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Repeatability of Some Agronomic Traits in Durum Wheat

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ABSTRACT

Analysis of variance (ANOVA), principal component (PC) method and Spearman's rank correlation (SRC), between single vs. multi-year trials, were used to study the repeatability of some agronomic traits i.e., grain yield (GY), day to heading (DH), thousand kernel weight (TKW) and plant height (PH) using 20 durum wheat genotypes grown during 2000-2006 cropping seasons in Iran. Repeatabilities calculated from ANOVA were in agreement with those obtained from PC and SRC methods. These results also clarified that the repeatability for PH is high and less influenced by the systematic factors such as climatic conditions. Repeatability estimates were low ($\hat{\rho}$ < 0.20) for GY, DH and TKW, whereas these estimates for PH were high based on ANOVA ($\hat{\rho}$ = 0.733) and PC ($\hat{\rho}$ = 0.920) methods. The highest coefficient of determination (R^2 = 94.3 up to 98.6%) was observed for PH, which indicates good precision of the procedure applied. Repeatability estimates using correlations derived from single and multiple year trials showed that PH had the highest repeatability, with correlations between years ranging from 0.54 (P <0.05) to 0.95 (P <0.01), whereas the correlations for the other traits (DH, TKW and GY) showed large fluctuations and were less predictable. A high magnitude of repeatability estimates indicated a predominant genetic control of the trait PH. However, the consistency of the repeatability estimates shown by the three procedures of estimation reinforces the regularity in the expression of the traits studied.

Keywords: ANOVA, coefficient of determination, principal components, repeatability, Spearman correlation

INTRODUCTION

Repeatability analysis has three main uses. One of them is to show the improvement in quantitative accuracy resulting from the temporal repetition of measurements. When the repeatability is high, multiple measurements do not significantly improve accuracy. Contrarily, if the repeatability is low, multiple measurements can substantially increase accuracy (Falconer and Mackay 1996). Second, the repeatability coefficient can be used in plant or animal breeding to determine the necessary number of assessment times to compare treatments with a certain accuracy and minimum resources (Dias and Kageyama 1998; Silveira *et al*. 1998; Ferreira *et al*. 1999; Di Renzo *et al*. 2000). Third, repeatability is used to predict future performance from past records and sets an upper limit to heritability (Falconer 1989). In many instances the repeatability coefficient provides knowledge about the heritability of a character, without the need for controlled crossing and progeny tests. The repeatability coefficient has been used in plant and animal breeding to determine the necessary number of assessment times to compare treatments with a certain accuracy and minimum resources (Dias and Kageyama 1998; Ferreira *et al*. 1999; Di Renza *et al*. 2000). Analysis of variance (ANOVA) and the principal component (PC) method are the methods used for estimating the repeatability coefficient. Both methods have been used by several researchers and for different crops, in cacao (Dias and Kageyama 1998), papaya (Liberato *et al*. 2004), sugarcane (Santos *et al*. 2004) and tomato (Abreu *et al*. 2006). ANOVA with two variation factors is an effective method for this type of studies because it excludes temporary environmental effects (Cruz *et al*. 2004; Abreu *et al*. 2006). The PC method can estimate the repeatability coefficient more efficiently when the genotype displays cyclic behavior like in perennial species. Because this effect may vary in different ways and intensities among genotypes, the ANOVA that estimates the usual repeatability coefficient may not eliminate the additional component of experimental error. Consequently, the repeatability may be underestimated (Cruz *et al.* 2004; Abreu *et al*. 2006).

Furthermore, the method of Spearman's rank correlation (SRC) among singles and singles vs. multi-year trials as widely used to assess repeatability (Eagles and Frey 1977; Virk *et al*. 1985; Kumar *et al*. 1998). Another method for estimating repeatability is based on the absence of interaction of genotypes with years. However, reports on the use of these methods for durum are not available in the literature and this study aimed at estimating the repeatability of some agronomic traits of durum pure lines using three different methods. The estimation of repeatability on the basis of the interaction genotype x years could not be used in this study since the interaction is used as the error.

MATERIALS AND METHODS

This study was carried out with 20 genotypes consisting of 18 improved pure line durum wheat genotypes along with two national checks, Zardak for durum wheat and Sardari as bread wheat. The trial was conducted at the Dryland Agricultural Research Institute (DARI) experimental station Sararood, Kermanshah during 2000-01 to 2005-06 cropping seasons. The experimental layout was a randomized complete block design with three replications in each year.

The growing conditions over years, total annual rainfall, average of minimum and maximum temperatures and other parameters are presented in **Table 1**. Sowing was done by an experimental drill in 1.2 m × 6 m plots, consisting of six rows with 20 cm apart. Seeding rate was 350 seeds m^{-2}. Fertilizer application was 41 kg N ha^{-1} and 46 kg P_2O_5 ha^{-1} at planting. In assessing the genotypes, four characters important in breeding for rain-fed dry conditions: the grain yield (GY), plant height (PH), days to heading (DH) and thousand kernel weight (TKW).

Averages per genotype and per year were subjected to statistical analysis based on the model $Y_{ij} = m + g_i + a_j + e_{ij}$, where Y_{ij}=

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Table 1 Growing conditions of durum yield trial at Sararood experiment station during 2000-01 up to 2005-06 cropping seasons.

Cropping season	Rainfall (mm)	AT_{min} (°C)	AT_{max} (°C)	FD (day)	H%	Eva (mm)
2000-01	432.3	-11.2	39.6	79	43.4	1610
2001-02	441.8	-10	38.8	86	46.5	1741
2002-03	424.4	-9.8	41.4	76	47.9	1791
2003-04	587.6	-9.8	39.0	65	50.0	1562
2004-05	431.5	-15.0	41.6	98	49.7	2100
2005-06	505.0	-8.0	41.0	81	46.2	2021

AT_{min}: Absolute minimum temperature; AT_{max}: Absolute maximum temperature; Eva: Evaporation; FD: Freeze days; H%: Percentage of humidity.

the mean of three replications for each trait evaluated for the genotype *i* in year *j*; gi= effect of genotype *i*; aj= effect of year *j*; and e_{ij} = error associated with observation Y_{ij}. The effect of genotype and year were considered fixed and random, respectively. The individual methodology of ANOVA (Cruz *et al*. 2004), principal component (PC) method (Abeywardena 1972) and rank correlation (Eagles and Frey 1977; Virk *et al*. 1985; Kumar *et al*. 1998) used to estimate repeatability are described below:

1. ANOVA

From ANOVA, the overall repeatability ($\hat{\rho}$) was estimated as follows:

$$\hat{\rho} = \frac{\sigma_g^2}{\sigma^2 + \sigma_g^2}$$

where $\sigma^2 g$ = (MSg-MSe)/n; and σ^2 = MSe; MSg is the mean square for genotypes and MSe is the mean square for experimental error.

Then an approximate standard error of the repeatability, SE ($\hat{\rho}$), estimated from the ANOVA was calculated from the formula given by Becker (1984):

$$SE(\hat{\rho}) = \sqrt{\frac{2(1-\hat{\rho})^2[1+(\eta-1)\hat{\rho}]^2}{\eta(\eta-1)(g-1)}}$$

where η and g are the number of years and genotypes, respectively.

2. Principal-components method

The estimate of the coefficient of repeatability can be calculated either by means of a correlation matrix or by a matrix of phenotypical variances and covariances. The latter method is appropriate when genotypes display cyclic behavior in relation to the traits being studied. However, in this study we used the method based on correlation matrix.

Principal-components method - correlation matrix

A correlation matrix between genotypes must be obtained for each successive year pair. The correlation (rTj) between yield of year *j* and total yield (T), i.e., the sum of all years including year *j*, is related to $\hat{\rho}$, as:

$$r_{Tj} = \sqrt{\frac{1}{\eta}[1+(\eta-1)\hat{\rho}}$$

Using the above formula, the repeatability based on correlation matrix can be calculated (Turner and Young 1969) and the component can be written in terms of $\hat{\rho}_c$:

$$\hat{\rho}_c = \frac{(\eta r_{Tj}^2 - 1)}{(\eta - 1)}$$

The standard error in this method can be approximately estimated as follow:

$$SE(\hat{\rho}_c) = \sqrt{\frac{2(1-\hat{\rho}_c)^2[1+(\eta-1)\rho_c]^2}{\eta(\eta-1)(g-1)}}$$

Coefficient of determination (R^2)

Based on the average of the successive years (η = 6) and on the estimate of repeatability coefficients obtained by one of the methods (ANOVA or PC), the coefficient of determination (R^2) was calculated for each characteristic. This coefficient represents the certainty in predicting the real value of the individuals selected, through the following expression (Turner and Young 1969; Cruz *et al*. 2004):

$$R^2 = \frac{\eta\hat{\rho}}{1+\hat{\rho}(\eta-1)}$$

η = number of successive years

$\hat{\rho}$ = repeatability estimate calculated from the ANOVA table

Minimum number of years (measurements)

The number of measurements needed to predict the real value of genotypes based on pre-established coefficients of determination (i.e., R^2 = 0.80, 0.85, 0.90, 0.95 and 0.99) was obtained using the following expression:

$$\eta_y = \frac{R^2(1-\hat{\rho})}{(1-R^2)\hat{\rho}}$$

where η_y = number of years (measurements) necessary to predict the real value, R^2 = coefficient of determination and $\hat{\rho}$ = repeatability coefficient obtained by applying one of the methods. By this method the minimum number years, where 90% of R^2 is remaining, can be determined (Dias and Kageyama 1998).

3. Rank correlation method

To better test the repeatability of the studied traits, the Spearman's rank correlation coefficient was calculated between two values of each trait obtained from each pair of years. The repeatability of studied traits over years was also measured by rank correlating two values of single vs. multiple-year trials (Eagles and Frey 1977; Virk *et al*. 1985; Kumar *et al*. 1998). IRRISTAT software program (ver. 5.0) was employed to all analyses in this study.

RESULTS AND DISCUSSION

The results of the analysis of variance for the traits of 20 genotypes of durum wheat are presented in **Table 2**. The differences among genotypes for the traits were significant (P <0.01). Similarly, highly significant differences were observed among the years for each trait. This reveals that these years represented a range of agro-climatic conditions of the research station (Sararood) to assess the agronomic performance of the genotypes.

In **Table 2** the repeatability coefficient estimates for the traits are shown according to ANOVA and PC methods. These coefficients ranged from 0.118 (for GY) to 0.733 (for PH) based on ANOVA and from 0.182 (for GY) up to 0.920 (for PH) based on PC methods. The comparison of genotypes based on the mean of 6 years showed coefficients of determination varying from 44.5 to 94.3 (**Table 2**).

In the case of minimum number of years, the R^2-values for 3rd, 4th, 5th and 6th years was determined by repeatability analysis of all possible sets of three to six years. The R^2 estimated by ANOVA and PC methods showed that three years for PH are equally good for an accuracy of 89.4% < R^2 < 94.3% (**Table 2**).

In this study, all traits except for PH ($\hat{\rho}$ = 0.733) have

Table 2 Analysis of variance for the traits studied, estimates of the means, repeatability coefficient ($\hat{\rho}$), their corresponding standard error (SE) and coefficient of determination (R^2).

Sources	df	Mean Square			
		PH	DH	TKW	Yield
Year (Y)	5	1832.3**	712.5**	219.4**	9516996.2**
Genotype (G)	19	690.4**	13.6**	20.2**	97267.2*
Error	240	39.5	6.0	8.3	53941.8
Mean		94.6	192.0	34.3	2260.9
$\hat{\rho}$ ± SE		0.733 ± 0.074	0.175 ± 0.092	0.193 ± 0.094	0.118 ± 0.083
R^2 if n= 6		94.3	56.0	58.9	44.5
R^2 if n= 5		93.2	51.5	54.4	40.1
R^2 if n= 4		91.7	45.9	48.8	34.9
R^2 if n= 3		89.2	38.9	41.7	28.7
$\hat{\rho}$ ± SE		0.920 ± 0.059	0.250 ± 0.060	0.271 ± 0.060	0.182 ± 0.059
R^2 if n= 6		98.6	66.7	69.0	57.1
R^2 if n= 5		98.3	62.5	65.0	52.6
R^2 if n= 4		97.9	57.2	59.8	47.0
R^2 if n= 3		97.2	50.0	52.7	40.0

$\hat{\rho}$: Repeatabilities estimated from ANOVA.

$\hat{\rho}$: Repeatabilities estimated from PCA.

n: number of years.

*, ** significant at 5% and 1% probability levels, respectively

Table 3 Spearman's rank correlation coefficients between studied traits in single vs. multiple-year trials.

Subsets	Year	Traits			
		PH	DH	TKW	GY
Year-wise	2001 vs. 2002	0.84**	0.30	0.22	0.42
	2001 vs. 2003	0.89**	0.67**	0.49*	0.32
	2001 vs. 2004	0.82**	0.09	0.30	0.42
	2001 vs. 2005	0.82**	0.19	0.60**	0.28
	2001 vs. 2006	0.78**	0.42	0.21	0.14
	2002 vs. 2003	0.78**	0.24	0.23	-0.09
	2002 vs. 2004	0.63**	0.23	-0.03	0.23
	2002 vs. 2005	0.54*	0.42	0.09	0.45*
	2002 vs. 2006	0.62**	0.34	-0.04	-0.25
	2003 vs. 2004	0.84**	0.11	0.00	0.30
	2003 vs. 2005	0.79**	-0.36	0.46*	-0.04
	2003 vs. 2006	0.82**	0.07	0.23	0.17
	2004 vs. 2005	0.75**	-0.09	0.36	0.44
	2004 vs. 2006	0.74**	-0.03	-0.03	0.15
	2005 vs. 2006	0.66**	0.59**	0.38	-0.18
2001 vs. multi-year		0.97**	0.72**	0.77**	0.71**
2002 vs. multi-year		0.83**	0.77**	0.39	0.39
2003 vs. multi-year		0.95**	0.40	0.61**	0.36
2004 vs. multi-year		0.89**	0.33	0.45*	0.71**
2005 vs. multi-year		0.86**	0.61**	0.78**	0.55*
2006 vs. multi-year		0.85**	0.65**	0.56**	0.53*

poor repeatability values. This reflects the low stability of the characters over the years. Clearly, these characters are influenced by the environment, as demonstrated by the lower estimate of repeatability ($\hat{\rho} < 0.20$). In addition, these repeatability estimates were accurate, as demonstrated by their respective standard errors (**Table 2**). It is important to note that each repeatability value has a determination coefficient (R^2) associated with it. Thus, for the repeatability value of the plant height, the high R^2 values indicate good precision of the procedure applied.

In the case of good repeatability, any trait must show consistent results over different years. Eagles and Frey (1977) and Kumar *et al*. (1998) used the term 'repeatability' to measure this characteristic. Rank-correlation coefficients between the different traits from the year-pairs and over years are given in **Table 3**. From the results, the traits can be subdivided into two groups that showed different degrees of correlation. The PH values from different years were always highly correlated (from 0.54* to 0.97**), whereas the correlations of traits represented by DH, TKW and GY showed large fluctuations. For example the correlation coefficient for DH varied from -0.36 to 0.77**, for TKW from -0.04 to 0.78** and for GY from -0.25 to 0.71**. A non-significant correlation between the estimates of DH, TKW and GY suggests that the reliability of genotypes based on unfavorable years (environments) would not be useful in predicting their stability in favorable years (environments), and *vice versa*, unless the trials were conducted in wide range of favorable and unfavorable environments. This also suggests that the repeatability information derived from the traits and their interpretations are valid only for that specific set of years.

The similarity among the repeatability coefficients obtained by different analysis methods suggests consistency among estimations. High repeatability coefficients indicate a low chance of significant interaction between genotype and year; therefore, the real value of genotype performance can be predicted using less assessment years (environments). The R^2 value shows the accuracy of the analysis in predicting the real value of genotype performance (obtained with infinite assessment times) based on n assessment times (Cruz *et al*. 2004). In this study, a high accuracy level was obtained with n=6, but this number could be reduced to any successive three assessment times for PH, without affecting the accuracy of genotype comparison, thus saving labor and costs. When only three successive assessment times were considered, R^2 ranged from 89.4 to 94.3%, which indicates three year reduction of assessment years can reduce the accuracy of genotype performance estimates by only 5%.

Single year results for yield and other agronomic traits

can be used effectively in selection only if they are repeatable in other subsequent years. The estimates of PH, but not for the other traits studied, was consistent over years as indicated by positively significant correlation coefficients between all pair comparisons (**Table 3**). This suggests good repeatability for PH over years. In the six years studied, the same set of genotypes was tested in different conditions giving a fair representation of the next environmental conditions. The other three traits, however, were not repeatable over years when the number of years was six.

In the case of single vs. multi-year trials, a high repeatable correlation was found based on PH, whereas the correlations for other traits showed high fluctuations and poor repeatability (**Table 3**). However, these correlations were considerable than those from single years. A significant correlation between PH values from the two subsets of years, irrespective of the method of division, indicates that selection based on PH from multiple-year trials would be highly effective in producing durum wheat lines that would be superior at all yield levels. The traits GY, DH and TKW appear to be non-repeatable across years, therefore, may not be useful in selecting cultivars with predictable performance.

Fundamentally, the low magnitude of repeatability estimates indicates an importance influence of the environment on the expression of the trait. The causes for obtaining such low estimates are due to unpredictable environmental effects. High fluctuations in productivity of durum lines due to climatic adversity, particularly water deficit, have been observed.

In conclusion, repeatabilities calculated from the rank correlations among single and single vs. multiple-year trials were in agreement to those obtained from ANOVA and PC methods (**Tables 2** and **3**). Regarding the simplicity of rank correlation method, it can be used as a good alternative for other methods to estimate repeatability. The results of the three methods clarified that repeatability for the PH trait is high and less affected by environment factors. Furthermore, the consistency of the repeatability estimates shown by the three procedures of estimation reinforces the regularity in the expression of the characters studied.

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