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Repeatability, number of harvests, and phenotypic stability of dry matter yield and quality traits of *Panicum maximum* jacq.

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ABSTRACT. Selection of superior forage genotypes is based on agronomic traits assayed in repeated measures. The questions are how repeatable the performance of individual genotypes is and how many harvests are needed to select the best genotypes. The objectives were to estimate repeatability coefficients of dry matter yield (DMY) and forage quality, their phenotypic stability and the number of harvests needed for an accurate selection. Two randomized complete block design experiments data with 24 genotypes each, undergoing 12 and 16 harvests, over a period of 2 and 3 years, respectively, were used. The DMY repeatability estimates ranged from 0.42 to 0.55, suggesting a low heritability. The mean numbers of repeated measures were 5 and 7 harvests for 0.80 and 0.85 accuracy, respectively. The inclusion of the first two harvests negatively affects the estimates. Repeatability for quality traits ranged from 0.30 to 0.69, indicating low to moderate heritability.

Keywords: repeated measures, selection efficiency, selection effectiveness.

Repetibilidade, número de colheitas e estabilidade fenotípica da produção de matérica seca e de características de qualidade de *Panicum maximum* jacq.

RESUMO. A seleção de genótipos superiores em forrageiras é feita para características agronômicas analisadas em medições repetidas no tempo. As questões estão relacionadas à repetibilidade do desempenho dos genótipos e ao número necessário de colheitas para selecionar aqueles superiores. Os objetivos foram estimar coeficientes de repetibilidade da produção de matéria seca (PMS) e de características de qualidade da forragem, a estabilidade fenotípica e o número de colheitas necessárias para uma seleção mais precisa. Dois experimentos em blocos casualizados com 24 genótipos cada um, submetidos a 12 e 16 colheitas, durante um período de dois e três anos, respectivamente, foram utilizados para o estudo. As estimativas de repetibilidade de PMS variaram de 0,42 a 0,55, sugerindo baixa herdabilidade. Os números de colheitas foram cinco e sete para 0,80 e 0,85 de acurácia, respectivamente. A inclusão das duas primeiras colheitas afeta negativamente as estimativas de PMS. A repetibilidade para as características de qualidade variou de 0,30 a 0,69, indicando baixa à moderada herdabilidade.

Palavras-chave: medidas repetidas, eficiência de seleção, eficácia de seleção.

Introduction

Selection of a superior genotype of perennial forages is based on the analysis of agronomic traits in repeated measures, such as dry matter yield, over a number of harvests, seasons, and years. The main questions are 'How repeatable is the performance of a genotype along a number of harvests?' and 'How many harvests are necessary to select the best genotypes?' The repeatability coefficient, defined by Cruz and Regazzi (1994) as the correlation between repeated measures from the same individual over time and space, as well as its associated coefficient of determination (R^2), which measures accuracy in predicting the real value of an individual genotype, are the most used tools to address these questions. The higher the correlation between repeated measures, the lower may be the number of harvests needed to distinguish genotypes in a selection procedure. If the trait is of difficult assessment or its heritability is low, a higher number of harvests may be necessary. However, if the number of harvests increases largely, each selection phase will be longer, taking two or more full years, which substantially increases time and costs of selection.

Several studies estimate repeatability and the number of harvests required to select superior genotypes for DMY in *P. maximum*. Repeatability estimates from five harvests in two studies (Martuscello, Jank, Fonseca, Cruz, & Cunha, 2007, Martuscello et al., 2015) ranged from 0.51 to 0.86 in the first one and from 0.44 to 0.60 in the second one, with 0.80 and higher accuracy. Results from both studies concluded that at least five harvests are needed to have an effective selection with 0.85 accuracy. Repeatability estimates from 15 harvests (Lédo et al., 2008) ranged from 0.23 to 0.54 for DMY and for the same 0.85 level of accuracy it is necessary at least ten harvests. Torres et al. (2016) reported high repeatability from six harvests, ranging from 0.76 to 0.87 and accuracy levels from 0.94 and 0.98. They concluded that seven harvests are necessary to select the highest DMY cultivars at 0.85 accuracy level. These results suggest additional studies to better elucidate repeatable performance and number of harvests.

Although dry matter yield still is the focus of grass breeding programs, higher forage quality is becoming important target for forage grasses breeding (Stewart & Hayes 2011). High quality forage has been obtained by raising the leaf/stem ratio, crude protein, water soluble carbohydrates, dry matter digestibility, lower content of components of neutral detergent fiber as well as higher detergent neutral soluble fiber, such as pectin (Van Soest, 1994, Fonseca, Hansen, Thomas, Pell, & Viands, 1999, Casler et al. 2000). To our best knowledge, no information is available on repeatability estimates of quality traits for *P. maximum*. Thus, repeatability studies will better elucidate the nature of the phenotypic and genetic variations of quality traits as well.

The objectives were to estimate the repeatability coefficient of dry matter yield and forage quality traits, to determine the number of harvests required for an accurate selection of superior genotypes and to define the phenotypic stability of dry matter yield and quality traits in *P. maximum* genotypes grown in the Cerrado region of the Federal District, Brazil.

Material and methods

Two experiments evaluating forage yield and nutritive value of *P. maximum* were conducted in Planaltina, Federal District, Brazil (15° 35' S; 47° 42' W, 993 m a.s.l.) at Embrapa Cerrados. The local climate is Aw type (tropical savannah with dry winter and rainy summer) according to the Köppen and Geiger (1928) classification. The annual average temperature between 1974 and 2003 was 21.9°C with monthly averages ranging from 19.9 to 23.2°C; the mean relative humidity was 70%, with monthly averages ranging from 56 to 77%; and the total annual rainfall is about 1384 mm with monthly averages ranging from 5 mm (Jun) to 251 mm (Jan) (Silva, Evangelista, & Malaquias, 2014). Plants 0.5 m away from the row/plot borders were harvested at 0.2 m high and a randomly selected subsample of 1.5 kg fresh weight was separated into leaf (green blade), stem (stem plus leaf sheath) and dead material (brown leaves and brown stems). All samples were dried in a forced air oven at 55°C for 72 hours. Total dry matter yield was estimated and quality traits were analyzed via Near Infrared Reflectance Spectroscopy (NIRS).

The first experiment (Exp. 1) was conducted in 2003 and 2004. Twenty-four *P. maximum* genotypes were evaluated: 14 advanced accessions tagged PM30 to PM43; four artificial intraspecific hybrids PM44 to PM47; and six cultivars Aruana, Vencedor, Milênio, Mombaça, Tanzânia and Massai. Accessions and hybrids belong to the breeding program of Embrapa Beef Cattle located in Campo Grande, state of Mato Grosso do Sul (20° 26’ S; 54° 38’ W), Brazil. The experiment was laid out in a three-replicate randomized complete block design with 24 treatments, in plots consisting of 6 rows of 4 m (0.5 m spacing), with 3 × 4 m size and 12 m^2 total area and 6 m^2 useful area. An equivalent of 1 t ha^{-1} dolomitic lime was applied to the soil in October 2002. Plots were sown on November 21st, 2002, at a rate of 3000 g ha^{-1} of viable seeds, and harvested six times in both years 2003 and 2004, as described by Fernandes et al. (2014). Forty nine days after sowing, 50 kg ha^{-1} Nitrogen (N) and 21 kg ha^{-1} Potassium (K) were applied as urea and potassium chloride, respectively. Genotypes were harvested and sampled six times each year: Feb 5th, 2003; Mar 12nd, 2003; Apr 16th, 2003; June 25th, 2003; Oct 27th, 2003; Dec 1st, 2003; Jan 05th, 2004; Feb 09th, 2004; Mar 15th, 2004; Apr 19th, 2004; June 28th, 2004 and Nov 12nd, 2004. A total of 250 kg ha^{-1} N and 207.5 kg ha^{-1} K per year, as urea and potassium chloride, were split into five applications after each harvest, except for June, 2003 and 2004, and November, 2004, corresponding to dry season harvests.

The second experiment (Exp. 2 from now on) was conducted from 2013 to 2015. Twenty hybrid genotypes and four cultivars of *P. maximum* from the same breeding program at Embrapa Beef Cattle were evaluated: A105, A124, A125, A51, A62, A78, B11, B126, B16, B44, B46, B53, B55, B57, B97, C10, C12, C53, C55, DE6, Colonião, Massai, Mombaça and BRS Zuri. The experiment was a four-replicate randomized complete block design with 24 treatments, each in a three meter-length row spaced one meter and a half apart. Plots were sown on December 6th, 2012 at a seeding rate of 0.15 g m^{-1}, and harvested five times in 2013, seven in 2014, and 4 in 2015.The establishment fertilization consisted of 100 kg ha^{-1} P_2O_5as simple superphosphate, 50 kg ha^{-1} K_2O_5 as potassium chloride, and 30 kg ha^{-1} FTE 12 micronutrient mixture. On January 10th, 2013 50 kg ha^{-1} N as ammonium sulfate was applied on the row. Genotypes were harvested and sampled five times in 2013, seven times in 2014, and 4 times in 2015: Feb 20th, 2013; Mar 27th, 2013; Jun 10th, 2013; Oct 15th, 2013; Dec 2nd,2013; Jan 8th, 2014; Feb 10th, 2014; Mar 17th, 2014; Apr 22nd, 2014; May 27th, 2014; Sep 9th, 2014; Nov 2nd, 2014; Jan 15th, 2015; Feb 19th, 2015; Apr 1st, 2015; and May 7th, 2015. Ammonium sulfate and potassium chloride doses of 50 kg ha^{-1} N and 42 kg ha^{-1} K were applied after each harvest, except for June 2013, April 2014, May 2014, September 2014 and May 2015, corresponding to dry season harvests.

Dry leaf and stem samples were weighed, ground through a 1-mm screen Wiley mill, and stored in plastic bags. Spectra for all samples were collected on a NIRS model NR5000 Systems Inc., USA, in a wavelength range of 1100-2500 μm. The coefficient of determination for quality curves ranged from 0.87 to 0.99 and validation residues were lower than 5%. In Exp. 1, four and three harvests were sampled in 2003 and 2004, respectively, for quality analysis.

The repeatability coefficients (r) were estimated by four statistical procedures (Cruz & Regazzi, 1994) - analysis of variance based on variance components, principal component based on the covariance and correlation matrix, and structural analysis based on the correlation matrix. The repeatability coefficient based on ANOVA was estimated by using the statistical model with two factors. The phenotypic stability was evaluated by principal component analysis obtained from the intra class correlation matrix for 2, 3, and up to ‘n’ successive repeated measures. All repeatability analyses were developed using Genes – Quantitative Genetics and Experimental Statistics software, version 2013.5.1 (Cruz, 2007).

Results and discussion

The repeatability coefficient indicates the ability of genotypes to repeat the expression of a trait over repeated measures and the coefficient of determination (R^2) denotes the accuracy in predicting the real value of an individual genotype (Table 1). According to Falconer (1981), repeatability shows how much is to be gained by repeated measures, to set the upper limit to the broad-sense heritability and to predict future performance from past records. Repeatability coefficients for DMY were low to moderate in magnitude and higher for Exp. 2. The repeatability estimates for DMY ranged from 0.26 to 0.42 in Exp. 1 and from 0.39 to 0.55 in Exp. 2, through ANOVA and Principal Component Correlation (PCCor) methods, respectively. ANOVA consistently came up with the lowest estimates while PCCor the highest, similar to other reports (Martuscello et al., 2007, Lédo et al., 2008, Braz, Fonseca, Jank, Cruz, & Martuscello, 2015). The magnitude of the estimates in Exp. 1 and Exp. 2 suggested that DMY has a multigene genetic control, largely influenced by environmental conditions, which may lead to moderate gains in selection due to a low to medium heritability. In addition, it may require a high number of repeated measures to be more effective. The magnitude of repeatability estimates in Exp. 1 (12 harvests) were similar to those obtained by Lédo et al. (2008, 15 harvests) and Martuscello et al. (2015, 5 harvests). In Exp.2 (16 harvests), the magnitude of the repeatability estimates were similar to those reported by Martuscello, Jank, Fonseca, Cruz, and Cunha (2007).

Table 1. Estimates of repeatability coefficient (r) and coefficient of determination (R^2) of total dry matter yield (DMY) for 12 harvests in Exp.1 and 16 harvests in Exp.2, by analysis of variance (ANOVA), principal component covariance (PCCov), principal component correlation (PCCor) and structural analysis (STCor).

Method	DMY (Exp.1)		DMY (Exp.2)	
	r	R^2	r	R^2
ANOVA	0.259	0.81	0.391	0.91
Principal Component Covariance	0.413	0.89	0.537	0.95
Principal Component Correlation	0.421	0.90	0.547	0.95
Structural Correlation	0.394	0.89	0.480	0.94

Values of R^2 were high in magnitude, ranging from 0.81 to 0.90 in Exp. 1, and from 0.91 to 0.95 in Exp. 2, suggesting the number of harvests was enough to provide high accuracy in selecting genotypes. However, 12 and 16 harvests may not be feasible in practice, since high number of harvests is time-consuming and it would lead to high costs. In this study, the high number of harvests as well as the high R^2 estimates may allow for a more robust estimation of the minimum number of harvests, at similar accuracy levels, than those with much lower number of harvests and lower R^2.

The number of harvests estimated for DMY ranged from 6 to 11 in Exp. 1 and from 4 to 7 in Exp. 2 at 0.80 accuracy, and from 8 to 16 in Exp. 1 and from 5 to 9 in Exp. 2 at 0.85 accuracy (Table 2). The number of harvests was more consistent and lower for Exp. 2. The magnitudes of the estimates were higher for ANOVA when compared with the other methods in both experiments. The principal component and structural analyses resulted in lower, closer, and more consistent estimates. The estimates of four methods may be used to set an interval in which the real value of a parameter is most likely to be found (Martuscello et al., 2007). An approach can be the use of the average over all four methods to generate an estimate of the number of repeated measures. In this sense, estimates of the number of harvests combined over methods for each experiment would fall between 4 and 7 at an accuracy of 0.80 and between 6 and 10 at an accuracy of 0.85 for Exp. 1 and Exp. 2, respectively (Figure 1). Yet, the estimated number of harvests combined over Exp. 1 and Exp. 2 would be 6 for an accuracy of 0.80 and 8 for an accuracy of 0.85.

Table 2. Number of repeated measures of dry matter yield of *P. maximum* for Exp.1 and Exp.2 estimated by four methods of repeatability coefficient and four levels of accuracy (R^2).

R^2	Exp. 1				Exp.2			
	ANOVA[1]	PCCov	PCCor	STCor	ANOVA	PCCov	PCCor	STCor
0.80	11.5	5.7	5.6	6.1	6.2	3.5	3.3	4.3
0.85	16.25	8.7	7.8	8.7	8.8	4.9	4.7	6.2
0.90	26.8	12.8	12.4	13.8	14.0	7.8	7.5	9.8
0.95	54.4	27.0	26.2	29.2	29.6	16.4	15.7	20.6

[1]Methods of estimation: ANOVA – Analysis of Variance; PCCov - Principal Component Covariance; PCCor - Principal Component Correlation; STCor - Structural Correlation.

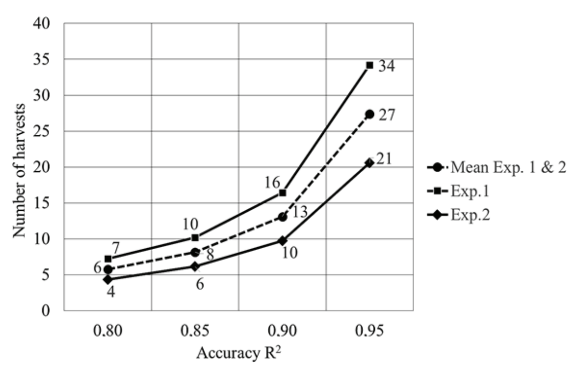

Figure 1. Mean number of repeated measures for dry matter yield of *P. maximum* for Exp.1, Exp.2, and the average over four methods for four levels of accuracy.

No individual repeatability method gives precise estimation of the number of repeated measures for DMY over wide ranges of conditions, such as years and experimental sites, different genetic composition of the populations and time of harvest. The differences between methods in six studies (Martuscello et al., 2007, Lédo et al., 2008, Martuscello et al., 2015, Torres et al., 2016, and Exp. 1 and Exp. 2 from this study) resulted in different estimates of the number of repeated measures for DMY (Figure 2). Since the estimates are likely an integration of many factors, the definition of a range considering the outcome of many studies may be more robust than a specific number of repeated measures estimated for individual studies. For the above experiments, there is a cluster of estimates ranging from 4 to 7 at 0.80 accuracy and 5 to 9 at 0.85 accuracy (Figure 2). Thus, the expected range of harvests for DMY in *P. maximum* would likely be within those ranges. The environmental span variability affecting the experiments would direct the decision to lower or higher number of harvests. Some similar estimates of repeated measures are also reported for DMY of other forages such as elephant-grass (*Pennisetum purpureum* Schum., Shimoya, Pereira, Depaula, Damião, & Souza, 2002) and alfalfa (*Medicago sativa* L., Souza-Sobrinho et al., 2004).

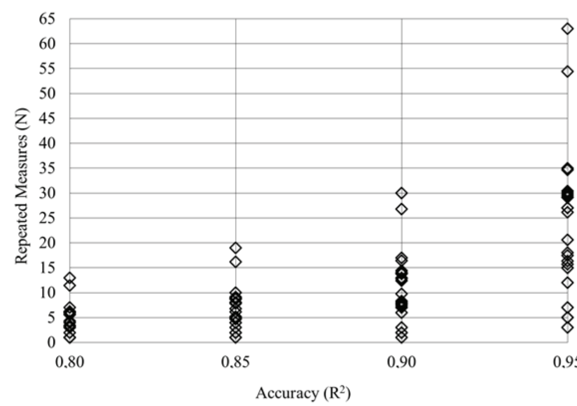

Figure 2. Estimated number of repeated measures for total dry matter yield (DMY) of *P. maximum* for four levels of accuracy with data from 6 different studies by Martuscello et al. (2007, 2015), Lédo et al. (2008), Torres et al. (2016), Exp. 1, and Exp. 2.

The phenotypic stability analysis for DMY resulted in low to moderate repeatability coefficients, 0.14 to 0.49 in Exp. 1 and 0.09 to 0.78 in Exp. 2 (Table 3). The magnitude of the repeatability estimates was consistently higher in Exp. 2. Yet, the accuracy estimates were moderate to high in magnitude, ranging from 0.40 to 0.90 in Exp. 1 and from 0.28 to 0.96 in Exp. 2, regardless of the method, ANOVA or PCA. The highest repeatability and accuracy coefficients for DMY were observed when harvest 1 and 2 clusters were excluded from analysis (Table 3). Probably, gene expression is not stable during early stages of the plant development. Martuscello et al. (2007) and Torres et al. (2016) reported higher estimates when harvests 1 and 2 were not used in the analysis.

The highest repeatability and accuracy estimates for Exp. 1 were 0.47 and 0.78, respectively for 4 harvests based on the combination of harvests 6 to 9. The trend was similar until harvest 5, in which the best harvest combination was 5 to 9. This may be because harvest 3 was done at the end of the rainy season, harvest 4 in the dry season, and harvest 5 at the end of the dry season. Both, Braz, Fonseca, Jank, Cruz, and Martuscello (2015) and Martucscello et al. (2015) also reported similar results, in which dry season harvests decrease the repeatability coefficient estimates. In Exp. 1, the phenotypic stabilization occurred with 6 harvests where the best harvest combinations were 3 to 8 and 4 to 9. From harvest 7 onward, the estimates were very close to those from harvest 6 for cluster with 3 and more harvests.

Table 3. Phenotypic stabilization - estimates of repeatability (r) and coefficients of determination (R^2) of dry matter yield for different groups of 12 successive harvests for Exp. 1 and 16 successive harvests for Exp. 2 in *P. maximum* using ANOVA and principal component analysis (PCA).

Exp. 1						Exp. 2					
Evaluations	N	ANOVA		PCA		Evaluations	N	ANOVA		PCA	
		r	R^2	r	R^2			r	R^2	r	R^2
1-4	4	0.14	0.40	0.26	0.58	1-4	4	0.28	0.61	0.29	0.63
2-5	4	0.26	0.58	0.39	0.72	2-5	4	0.36	0.69	0.50	0.80
3-6	4	0.19	0.49	0.43	0.75	3-6	4	0.74	0.92	0.78	0.93
4-7	4	0.30	0.63	0.47	0.78	4-7	4	0.66	0.88	0.66	0.89
5-8	4	0.32	0.65	0.43	0.75	8-11	4	0.09	0.28	0.19	0.49
6-9	4	0.47	0.78	0.47	0.78	1-5	5	0.31	0.69	0.38	0.76
7-10	4	0.30	0.63	0.40	0.73	2-6	5	0.42	0.78	0.58	0.88
9-12	4	0.32	0.66	0.47	0.78	3-7	5	0.68	0.92	0.74	0.93
1-5	5	0.12	0.40	0.29	0.67	4-8	5	0.73	0.93	0.77	0.94
2-6	5	0.31	0.69	0.45	0.80	12-16	5	0.57	0.87	0.68	0.91
3-7	5	0.23	0.60	0.40	0.77	1-6	6	0.35	0.77	0.47	0.84
4-8	5	0.32	0.70	0.43	0.79	2-7	6	0.41	0.81	0.59	0.90
5-9	5	0.37	0.75	0.44	0.80	3-8	6	0.66	0.92	0.73	0.94
6-10	5	0.31	0.69	0.41	0.78	4-9	6	0.46	0.84	0.56	0.88
7-11	5	0.34	0.72	0.44	0.80	11-16	6	0.46	0.84	0.55	0.88
8-12	5	0.36	0.74	0.49	0.83	1-7	7	0.35	0.79	0.49	0.87
1-6	6	0.18	0.56	0.36	0.77	2-8	7	0.43	0.84	0.61	0.92
2-7	6	0.30	0.72	0.41	0.81	3-9	7	0.63	0.92	0.72	0.95
3-8	6	0.30	0.72	0.41	0.81	4-10	7	0.43	0.84	0.55	0.89
4-9	6	0.37	0.78	0.45	0.83	1-8	8	0.37	0.83	0.53	0.90
5-10	6	0.26	0.68	0.42	0.81	2-9	8	0.44	0.86	0.61	0.93
6-11	6	0.34	0.76	0.43	0.82	3-10	8	0.59	0.92	0.71	0.95
7-12	6	0.32	0.74	0.43	0.82	4-11	8	0.37	0.82	0.48	0.88
1-7	7	0.20	0.64	0.35	0.79	1-9	9	0.38	0.85	0.54	0.91
2-8	7	0.35	0.79	0.42	0.84	2-10	9	0.42	0.87	0.62	0.94
3-9	7	0.35	0.79	0.44	0.85	3-11	9	0.52	0.91	0.62	0.94
4-10	7	0.27	0.72	0.44	0.85	1-10	10	0.37	0.85	0.55	0.93
5-11	7	0.30	0.75	0.43	0.84	2-11	10	0.37	0.86	0.55	0.93
6-12	7	0.34	0.78	0.45	0.85	3-12	10	0.53	0.92	0.63	0.95
1-8	8	0.23	0.70	0.37	0.82	1-11	11	0.33	0.85	0.50	0.92
2-9	8	0.39	0.83	0.46	0.87	2-12	11	0.40	0.88	0.57	0.94
3-10	8	0.32	0.79	0.44	0.86	3-13	11	0.53	0.92	0.63	0.95
4-11	8	0.31	0.78	0.45	0.87	1-12	12	0.36	0.87	0.52	0.93
5-12	8	0.30	0.77	0.45	0.87	2-13	12	0.40	0.89	0.57	0.94
1-9	9	0.25	0.75	0.40	0.86	3-14	12	0.52	0.93	0.64	0.95
2-10	9	0.35	0.83	0.45	0.88	1-13	13	0.36	0.88	0.52	0.93
3-11	9	0.34	0.82	0.45	0.88	2-14	13	0.40	0.89	0.58	0.95
1-10	10	0.24	0.76	0.40	0.87	1-14	14	0.36	0.89	0.54	0.94
2-11	10	0.36	0.85	0.46	0.89	1-15	15	0.38	0.90	0.54	0.95
2-12	11	0.35	0.86	0.46	0.90	1-16	16	0.39	0.91	0.55	0.95

The highest repeatability estimates for Exp. 2 were 0.78 for 4 harvests and 0.74 and 0.77 for 5 harvests, based on the combination of harvests 3 to 6, 3 to 7 and 4 to 8, respectively. In Exp. 2, higher repeatability estimates are more evident when harvests 1 and 2 were not in the clusters. The trend was similar up to harvest 7, in which the best harvest combination was 3 to 9. In Exp. 2, the phenotypic stabilization occurred between 4 and 5 harvests, where the best harvest combinations were 3 to 6, 3 to 7, and 4 to 8. In Exp. 2, dry season harvest did not affect the estimates of repeatability as did in Exp. 1. This may be due to the rainfall in May (18.9 mm), June (51.1 mm), August (1.6 mm) and September (55.9 mm), during the dry season of 2013.

All accuracy estimates in both Exp. 1 and Exp. 2, for the above clusters, were higher than 0.92, suggesting high accuracy in selecting genotypes based on the designated number and clusters of harvests.

The highest estimates of repeatability coefficient were moderate in magnitude, especially for ADF, cellulose and lignin-H_2SO_4 that ranged from 0.61 to 0.69. The estimates of repeatability coefficient were lower, but still moderate in magnitude for OM, CP, and IVOMD and, regardless of the method, ranged from 0.54 to 0.61. The lowest estimates were found for NDF, Lig-$KMnO_4$, hemicellulose, and silica, ranging from 0.30 to 0.54 (Table 4).

Table 4. Estimates of repeatability coefficient (r) and coefficient of determination (R^2) of *P. maximum* leaf quality traits for 7 harvests, by analysis of variance (ANOVA), principal component covariance (PCCov), principal component correlation (PCCor) and structural analysis (STCor).

Method	OM[1]		CP	
	r	R^2	r	R^2
ANOVA	0.54	0.89	0.54	0.89
PCCov	0.62	0.92	0.61	0.92
PCCor	0.62	0.92	0.57	0.90
STCor	0.61	0.92	0.56	0.89
	NDF		ADF	
	r	R^2	r	R^2
ANOVA	0.30	0.75	0.61	0.92
PCCov	0.34	0.78	0.65	0.93
PCCor	0.33	0.77	0.69	0.94
STCor	0.30	0.75	0.68	0.94
	Lig-$KMnO_4$		Lig-H_2SO_4	
	r	R^2	r	R^2
ANOVA	0.40	0.82	0.65	0.93
PCCov	0.54	0.89	0.65	0.93
PCCor	0.48	0.87	0.66	0.93
STCor	0.45	0.85	0.65	0.93
	Hemicellulose		Cellulose	
	r	R^2	r	R^2
ANOVA	0.36	0.79	0.66	0.93
PCCov	0.40	0.82	0.68	0.94
PCCor	0.47	0.86	0.68	0.94
STCor	0.43	0.84	0.67	0.93
	OMD		Silica	
	r	R^2	r	R^2
ANOVA	0.57	0.90	0.38	0.81
PCCov	0.61	0.92	0.54	0.89
PCCor	0.58	0.91	0.46	0.85
STCor	0.58	0.91	0.43	0.84

[1]OM - Leaf Organic Matter; CP – Leaf Crude Protein; NDF – Leaf Neutral Detergent Fiber; ADF – Leaf Acid Detergent Fiber; Lig-$KMnO_4$ – Leaf Lignin in potassium permanganate; Lig–H_2SO_4 - Lignin in sulfuric acid; OMD – *In vitro* organic matter digestibility.

This magnitude clearly indicates low to moderate heritability for the quality traits, mainly due to the environmental variability over the harvests in different seasons and years. These repeatability estimates were associated with high R^2 that reflects the accuracy in predicting the trait value of an individual genotype. The accuracy levels were 0.80 and higher for all quality traits except for NDF that falls into the 0.75 to 0.78 range, which, however, is still somewhat high in magnitude. Thus, all estimates above suggested high effectiveness of seven harvests when selecting the best *P. maximum* genotypes for quality traits (Table 4).

The number of harvests estimated for OM, CP, ADF, lignin-H_2SO_4, cellulose, and IVOMD for all four methods, ranged from 2 to 4 at 0.80 accuracy level and from 4 to 5 at 0.85 accuracy level (Table 5).

Table 5. Number of repeated measures for quality traits of *P. maximum* estimated by four methods of repeatability coefficient and four levels of accuracy (R^2).

R^2	Leaf Organic Matter				Leaf Crude Protein			
	ANOVA[1]	PCCov	PCCor	STCor	ANOVA	PCCov	PCCor	STCor
0.80	3.5	2.5	2.4	2.5	3.4	2.6	3.0	3.1
0.85	4.9	3.5	3.4	3.6	4.8	3.7	4.2	4.4
0.90	7.8	5.5	5.5	5.7	7.6	5.9	6.7	7.0
0.95	16.5	11.7	11.5	12.0	16.1	12.4	14.2	14.8
R^2	Leaf NDF				Leaf ADF			
	ANOVA	PCCov	PCCor	STCor	ANOVA	PCCov	PCCor	STCor
0.80	9.4	7.9	8.2	9.2	2.5	2.2	1.8	1.9
0.85	13.2	11.2	11.5	13.0	3.6	3.1	2.6	2.7
0.90	21.0	17.8	18.3	20.6	5.7	4.9	4.1	4.3
0.95	44.4	37.6	38.7	43.6	12.0	10.4	8.7	9.1
R^2	Leaf Lignin $KMnO_4$				Leaf Lignin H_2SO_4			
	ANOVA	PCCov	PCCor	STCor	ANOVA	PCCov	PCCor	STCor
0.80	6.1	3.5	4.3	4.9	2.2	2.1	2.1	2.1
0.85	8.7	4.9	6.1	6.9	3.1	3.0	2.9	3,0
0.90	13.8	7.8	9.6	10.9	5.0	4.8	4.6	4.7
0.95	29.2	16.4	20.4	23.1	10.5	10.0	9.8	9.9
R^2	Leaf Hemicellulose				Leaf Cellulose			
	ANOVA	PCCov	PCCor	STCor	ANOVA	PCCov	PCCor	STCor
0.80	7.3	6.1	4.5	5.1	2.0	1.9	1.9	2.0
0.85	10.3	8.7	6.4	7.3	2.9	2.7	2.7	2.8
0.90	16.3	13.8	10.2	11.5	4.6	4.2	4.3	4.4
0.95	34.4	29.0	21.5	24.3	9.7	8.9	9.1	9.3
R^2	Leaf IVOMD				Leaf Silica			
	ANOVA	PCCov	PCCor	STCor	ANOVA	PCCov	PCCor	STCor
0.80	3.0	2.5	2.9	2.9	6.6	3.5	4.8	5.2
0.85	4.2	3.6	4.0	4.2	9.3	4.9	6.8	7.4
0.90	6.7	5.7	6.5	6.6	14.8	7.8	10.8	11.8
0.95	14.1	12.0	13.6	13.9	31.3	16.4	22.7	24.9

[1]Methods of estimation: ANOVA – Analysis of Variance; PCCov - Principal Component Covariance; PCCor - Principal Component Correlation; STCor - Structural Correlation.

The estimated number of harvests for NDF was the highest among all of them and ranged from 8 to 10 for 0.80 accuracy level. Intermediate estimates was obtained for lignin $KMnO_4$, hemicellulose, and silica and ranged from 4 to 8 for 0.80 accuracy and from 5 to 11 for 0.85 accuracy. Thus, selection for NDF, hemicellulose, lignin $KMnO_4$ and silica may require a higher number of repeated measures than for OM, CP, ADF, lignin-H_2SO_4, and IVOMD.

Repeatability estimates and associated determination coefficients for all quality traits were similar in magnitude when considering the first and second harvests in the clusters (Table 6). In brief, the first and the second harvests did not have a significant negative effect on the repeatability estimates for all quality traits as they did for DMY. Thus, all clusters containing harvests 1 and 2 may be used to estimate repeatability of quality traits. Phenotypic stability occurred at the third harvest for CP, ADF, Lig-H_2SO_4, and cellulose; at the fourth harvest for OM, Lig-$KMnO_4$, and IVOMD; at the fifth harvest for silica; and at the sixth for NDF (Table 6). In general, the number of harvests for quality traits may be within the range 3 to 6.

Table 6. Phenotypic stabilization in *P. maximum* - estimates of repeatability and determination coefficients of quality traits for different groups of 7 harvests using ANOVA and PCA.

Trait	Eval.	N	ANOVA		PCA		Trait	Eval.	N	ANOVA		PCA	
			r	R^2	r	R^2				r	R^2	r	R^2
Organic Matter	2-5	4	0.52	0.82	0.63	0.87	Crude Protein	1-3	3	0.66	0.85	0.68	0.86
	3-6	4	0.51	0.81	0.64	0.88		2-4	3	0.58	0.81	0.58	0.81
	4-7	4	0.49	0.80	0.55	0.83		3-5	3	0.49	0.74	0.52	0.76
	1-5	5	0.53	0.85	0.65	0.90		4-6	3	0.59	0.81	0.60	0.82
	2-6	5	0.56	0.86	0.67	0.91		5-7	3	0.58	0.81	0.59	0.81
	3-7	5	0.52	0.84	0.58	0.87		1-4	4	0.55	0.83	0.56	0.84
	1-6	6	0.57	0.89	0.69	0.93		2-5	4	0.56	0.83	0.58	0.85
	2-7	6	0.54	0.88	0.61	0.90		3-6	4	0.60	0.86	0.62	0.87
	1-7	7	0.54	0.89	0.62	0.92		4-7	4	0.59	0.85	0.60	0.86
NDF	2-5	4	0.15	0.42	0.17	0.45	ADF	1-3	3	0.59	0.81	0.64	0.84
	3-6	4	0.33	0.66	0.34	0.68		2-4	3	0.52	0.77	0.58	0.80
	4-7	4	0.46	0.77	0.48	0.79		3-5	3	0.67	0.86	0.70	0.87
	1-5	5	0.16	0.48	0.17	0.50		4-6	3	0.81	0.93	0.82	0.93
	2-6	5	0.29	0.67	0.30	0.69		5-7	3	0.82	0.93	0.83	0.94
	3-7	5	0.38	0.75	0.39	0.77		1-4	4	0.56	0.84	0.62	0.87
	1-6	6	0.28	0.70	0.31	0.73		2-5	4	0.52	0.81	0.60	0.86
	2-7	6	0.32	0.74	0.34	0.76		3-6	4	0.75	0.92	0.77	0.93
	1-7	7	0.30	0.75	0.33	0.77		4-7	4	0.83	0.95	0.84	0.95
Lig-K	4-6	3	0.65	0.85	0.67	0.86	Lig-H	1-3	3	0.67	0.86	0.70	0.87
	5-7	3	0.67	0.86	0.67	0.86		2-4	3	0.63	0.83	0.66	0.85
	1-4	4	0.30	0.64	0.34	0.67		3-5	3	0.52	0.77	0.55	0.78
	2-5	4	0.35	0.68	0.44	0.76		4-6	3	0.64	0.84	0.64	0.84
	3-6	4	0.58	0.85	0.60	0.86		5-7	3	0.63	0.84	0.63	0.84
	4-7	4	0.66	0.88	0.67	0.89		1-4	4	0.63	0.87	0.66	0.88
	1-5	5	0.30	0.68	0.36	0.73		2-5	4	0.64	0.87	0.66	0.89
	2-6	5	0.42	0.79	0.53	0.85		3-6	4	0.57	0.84	0.59	0.85
	3-7	5	0.59	0.88	0.61	0.89		4-7	4	0.68	0.89	0.68	0.89
Hemicellulose	1-5	4	0.25	0.57	0.30	0.63	Cellulose	1-3	3	0.71	0.88	0.71	0.88
	2-5	4	0.34	0.67	0.42	0.75		2-4	3	0.65	0.85	0.65	0.85
	3-6	4	0.54	0.82	0.58	0.85		3-5	3	0.57	0.80	0.60	0.82
	4-7	4	0.56	0.83	0.60	0.85		4-6	3	0.70	0.88	0.73	0.89
	1-5	5	0.28	0.66	0.36	0.74		5-7	3	0.68	0.86	0.71	0.88
	2-6	5	0.34	0.72	0.46	0.81		1-4	4	0.62	0.87	0.63	0.87
	3-7	5	0.58	0.87	0.61	0.89		2-5	4	0.64	0.88	0.67	0.89
	1-6	6	0.31	0.73	0.42	0.82		3-6	4	0.68	0.89	0.70	0.90
	2-7	6	0.39	0.79	0.51	0.86		4-7	4	0.72	0.91	0.74	0.92
OMD	1-3	3	0.57	0.80	0.57	0.80	Silica	1-4	4	0.32	0.66	0.42	0.74
	2-4	3	0.60	0.82	0.61	0.82		2-5	4	0.39	0.72	0.53	0.82
	3-5	3	0.47	0.73	0.48	0.73		3-6	4	0.43	0.75	0.57	0.84
	4-6	3	0.58	0.81	0.62	0.83		4-7	4	0.40	0.73	0.46	0.77
	5-7	3	0.52	0.76	0.54	0.78		1-5	5	0.34	0.72	0.45	0.80
	1-4	4	0.59	0.85	0.59	0.85		2-6	5	0.46	0.81	0.60	0.88
	2-5	4	0.52	0.81	0.53	0.82		3-7	5	0.41	0.77	0.48	0.82
	3-6	4	0.56	0.83	0.57	0.84		1-6	6	0.40	0.80	0.51	0.86
	4-7	4	0.62	0.86	0.62	0.87		2-7	6	0.42	0.81	0.51	0.86

Conclusion

The overall mean number of harvests required for an effective selection for DMY in *P. maximum* would fall within the 5-7 range for 0.80 and 0.85 accuracy levels, respectively. The inclusion of DMY from the first two harvests negatively affects the estimate of the repeatability coefficient and its associated accuracy. The inclusion of the dry season harvests may decrease these estimates as well. Phenotypic stability for DMY occurs from the fourth harvest onward, when the repeatability coefficients and associated accuracy reach the highest and stable values.

The mean number of harvests needed for a reliable selection for OM, CP, ADF, lignin-H_2SO_4, cellulose, and IVOMD would be 3 and 5 at 0.80 and 0.85 accuracy levels, respectively. For NDF, Lig-KMnO4, hemicellulose and silica, the mean values would be 6 and 8 at the same accuracy levels. The first and second harvests do not remarkably affect the repeatability estimates of all quality traits, suggesting their use on the estimations. Phenotypic stability first occurred for CP, ADF, Lig-H_2SO_4 and cellulose at the third harvest, followed by OM, Lig-$KMnO_4$, and IVOMD at the fourth, silica at the fifth, and NDF at the sixth harvest.

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