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Determination of the optimal number of evaluations in half-sib progenies of kale by Bayesian approach

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ABSTRACT

Kale has a long vegetative cycle, requiring a lot of labor, due to the need for tutoring, thinning and multiple harvests, leading to difficulties in the maintenance and evaluation of experiments. Thus, the objective was to estimate the minimum number of evaluations for the assertive selection of half-sib progenies of kale by means of a repeatability study by Bayesian approach. Twenty four half-sib progenies were evaluated in a randomized block design with four replicates and five plants per plot. The number of shoots, number of marketable leaves, fresh mass of marketable leaves and fresh mass per leaf were measured throughout 15 harvests. All traits showed high estimates of the repeatability, indicating high regularity in the expression of the traits during the harvesting period. With eight harvests it is possible to evaluate all the traits with a coefficient of determination superior to 85% in half-sib progenies of kale.

Keywords: *Brassica oleracea* var. *acephala*, repeatability, genetic improvement, production of leaves.

RESUMO

Determinação do número ótimo de avaliações em progênies de meios-irmãos de couve por abordagem Bayesiana

A couve possui ciclo vegetativo longo, requerendo muita mão-de-obra, pela necessidade de tutoramento, desbrotas e colheitas múltiplas, trazendo dificuldades na manutenção e avaliação de experimentos. Assim, objetivou-se estimar o número mínimo de avaliações para a seleção assertiva de famílias de meios-irmãos de couve por meio do estudo de repetibilidade, utilizando-se para isso a abordagem bayesiana. Foram avaliadas 24 famílias de meios-irmãos de couve no delineamento em blocos casualizados com quatro repetições e cinco plantas por parcela. O número de brotações, número de folhas comerciais, massa fresca de folha e massa fresca por folha foram avaliadas em 15 colheitas. Todas as características tiveram altas estimativas do coeficiente de repetibilidade, indicando elevada regularidade na expressão das características avaliadas ao longo do período de colheitas. Com 8 colheitas é possível avaliar todas as características com um coeficiente de determinação superior a 85% em progênies de meios-irmãos de couve.

Palavras-chave: *Brassica oleracea* var. *acephala*, repetibilidade, melhoramento genético, produção de folhas.

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Kale (*Brassica oleracea* var. *acephala*) is consumed worldwide. Due to its high nutritional potential, high contents of carotenoids, vitamins C and K, phenolic compounds and organic acids is considered a “superfood” (Sikora *et al.*, 2008; Null & Feldman, 2011). These traits allow it to show antioxidant and antimicrobial properties (Ayaz *et al.*, 2008; Korus, 2011; Soengas *et al.*, 2011; Kaulmann *et al.*, 2014) and some phytotherapeutic properties, such as acting on gastric ulcers and many types of cancer (Vilar *et al.*, 2008; Agbaje & Okpara, 2013).

The crop is considered as an annual

or biennial plant and leaf harvesting is carried out periodically throughout its vegetative cycle. It also demands a lot of labor, as it needs staking, sprout removal and multiple harvests, which brings difficulties in the maintenance and evaluation of experiments. According to Patcharin *et al.* (2013), the lack of information about the minimum number of harvests to adequately evaluate an experiment can lead the researcher to carry out more harvests than necessary to differentiate treatments (Della Bruna *et al.*, 2012). This can cause waste in the utilization of labor and financial resources (Martuscello *et al.*, 2007).

The reliability of the good performance of a genotype throughout successive evaluations can be proven by the repeatability coefficient (Neves *et al.*, 2010), from which the ideal number of harvests can be estimated.

In order to estimate genetic parameters such as repeatability, it is necessary to obtain variance components (Della Bruna *et al.*, 2012; Tenkouano *et al.*, 2012). These are unknown, and generally are estimated by the method of moments, maximum likelihood (ML) or restricted maximum likelihood (REML) (Azevedo *et al.*, 2017). However, Bayesian inference

can be used advantageously, since it allows obtaining the *a posteriori* distribution and credibility intervals of the estimated parameters (Gonçalves-Vidigal *et al.*, 2008; Mathew *et al.*, 2012; Rodvalho *et al.*, 2014). This makes the technique very informative (Mathew *et al.*, 2012) and facilitates hypothesis testing. In addition, Bayesian inference enables the evaluation of experiments with unbalanced data and the study of complex statistical models (Waldmann & Ericsson, 2006; Bink *et al.*, 2007). Consequently, its use is increasing among breeders, not only for the analysis of molecular data, but also for phenotypic data (Waldmann & Ericsson, 2006; Omer *et al.*, 2016). In this sense, the objective was to estimate the minimum number of evaluations for assertive selection of half-sib progenies of kale by means of a repeatability study, using the Bayesian approach.

MATERIAL AND METHODS

The experiment was carried out in field conditions, in the research vegetable garden of the Federal University of Viçosa (UFV), in Viçosa-MG. Twenty-four half-sib progenies of kale were evaluated in a randomized block design with four replicates and five plants per plot. The plants were sown in expanded polystyrene trays. After 60 days, the seedlings were transplanted to seedbeds with approximately 2.5 m width and 0.3 m height, using a 1 m x 0.5 m spacing. From 30 days after transplantation, fifteen harvests were carried out each fourteen days. In five plants per plot the number of shoots (which were removed in the occasion), number of marketable leaves, fresh mass of marketable leaves and fresh mass per leaf were evaluated. The completely expanded leaves with leaf blade length greater than 15 cm and without signs of senescence were considered as marketable ones.

For the repeatability study, the statistical model was used, proposed by Cruz *et al.* (2012): $Y_{ij} = m + g_i + a_j - e_{ij}$, in which: Y_{ij} : observation referring to the *i*-th progenies ($i = 1, 2, \dots, 24$ progenies) in the *j*-th harvest ($j = 1, 2, \dots, 15$ harvests); m : general means; g_i : aleatory

effect of the *i*-th progeny on the influence of permanent environment; a_j : effect of the *j*-th harvest, and, e_{ij} : experimental error associated to the observation Y_{ij} . The Bayesian theorem was used to estimate the variance components. This theorem admits that the *a posteriori* joint distribution of all unknown parameters is proportional to the product of the function with maximum likelihood with the *a posteriori* distribution (Azevedo *et al.*, 2017). Assuming that $e | \sigma_e^2 \sim N(0, \sigma_e^2)$, the sampling distribution of the observed data (function of maximum likelihood) is: $y_{ij} | m, g_i, a_j, \sigma_e^2 \sim N(m + g_i + a_j, \sigma_e^2)$.

For the location parameters, the following *a priori* distributions were considered:

$$m | u_m, \sigma_m^2 \sim N(u_m, \sigma_m^2),$$

$$g_i | u_g, \sigma_g^2 \sim N(u_g, \sigma_g^2) \text{ and}$$

$$a_j | u_a, \sigma_a^2 \sim N(u_a, \sigma_a^2)$$

For the variance components, the inverse chi-squared distribution was considered as the *a priori* distribution:

$$\sigma_g^2 | v_g, s_g \sim v_g s_g X_{v_g}^{-2},$$

$$\sigma_a^2 | v_a, s_a \sim v_a s_a X_{v_a}^{-2} \text{ and}$$

$$\sigma_e^2 | v_e, s_e \sim v_e s_e X_{v_e}^{-2}.$$

Therefore, the joint *a posteriori* distribution can be represented by:

$$P(\theta | y_{ij}) \propto \prod_{i=1}^{24} \prod_{j=1}^{15} \exp\left\{-\frac{[y_{ij} - (m + g_i + a_j)]^2}{2\sigma_e^2}\right\} (\sigma_e^2)^{-0.5} \exp\left\{-\frac{[m - u_m]^2}{2\sigma_m^2}\right\} (\sigma_m^2)^{-0.5} \exp\left\{-\frac{[g_i - u_g]^2}{2\sigma_g^2}\right\} (\sigma_g^2)^{-0.5} (\sigma_g^2)^{-\frac{(v_g+1)}{2}} \exp\left(-\frac{v_g s_g}{2\sigma_g^2}\right) \exp\left\{-\frac{[a_j - u_a]^2}{2\sigma_a^2}\right\} (\sigma_a^2)^{-0.5} (\sigma_a^2)^{-\frac{(v_a+1)}{2}} \exp\left(-\frac{v_a s_a}{2\sigma_a^2}\right) (\sigma_e^2)^{-\frac{(v_e+1)}{2}} \exp\left(-\frac{v_e s_e}{2\sigma_e^2}\right)$$

For the statistical analysis, the rJags package of the R software (R Core Team, 2020) was employed. Since there is no previous work with half-sib progenies of kale to obtain informative *priori*, vague (less informative) *priori* were used. Thus, for the location effects, it was considered $u_i = 0 (i = m, g, a, e)$. For the general means variance component, it was stipulated $\sigma_m^2 = 0,001$. For the other variance components, it was considered $v_i/2 = s_i/2 = 0,001 (i = a, g, e)$. In order to obtain the MCMC chains, 1,000,000 iterations per characteristic were established. Burnin of 100,000 iterations and thin of 500 iterations was used, resulting in a total sample of 1,800 iterations for each characteristic. After confirming the convergence by the Geweke test ($p > 0.05$) and the absence of autocorrelation, the residual coefficient of variation

$$\left(CV_e = \frac{100\sqrt{\sigma_e^2}}{m}\right),$$

coefficient of repeatability

$$(r = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2)),$$

coefficient of determination

$$(R^2 = 100 * 15 * r / [1 + r(15 - 1)])$$

and the optimal number of harvests

$$(no = R_p^2(1 - r) / [1 + r(15 - 1)])$$

were estimated. For all the parameters

$$(\sigma_a^2, \sigma_g^2, \sigma_e^2, m, CV_e, r, R^2)$$

the HPD 95% (high probability density) interval and mode were estimated with the aid of the Bayesian Output Analysis (BOA) package of the software R.

RESULTS AND DISCUSSION

Only the characteristic number of shoots showed overlapping of the HPD intervals for the variance components due to the effects of family and evaluation (Table 1). For the other traits, there were higher estimates of the variance components due to the effects of the evaluations in relation to the family effects, without overlapping the HPD intervals. The obtained asymmetric HPD for the variance components and genetic parameters are a peculiarity

of Bayesian inference and facilitate hypothesis testing (Azevedo *et al.*, 2017).

The higher magnitudes of the variance components of the effects of the evaluations compared to the genetic effects (progenies), without overlapping the credibility interval, indicates the predominance of the evaluation effects when compared to the genetic effects. This happened for number of leaves,

fresh leaf mass and fresh mass per leaf. The higher magnitudes of the variance components of the effects of the evaluations compared to the genetic effects were also verified by Brito *et al.* (2019) when evaluating half-sib kale. The higher magnitudes of the residual coefficient of variation for number of shoots and fresh leaf mass shows that these traits are more influenced by random effects of the environment

(experimental error).

The highest mode for the coefficient of residual variation was found for the number of shoots (19.10%), and its HPD interval only did not overlap those values found for the number of leaves and fresh mass per leaf, which presented the lower modes (8.17 and 10.48%, respectively). There were overlapping HPD intervals between the coefficients of determination of all the traits. The

Table 1. HPD (high probability density) interval, mean, median and mode for the variance components of the evaluations (σ_a^2), progenies (σ_g^2), error (σ_e^2), for the general means (m), residual coefficient of variation (CV_e) and coefficient of determination (R^2) in half-sib progenies of kale. Viçosa, UFV, 2020.

Characteristics	Parameters	HPD		Means	Median	Mode
		2.50%	97.50%			
Number of shoots	σ_a^2	0.30	1.53	0.70	0.63	0.55
	σ_g^2	0.37	1.28	0.70	0.66	0.57
	σ_e^2	0.47	0.64	0.55	0.55	0.54
	m	3.26	4.35	3.80	3.80	3.88
	CV_e (%)	16.73	23.32	19.60	19.43	19.10
	R^2 (%)	90.89	97.28	94.58	94.74	95.31
Number of leaves	σ_a^2	0.58	2.67	1.25	1.13	0.93
	σ_g^2	0.17	0.56	0.31	0.29	0.26
	σ_e^2	0.14	0.18	0.16	0.16	0.16
	m	4.19	5.42	4.82	4.83	4.88
	CV_e (%)	7.15	9.68	8.27	8.22	8.17
	R^2 (%)	93.97	98.18	96.42	96.54	96.67
Fresh mass of leaves	σ_a^2	8344.89	44494.34	19404.11	17081.81	13125.45
	σ_g^2	955.14	3387.68	1852.92	1738.61	1616.06
	σ_e^2	1672.24	2292.04	1950.32	1939.04	1922.51
	m	225.76	373.76	308.16	310.71	314.87
	CV_e (%)	11.59	19.84	14.56	14.19	13.75
	R^2 (%)	87.66	96.44	92.82	93.07	93.28
Fresh mass per leaf	σ_a^2	198.53	950.31	431.65	384.70	345.01
	σ_g^2	20.29	73.49	39.46	36.91	31.77
	σ_e^2	48.72	66.30	57.05	56.86	56.95
	m	61.39	83.11	71.71	71.49	71.12
	CV_e (%)	8.86	12.65	10.59	10.53	10.48
	R^2 (%)	83.82	95.10	90.39	90.72	91.37

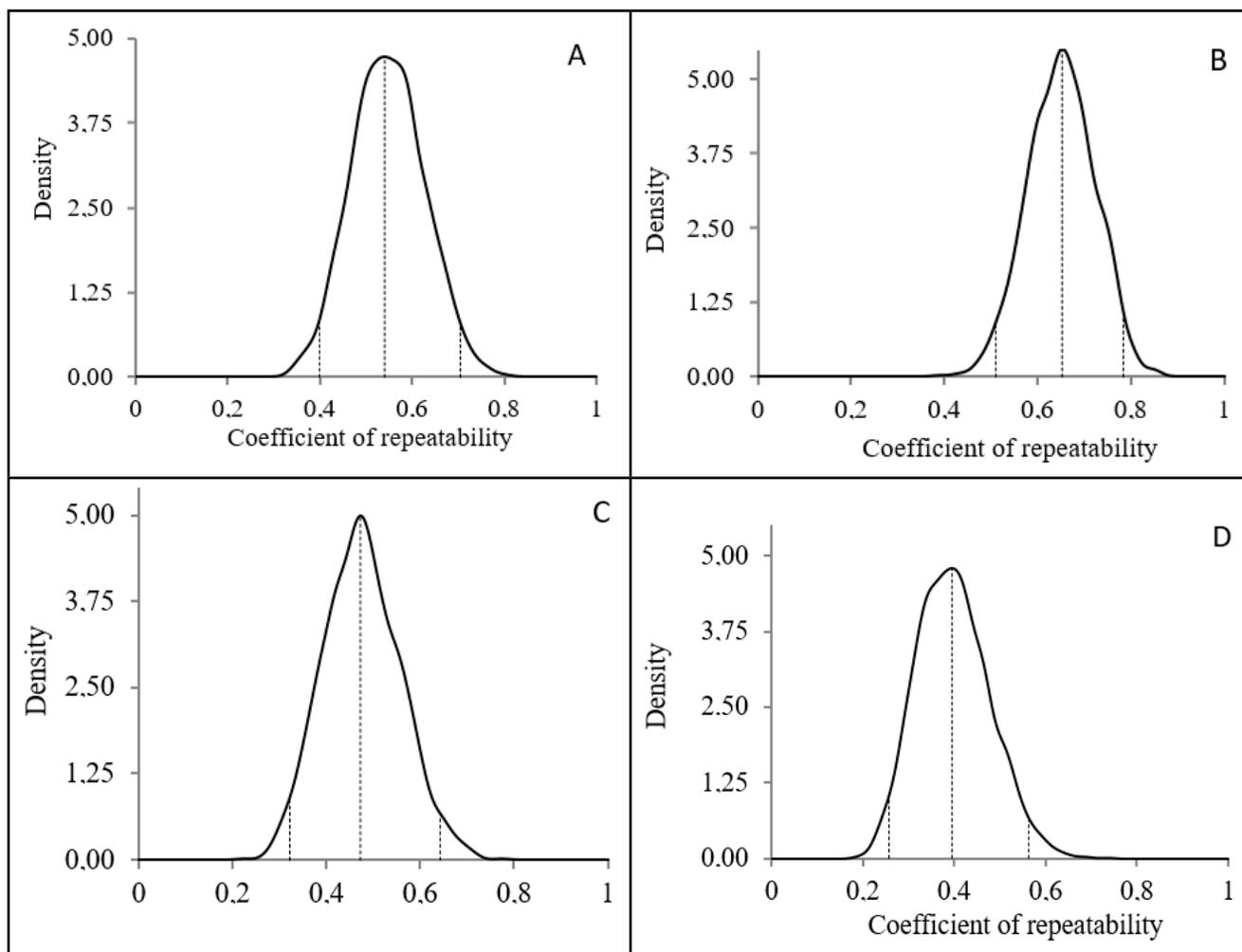


Figure 1. Graphical representation of HPD 95% (high probability density) interval, mode and density of the *a posteriori* distribution of coefficients of repeatability for the number of shoots (A), number of leaves (B), fresh mass of leaves (C) and fresh mass per leaf (D) in half-sib progenies of kale. Viçosa, UFV, 2020.

traits with higher mode values for this parameter were number of leaves and number of shoots (96.67 and 95.31%, respectively).

The lowest mode for repeatability was found for fresh matter per leaf, followed by fresh matter of leaves, with mode values of 0.40 and 0.47 (Figure 1). However, the number of leaves and number of shoots showed the highest estimates of repeatability, with mode values of 0.65 and 0.55, respectively. However, there was an overlap of the HPD interval of repeatability in all traits. The repeatability estimation can vary from 0 to 1, and high coefficients allow to predict the real value for a given characteristic with few measurements (Oliveira & Moura, 2010). The highest estimates for the repeatability coefficient of the number of marketable leaves

(Figure 1) were also found by Brito *et al.* (2019). This indicates for this trait, a smaller increase in the experimental accuracy due to the increase in the number of evaluations (Della Bruna *et al.*, 2012). On the other hand, the trait fresh mass per leaf, with smaller estimates, requires a greater number of harvests for a selection with greater efficiency and reliability.

The number of wished harvests according to the used coefficient of determination indicates that the traits mass of fresh matter per leaf and mass of fresh leaves require a greater number of evaluations for the efficient selection of progenies (Figure 2). This can be justified by the percentage of water in the leaves that can vary at the time of harvest, due to variations in soil moisture, relative humidity or temperature. For this, 13

harvests are required to guarantee the coefficient of determination of 90%, and eight harvests to reach a coefficient of determination of 85%. This information is important, and indicates that in future experiments with half-sib progenies of kale, it is possible to have considerable precision with only eight harvests. This number of harvests is much higher than that found by Azevedo *et al.* (2012), which suggest only three harvests to obtain a coefficient of variation higher than 95%. Among the justifications for the need of a smaller number of harvests found by these authors stands out the fact that they evaluated kale clones. In the present work, the genetic variability within each treatment (half-sib progenies) may have contributed to the lower repeatability coefficients. This justification agrees with the work

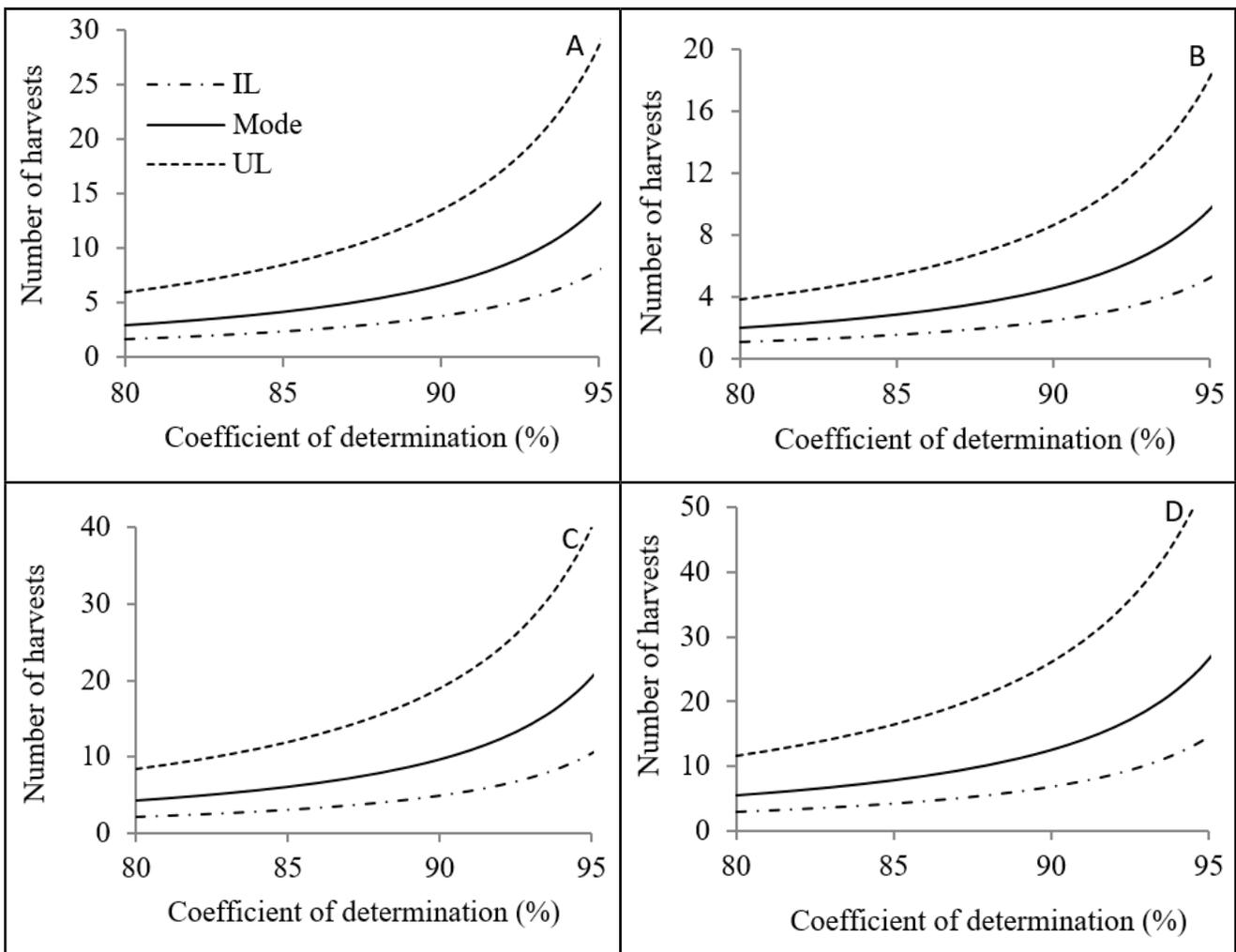


Figure 2. Estimation of mode, lower (IL) and upper (US) limits of the HPD 95% (high probability density) interval of the *a posteriori* distribution of the ideal number of harvests as a function of the different coefficients of determination required for number of shoots (A), number of leaves (B), fresh mass of leaf (C) and fresh mass per leaf (D) in half-sib progenies of kale. Viçosa, UFV, 2020.

done by Cruz *et al.* (2012), in which the authors state that the repeatability coefficient may vary according to the genetic structure of the studied population (clones, half-sib progenies, complete-sib progenies). The higher number of measurements required for the evaluation of fresh mass per leaf may be due to a higher interaction between genotypes and temporary environment in these traits. A possible cause for this interaction may be the regulation of the character by different gene sets, which may be more or less active, depending on the developmental state of the individual (Cruz *et al.*, 2012). More than 8 harvests were also necessary to obtain the determination coefficient greater than 85% by Brito *et al.* (2019) to half-sib progeny.

On the other hand, the number of

leaves is the characteristic that requires a smaller number of evaluations, followed by the number of shoots. From the mode of the *a posteriori* distribution, it is estimated that three, two, four and six evaluations of the traits number of shoots, number of marketable leaves, fresh matter of leaves and fresh matter per leaf are required, respectively, if a coefficient of determination of 80% is desired (Figure 2). To obtain a coefficient of determination of 85%, four, three, six and eight evaluations are required for the number of shoots, number of marketable leaves, fresh matter of leaves and fresh matter per leaf, respectively. To achieve the 90% coefficient of determination, seven, five, ten and 12 evaluations are required, respectively. To obtain the coefficient of 95%, 14, 10, 20 and 26 evaluations

are necessary, respectively.

In breeding programs, this information is important, as it permits knowing the minimum number of evaluations to compare genotypes (Patcharin *et al.*, 2013). This allows avoiding the loss of time with evaluations beyond necessary, also avoiding evaluation for a very short period, which can lead to errors in the identification of the superior genotypes (Neves *et al.*, 2010).

Therefore, it can be concluded that the number of leaves is the characteristic with higher repeatability, as opposed to the fresh mass per leaf, which requires a higher number of harvests for the selection of better half-sib progenies of kale. With eight harvests it is possible to evaluate all the traits with a coefficient of determination superior to 85% in

half-sib progenies of kale.

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