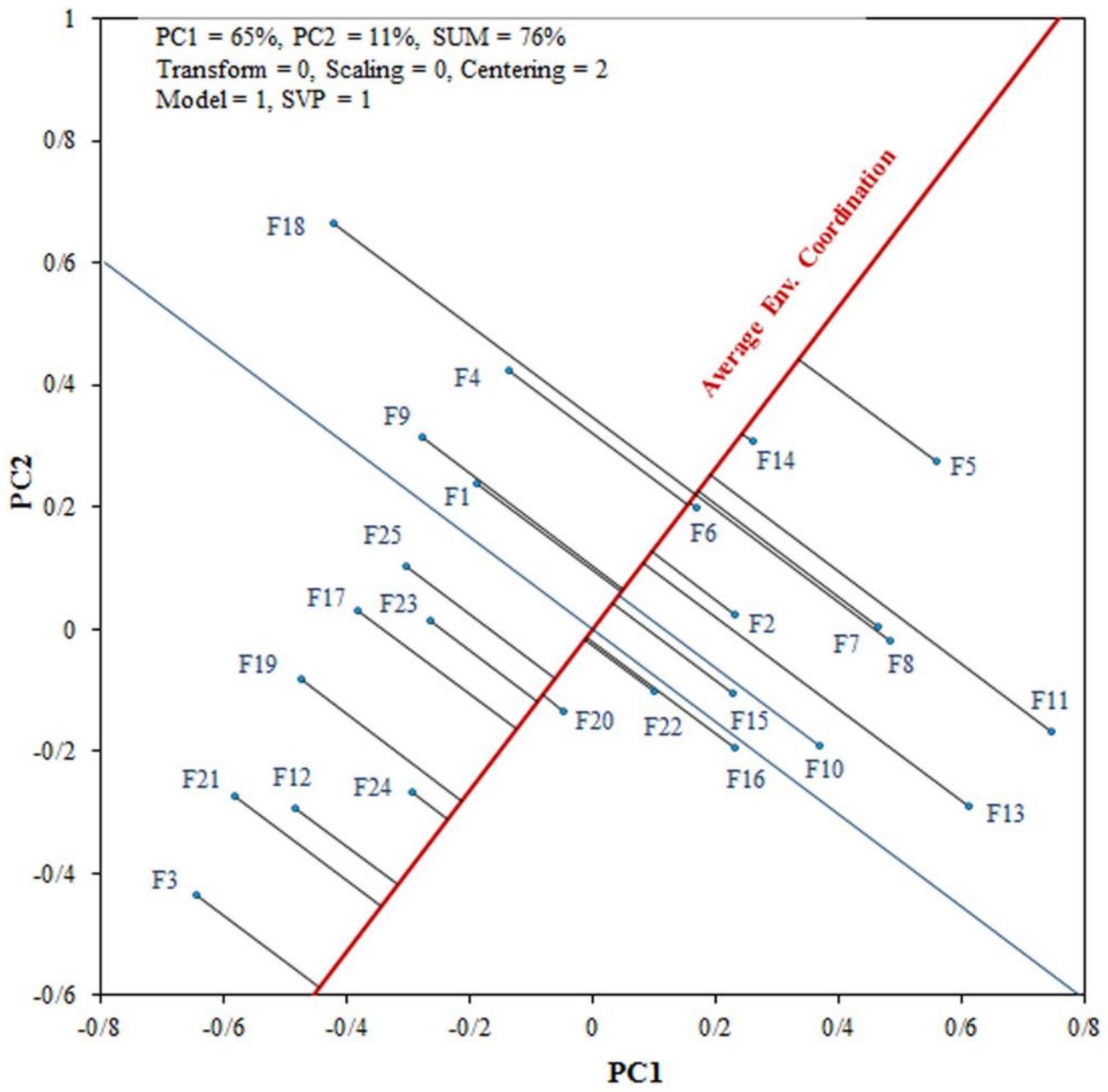


Figure 3

GGE biplot showing the ranking of HS families of orchardgrass based on yield performance and stability

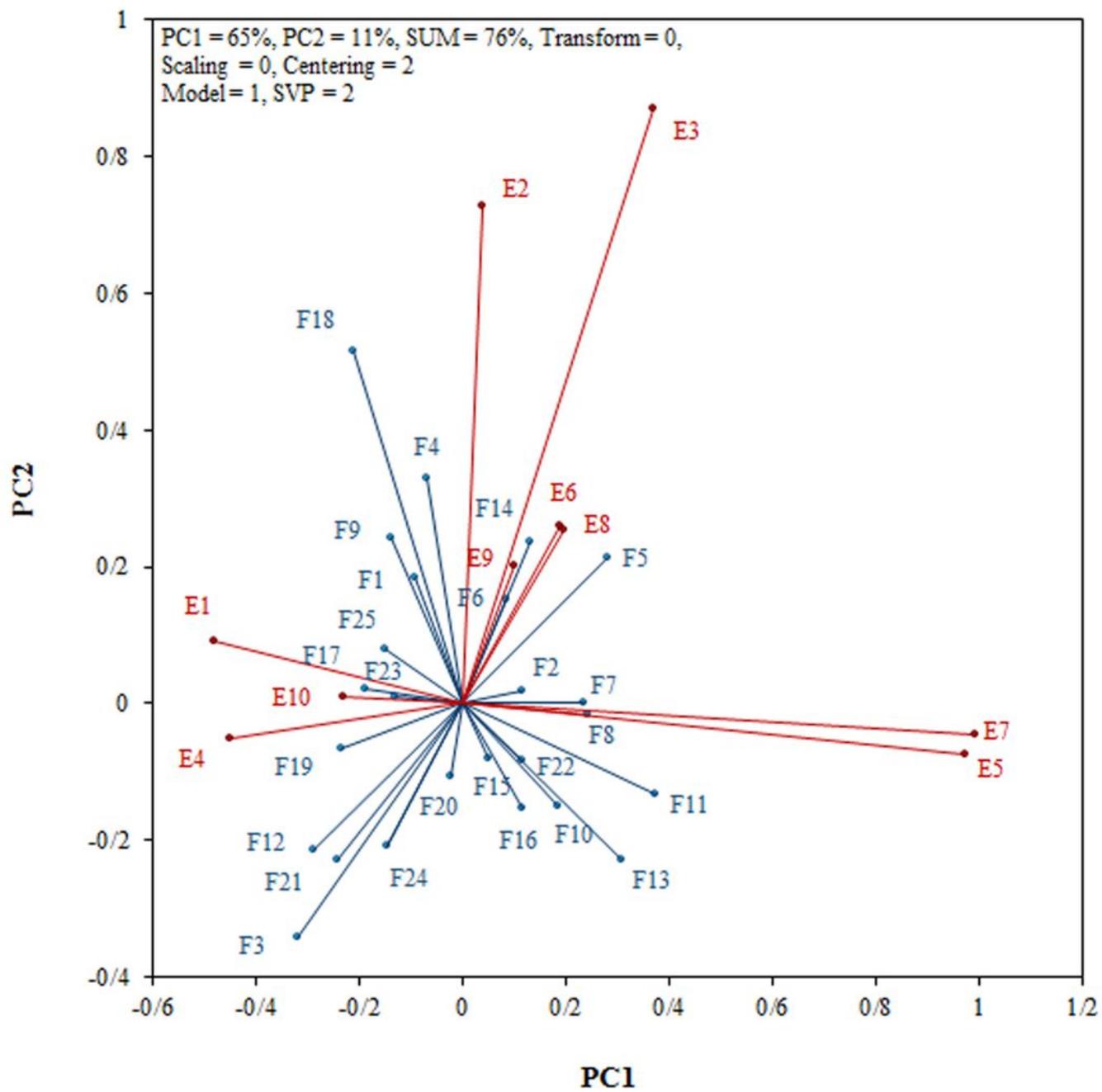


Figure 4

GGE biplot showing the performance of each HS families of orchardgrass in each environment

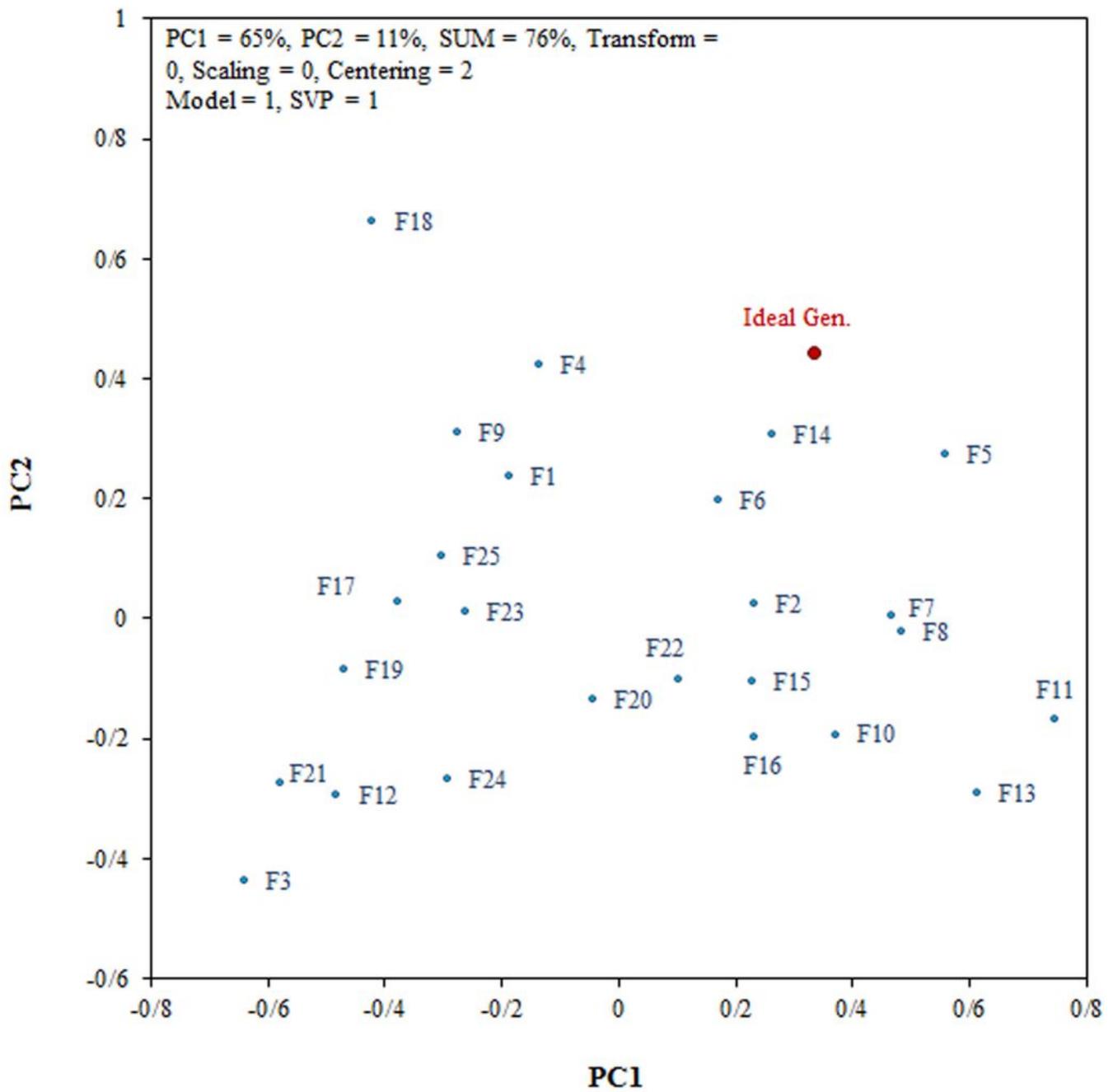


Figure 5

Comparison of HS families of orchardgrass against the position of an 'ideal' genotype for forage yield and stability of performance across the environments

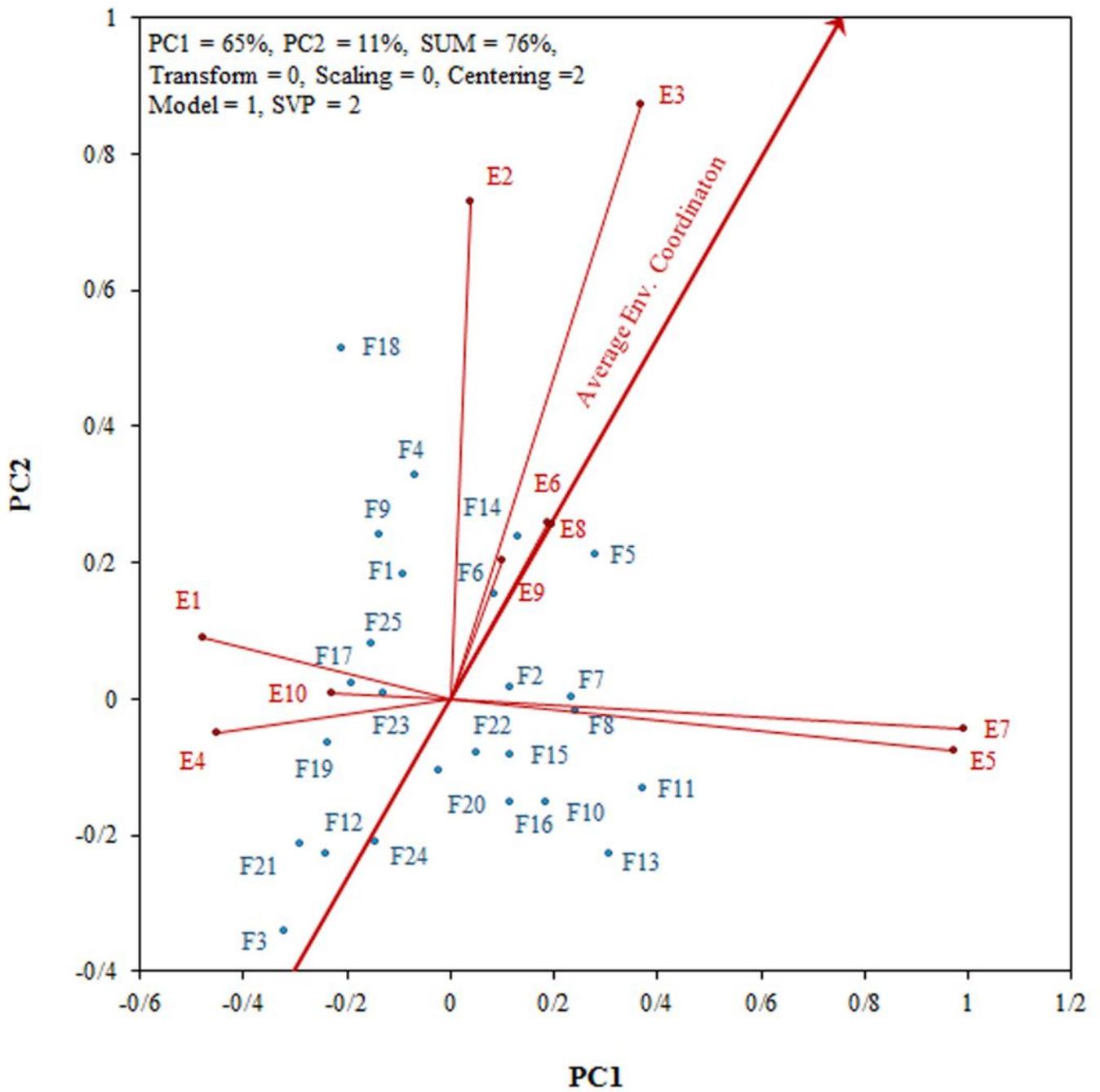


Figure 6

GGE biplot showing the ranking of HS families of orchardgrass based on yield performance and stability

Supplementary Files

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- [SupplementaryInformation.doc](#)