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Selection of *Artemisia annua* genotypes based on agronomic traits

Abstract – The objective of this work was to select *Artemisia annua* genotypes based on the agronomic characteristics of half-sib families. The experiment consisted of 24 treatments, of which 23 with half-sib families of *A. annua* germplasm and one control with Artemis cultivar, in a randomized block design. Data analysis used the restricted maximum likelihood/best linear unbiased predictor methodology and the correlation among agronomic traits. The half-sibs of *A. annua* present high phenotypic variability in agronomic traits. The classification of the ten half-sibs with the greatest results based on the estimated mean of the best linear unbiased predictor for dry mass of the leaves as follows: 1005_2-1; 2713_3-2; 2612_4-1; 1315_2-2; 1107_3-1; 213_4-3; 2612_3-1; 213_1-1; 2612_4-2; and 2417_4-2. The variation of genotypes within half-sib families in leaf dry mass, diameter of the middle portion of the canopy, and the total number of branches enables the selection of specific plant architectures. The agronomic traits branch and leaf dry mass, diameter of the middle portion of the canopy, and total branches show higher heritability in *A. annua* and are suitable for field selection.

Index terms: Artemisinin, plant breeding, REML/BLUP.

Seleção de genótipos de *Artemisia annua* com base em caracteres agronômicos

Resumo – O objetivo deste trabalho foi selecionar genótipos de *Artemisia annua* com base nas características agronômicas de famílias de meios-irmãos. O experimento consistiu-se em 24 tratamentos, dos quais 23 com famílias de meios-irmãos do germoplasma de *A. annua* e um controle com a cultivar Artemis, em delineamento em blocos casualizados. A análise dos dados utilizou a metodologia de máxima verossimilhança restrita/melhor previsor linear não viesado e a correlação entre características agronômicas. Os meios-irmãos de *A. annua* apresentam alta variabilidade fenotípica em caracteres agronômicos. A classificação dos dez meios-irmãos com os resultados mais promissores com base na média estimada do melhor preditor linear não enviesado para massa seca das folhas é a seguinte: 1005_2-1; 2713_3-2; 2612_4-1; 1315_2-2; 1107_3-1; 213_4-3; 2612_3-1; 213_1-1; 2612_4-2; e 2417_4-2. A variação na massa seca das folhas, no diâmetro do caule na seção mediana e no número total de ramos de genótipos de famílias de meios-irmãos permite a seleção de arquiteturas vegetais específicas. As características agronômicas massa seca de ramos e folhas, diâmetro do caule na seção mediana e ramos totais apresentam maior herdabilidade em *Artemisia annua* e são adequadas para seleção em campo.

Termos para indexação: Artemisinina, melhoramento vegetal, REML/BLUP.

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Introduction

Artemisia (*Artemisia annua* L.), a plant from the Asteraceae family with a diversity center in China, was acclimatized for cultivation in various countries (Acquaviva et al., 2023). *A. annua* is the main source of artemisinin, the primary compound used in the treatment of malaria (Wetzstein et al., 2019), an endemic tropical disease caused by a haemosporidian blood parasite of the genus *Plasmodium*.

Bolina et al. (2013, 2014) conducted studies on the genetic variability of the species and observed that plant height contributes significantly to variability among *A. annua* plants. Paul et al. (2014) reported a correlation between taller plants and a pyramidal canopy shape. However, there is no specific classification for morphological descriptors of the species, only a phenological scale is available (Marchese et al., 2023). According to Wetzstein et al. (2018), *A. annua* open-pollinated cultivars produced by mass selection and hybrid cultivars from the intercrossing of heterozygous lines exhibit high variability from plant to plant when propagated by seeds.

The methodology of selecting genotypes through mixed models has been widely used in genetic improvement (Sá et al., 2021). The use of restricted maximum likelihood (REML) methodology (Patterson & Thompson, 1971), for estimating variances (Resende & Alves, 2021), and best linear unbiased predictor (BLUP) (Henderson, 1975), for predicting genetic values in mixed models, is an alternative for the genotypic evaluation of plants, especially with unbalanced data (Resende, 2016). Accurate predictions can be made with unbalanced data with mixed models, enabling the evaluation of individual characteristics, such as family and repeated measures over time simultaneously (Muth et al., 2016). Selection based on half-sib progeny tests captures the entire additive genetic variance, enabling selection within and among families. BLUP selection determines the number of individuals to be selected within families, the total number of individuals to be chosen, and the number of families to be selected (Abu-Ellail et al., 2018).

Mixed models have been used in the genetic study of various medicinal and aromatic plants, such as spearmint (*Mentha spicata* L.) (Joshi et al., 2024), annual caraway (*Carum carvi*) (von Maydell et al., 2021), *Curcuma caesia* (Munda et al., 2023), and Greek oregano (*Origanum vulgare* L. subsp. *hirtum*) (Sarrou et al., 2023). However, only Townsend et al. (2013) published a work employing the combination of REML and BLUP methodologies in *A. annua*, to estimate general combining ability in diallel crosses and identify quantitative trait loci for the species.

The objective of this work was to select *Artemisia annua* genotypes based on the agronomic characteristics of half-sib families.

Materials and Methods

The research was conducted in the research center of Universidade Tecnológica Federal do Paraná (UTFPR), located in the municipality of Pato Branco, in the state of Paraná, Brazil (26°10'32"S, 52°41'24"W, 760 m of altitude). According to the Köppen classification updated by Kottek et al. (2006), the climate in the region is classified as Cfa subtropical. Temperature, humidity, and precipitation data were obtained from POWER Project (Nasa, 2023). During the period of 2020/2021, the plants remained in the field for 159 days, and the accumulated precipitation was 718 mm, with an average temperature of 22.49°C (Figure 1).

The experiment consisted of 24 treatments, with three plants each, of which one control and 23 half-sib families, arranged in a randomized complete block design with three replicates. The germplasm was selected from the *A. annua* breeding program of UTFPR, and the control treatment was carried out using Artemis cultivar.

The seedlings were obtained in a greenhouse and sowed in the last week of July in tubes under an artificially extended photoperiod of 16 hours of light and 8 hours of dark. Transplanting took place on October 15th, 2020, when the seedlings were approximately 0.2 m tall, and the day length was above the critical flowering photoperiod of the plants (13 hours and 2 minutes), according to the results of Marchese et al. (2023).

According to the Brazilian Soil Classification System (Santos et al., 2018), the soil was classified as Latossolo Vermelho distroférrico típico (Oxisol) with the following characteristics in the 0–20 cm soil depth: pH ($CaCl_2$ 1:2.5), 5.4; exchangeable Al ($cmol_c$ dm^{-3}), 0.0; Ca + Mg ($cmol_c$ dm^{-3}), 7.25, high; P (mg dm^{-3}), 16.81, high; K (cmolc dm^{-3}), 0.51, high; organic matter (g dm^{-3}), 38.2, medium; base saturation (V, %), 69.59, medium; aluminum saturation (%), 0.0; sum of bases ($cmol_c$ dm^{-3}), 6.36, high; CEC ($cmol_c$ dm^{-3}), 11.82. The plants were irrigated and arranged in a 1x1 m spacing, with a border line around the experiment. For NPK fertilization, 140 kg ha^{-1} of urea (45% N), 135 kg ha^{-1} of P_2O_5, and 120 kg ha^{-1} of K_2O were used.

The selection of the half-sib families (HSFs) took place in March 2021, considering visual agronomic traits. Of the 23 HSFs initially installed, 19 completed the cycle until flowering, of which three plants were selected out of seven HSFs, two out of ten HSFs, and two 'Artemis' plants were selected as control, totaling 46 plants. The following agronomic traits were measured in the selected plants: plant height (PH), in meters; diameter of the middle portion of the canopy (CSM), in meters; diameter of the middle portion of the main branch (MBD), in millimeters; and total number of branches (NB).

From March 15th to 18th, 2021, when approximately 50% of the plants had initiated floral bud formation, the branches from the lower two-thirds of the plants were collected, leaving the upper portion of the plant for flowering and seed production. At the same time, phenotypic evaluation of the plants was performed using the proposed scale for morpho-agronomic descriptors (Table 1), using the parameters proposed by Paul et al. (2014).

Branches and leaves were separated and dried in an oven at 40°C, with air circulation to remove moisture and reach constant mass. Subsequently, the dry mass of the leaves (LDM) and the dry mass of the branches (BDM) were obtained to calculate the ratio of LDM/BDM (RLB) and the total biomass (BIOT), by LDM + BDM = BIOT.

Data analysis and genotype selection were performed using the mixed model approach, model 15, with the statistical program Selegen-Reml/Blup (Resende, 2016). The Individuals Evaluation in Half-Sibling Progenies, or Open Pollination in Allogamous Species,

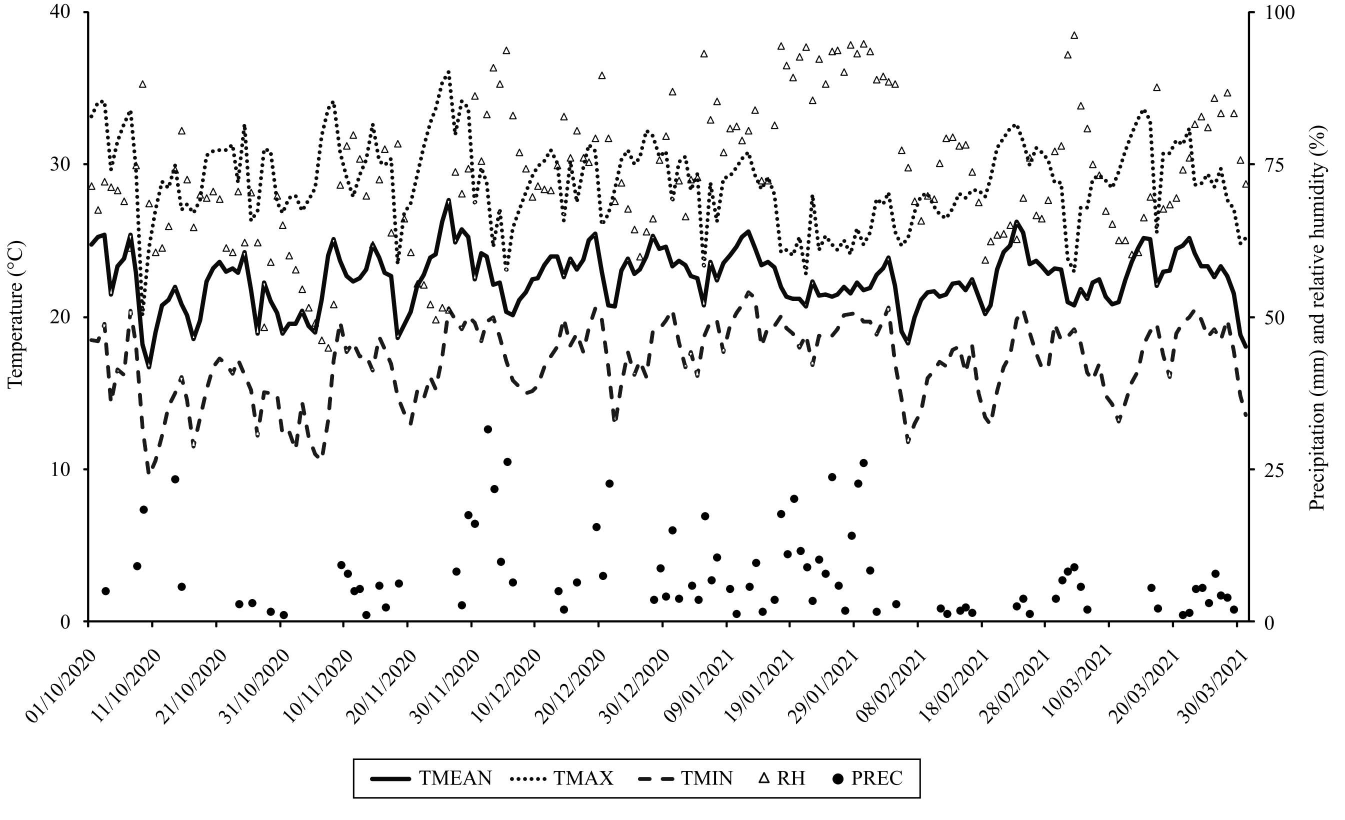

Figure 1. Climatic conditions: average mean temperature (TMEAN), maximum temperature (TMAX), minimum temperature (TMIN), relative humidity (RH), and precipitation (PREC) of the municipality of Pato Branco, in the state of Paraná, Brazil (26°10'32"S, 52°41'24"W) from October 1st, 2020, to March 31st, 2021. Source: Prediction of Worldwide Energy Resources (POWER) (Nasa, 2023).

One Observation per Plot, Incomplete Blocks (Model 15) were utilized. Iterative estimators of variance components by REML via Expectation-Maximization (EM) algorithms included additive genetic variance, residual variance, individual phenotypic variance, narrow-sense heritability within the block, individual additive genetic coefficient of variation, and progeny selection accuracy.

The correlation analysis between traits was conducted according to Pearson's methodology, utilizing the coorplot (Wei & Simko, 2021) and agricolae (Mendiburu, 2023), packages in the RStudio programming language (RStudio Team, 2020).

Table 1. Morpho-agronomic descriptors for the phenotypic classification of *Artemisia annua* plants(1).

	Canopy shape
Type 1	Pyramidal: The lower section of the canopy has a larger diameter than the others do, and the upper section has the smallest diameter.
Type 3	Rectangular: All sections of the canopy have similar diameters.
Type 5	Oval: The middle section has a larger diameter than the others do.
Type 7	Cup: The upper section has a larger diameter than the others do, and the lower section has the smallest diameter.
	Type of ramification
Type 1	The main branch stands out from the others, with lateral branches reaching up to the median section of the canopy.
Type 3	The main branch is still noticeable, but the secondary branches extend to the upper section of the canopy.
Type 5	The main branch needs to be clarified, and the secondary branches reach the upper section of the canopy.
	Intensity of branching
Type 1	Minimal branching, allowing easy visibility through the plant.
Type 3	A moderate number of secondary and tertiary branches partially obstruct the view through the plant.
Type 5	A high quantity of branches entirely obstructs the view through the plant.
	Canopy openness
Type 1	Basal branches at an angle of approximately less than 15º relative to the ground.
Type 3	Basal branches at an angle of approximately 45º relative to the ground.
Type 5	Basal branches at an angle greater than 60º relative to the ground.

(1)Descriptors proposed by the authors, using the parameters according Paul et al. (2014).

Results and Discussion

The high variability found among half-sibs for agronomic and phenotypic traits (Table 2) allows the selection of genotypes for different purposes and agronomic management strategies. Genetic variability is fundamental in breeding medicinal plants producing bioactive compounds to provide superior genotypes with high yield and adaptability; however, it is necessary to achieve stability in the characters of a hybrid for commercial purposes (Zutic et al., 2016). The present study observed genetic diversity among genotypes, which permits to identify and enhance traits related to the production of valuable bioactive compounds.

Phenotypic variance (Vf) was decomposed into additive variance (Va), block variance (Vbloc), and residual variance (Ve). The variance components BDM, LDM, RLB, BIOT, MBM, and NB showed that Va was 50% higher than Vf, indicating a lower environmental effect on these traits (Table 3). The genetic variance is critical in a plant breeding program to improve variability and select superior genotypes (Chaturvedi et al., 2022). The traits evaluated also exhibited high h^2a values, more significant than 0.5, which means that these traits in breeding cycles are more suitable for achieving significant genetic gains compared to other characteristics. Furthermore, the evaluated traits are crucial for cultivar selection, as the low concentration of artemisinin in leaves can be compensated by a high biomass input, resulting in a high yield per hectare. For all traits, the Vbloc showed low participation in the phenotypic variance, indicating low variability among genotypes of half-sib families.

The method genetic gain per unit of time (Lin et al., 2016), that evaluates the efficiency of a genetic improvement program, showed an inefficient selection of PH and CSM traits with low heritability (h^2a). Araújo et al. (2014) found similar results of plant dry mass with low heritability obtained with the medicinal plant billy goat weed (*Ageratum conyzoides* L); however, low heritability observed for some traits does not imply inefficiency in their selection. Assunção et al. (2015) showed that these traits that may result in lower genetic gains per selection cycle can still be utilized, since heritability can be increased by better environmental control. Narrow-sense heritability, the ratio of additive genetic variance to total phenotypic variance (Borém et al., 2021), is important when *A. annua* is propagated by seeds, as it proportionally expresses the variation that can be inherited; however, vegetative propagation of genotypes provides more significant genetic gains, since all additive, dominant, and epistatic genetic variances can be capitalized.

Estimates for CVgi were higher for BDM, MBD, and NB (Table 3), indicating the possibility of variation within families and the selection of genotypes with specific architecture for different population management practices in cultivation.

The distribution of observed field values and the BLUP-estimated means for BDM, LDM, BIOT, CSM, and NB were different and distant from the overall mean due to high genetic parameters, such as additive variance and heritability, thus avoiding erroneous selection based solely on the average without the application of BLUP (Figure 2). On the other hand, the distribution of observed field values and means for BDM, BIOT, and PH was similar and close to the

Table 2. Descriptive statistics for agronomic traits of *Artemisia annua* genotypes.

Agronomic trait	CV(1) (%)	Maximum	Average	Minimum
Branch dry mass (g)	37.95	1,467.40	813.30	41.90
Leaf dry mass (g)	30.48	486.60	278.60	79.40
Ratio leaf/branch	76.18	1.89	0.43	0.18
Total biomass (g)	34.22	1,846.60	1,091.96	121.30
Plant height	12.38	2.97	2.39	1.72
Diameter of the middle portion of the canopy (m)	29.79	2.20	1.06	0.48
Diameter of the middle portion of the main branch (mm)	29.10	20.77	14.56	5.26
Total number of branches	20.19	75.00	54.70	22.00

(1)CV, coefficient of variation.

Table 3. Variance components of the agronomic traits: branch dry mass (BDM), leaf dry mass (LDM), ratio leaf/branch (RLB), plant height (PH), diameter of the middle portion of the canopy (CSM), diameter of the middle portion of the main branch (MBD), and total number of branches (NB) of *Artemisia annua* genotypes.

	BDM	LDM	RLB	BIOT	PH	CSM	MBD	NB
Va(1)	94,147.76 (96.62%)	7,128.19 (110.30%)	0.60 (176.47%)	153,197.77 (111.04%)	0.00 (0.00%)	0.00 (0.00%)	14.52 (75.47%)	111.84 (97.92%)
Vbloc	1,975.01 (2.03%)	7.61 (0.12%)	0.19 (55.88%)	634.16 (0.46%)	0.00 (0.00%)	0.03 (25.00%)	1.28 (6.65%)	0.29 (0.25%)
Ve	1,314.93 (1.35%)	-672.96 (-10.41%)	-0.44 (-129.41%)	-15,867.22 (-11.50%)	0.07 (100.00%)	0.09 (75.00%)	3.43 (17.83%)	2.07 (1.81%)
Vf	97,437.69 (100.00%)	6,462.84 (100.00%)	0.34 (100.00%)	137,964.72 (100.00%)	0.07 (100.00%)	0.12 (100.00%)	19.24 (100.00%)	114.21 (100.00%)
h^2a	0.97±0.85	1.10±0.90	1.73±1.13	1.10±0.90	0.13±0.10	0.11±0.09	0.75±0.75	0.98±0.85
c^2bloc	0.02	0.00	0.54	0.00	0.00	0.27	0.07	0.00
h^2mp	0.98	1.14	44.48	1.16	0.01	0.01	0.76	0.98
Acprog	0.99	1.07	6.67	1.08	0.10	0.11	0.87	0.99
CVgi%	37.28	29.70	219.62	35.44	1.27	3.40	26.62	19.28
CVgp%	18.64	14.85	109.81	17.72	0.64	1.70	13.31	9.64
CVe%	32.59	24.05	28.52	28.49	11.02	27.36	26.44	16.90
PEV	430.30	-256.62	-6.49	-6,136.51	0.00	0.00	0.87	0.67
SEP	20.74	0.00	0.00	0.00	0.01	0.02	0.93	0.82
Average	823.04	284.23	0.35	1,104.53	2.35	1.09	14.31	54.87

(1)Va, additive genetic variance; Vbloc, environmental variance among blocks; Ve, residual variance; Vf, individual phenotypic variance; $h^2a = h^2$, individual heritability in the narrow sense, i.e., from additive effects; $c^2bloc = c^2$, determination coefficient of block effects; h^2mp, heritability of progeny means, assuming complete survival; Acprog, accuracy of progeny selection, assuming complete survival; CVgi%, individual additive genetic coefficient of variation; CVgp%, genetic coefficient of variation among progenies; CVe%, residual coefficient of variation; PEV, prediction error variance of genotypic values, assuming complete survival; SEP, standard error of predicted genotypic value, assuming complete survival.

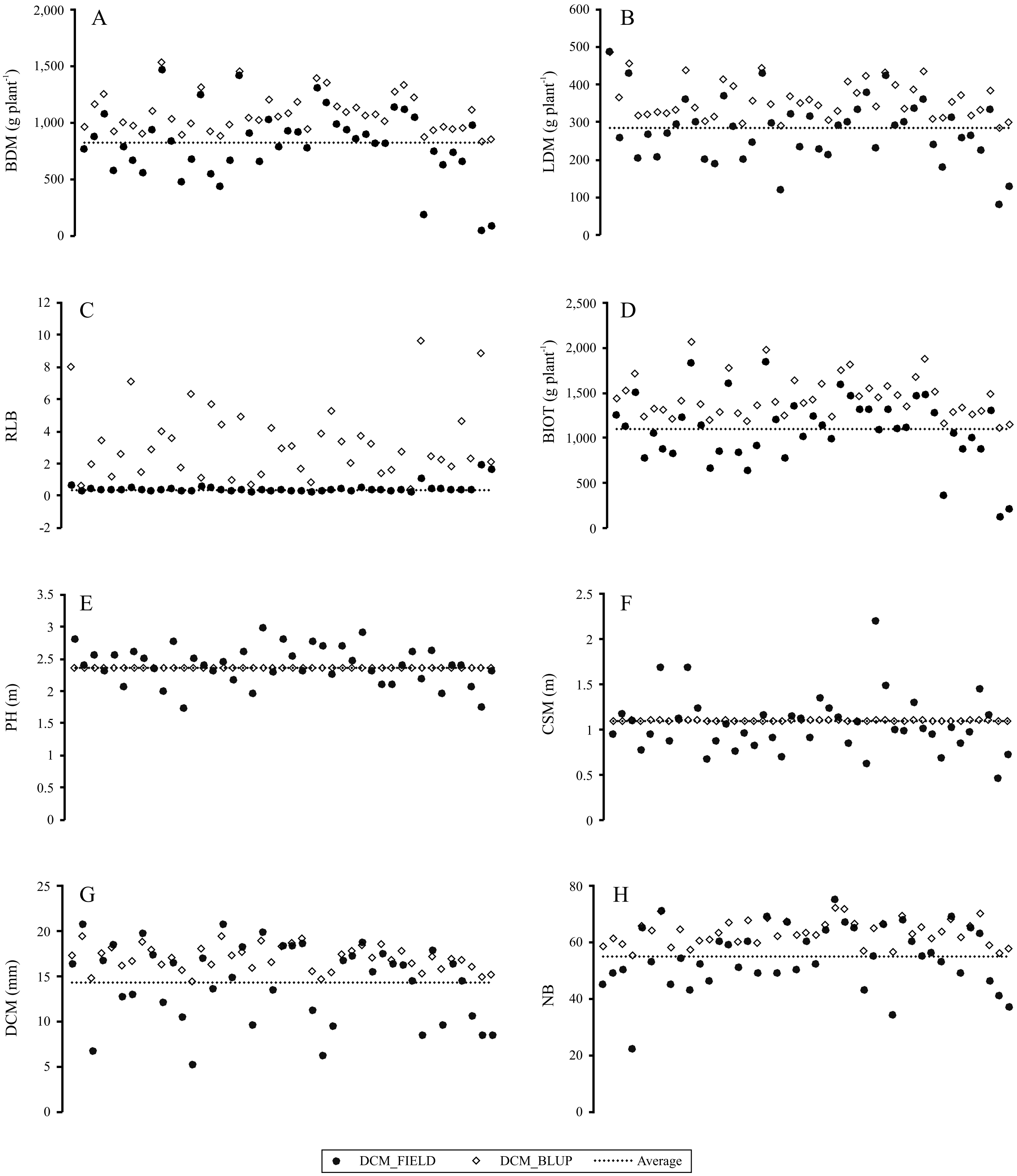

Figure 2. Best linear unbiased predictor (BLUP) and field original data collected distributed over the overall field mean (horizontal dashed line) for the agronomic traits: branch dry mass (A), leaf dry mass (B), ratio leaf/branch (C), total plant biomass (D), plant height (E), diameter of the middle portion of the canopy (F), diameter of the middle portion of the main branch (G), total number of branches (H) of *Artemisia annua* genotypes.

overall average due to shallow genetic parameters, such as heritability. In this case, selection becomes efficient as all genotype variation is attributed to environmental variation.

It was observed that the top seven genotypes for LDM were consistently ranked both by the BLUP-estimated means and field data (Figure 3); however, the ranking order between the two selection criteria

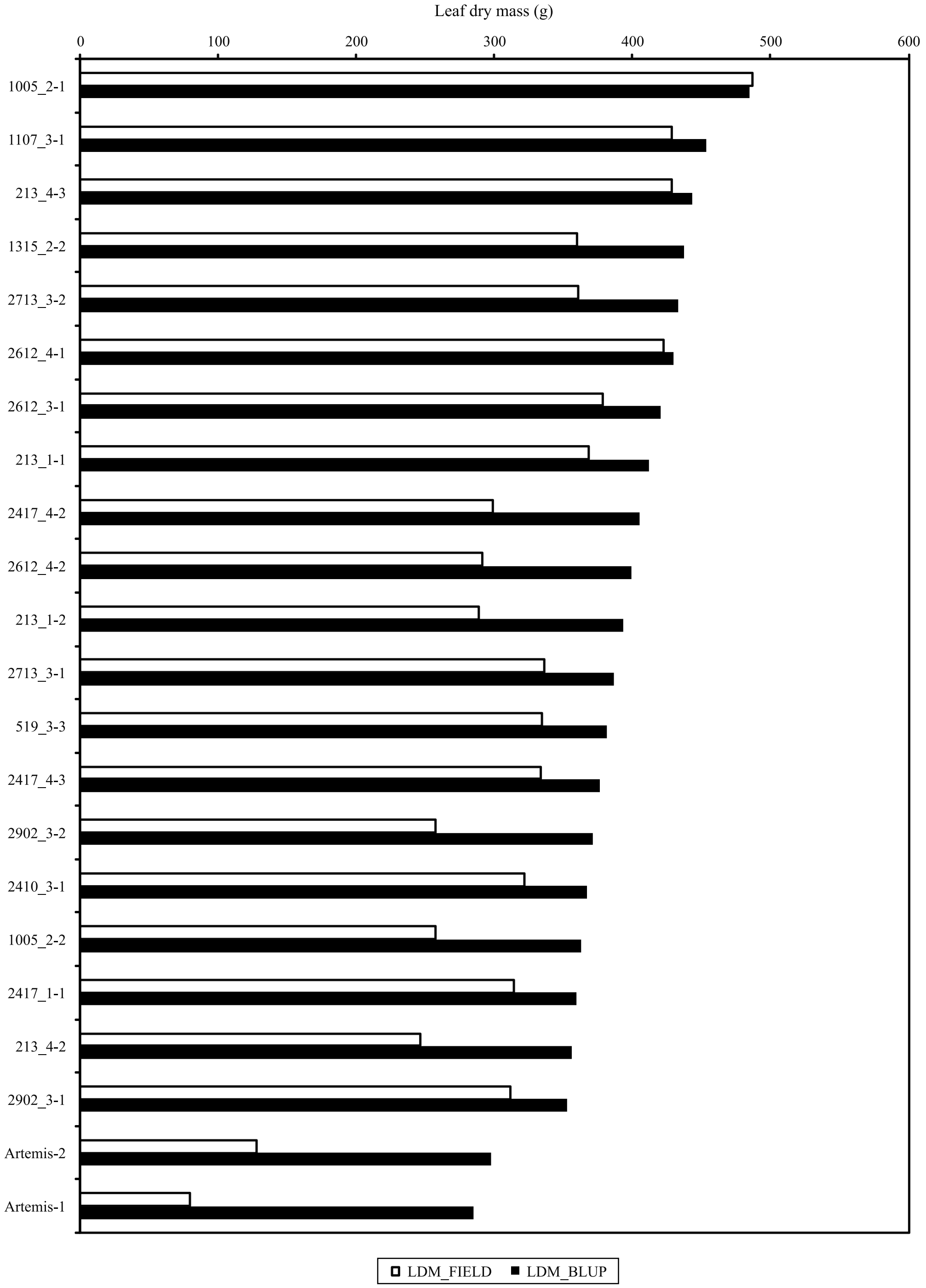

Figure 3. Classification of the 20 best genotypes and two controls (Artemis-1 and -2) for leaf dry mass (LDM) by the estimated best linear unbiased predictor (BLUP) mean compared to field original data.

differed significantly, as suitable genotypes with higher field averages did not necessarily have good BLUP means, as seen with genotypes 2417_4-2, 2612_4-2, and 213-1-2. Therefore, it was possible to identify superior genotypes for the LDM trait quickly. The selection of genotypes with high leaf production can increase the yield of artemisinin.

Phenotype variability among the genotypes was evident in the field, as an example, four genotypes were selected (Figure 4). While genotypes 1315_2-2, 1005_2-2, and 2713_3-3 (Figures 4 B, E, and F, respectively) exhibit a canopy resembling an inverted pyramid, Type 7, genotype 2612_4-1 (Figure 4 A) displays a pyramid-shaped canopy. In contrast, the 'Artemis' plants (Figures 4 C and D) have a cylindrical canopy shape that corresponds to Type 3. Genotypes 2612_4-1 and Artemis_2-2 branches are Type 1, while the other branches are closer to Type 5 (Table 1).

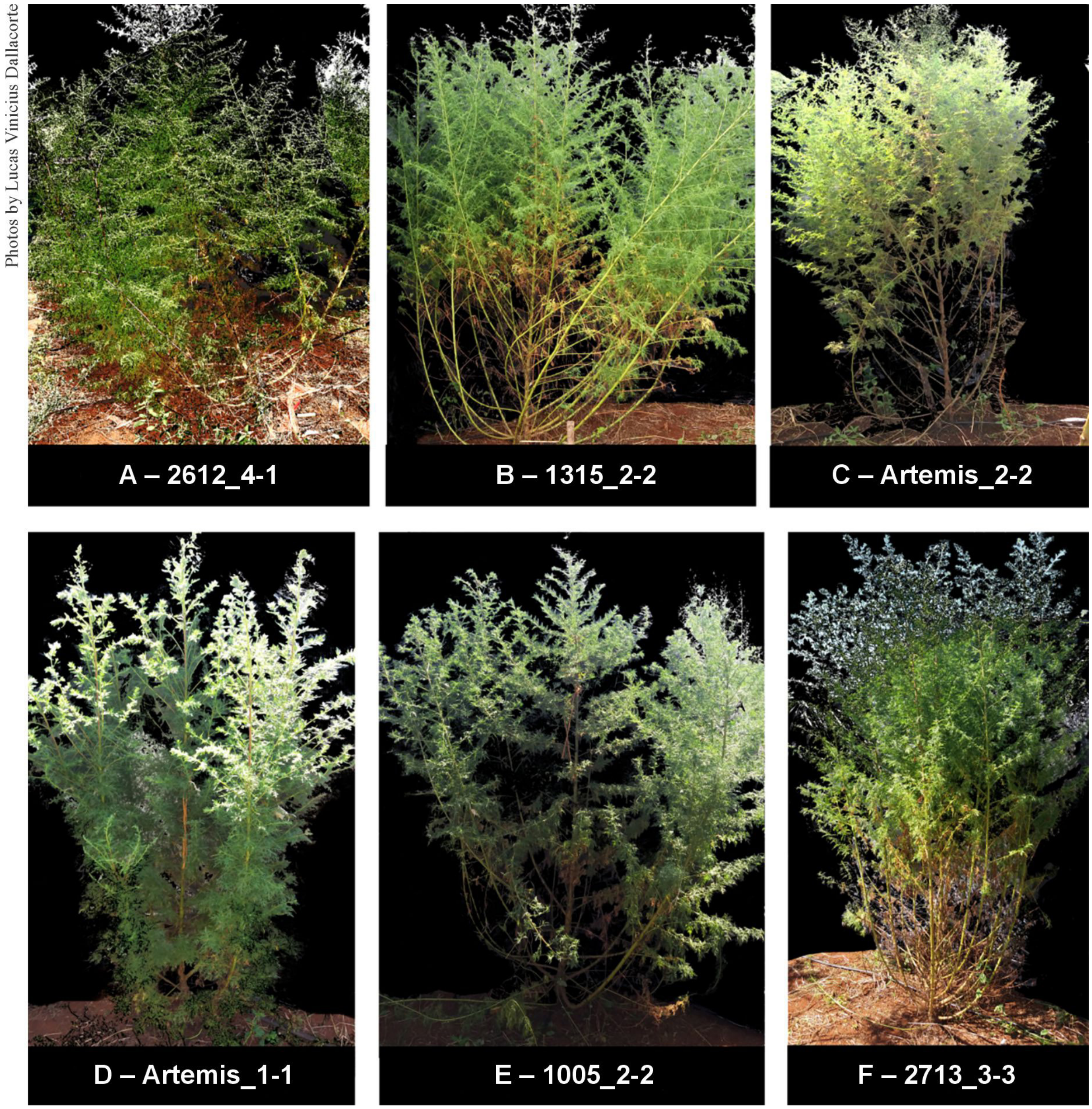

Figure 4. The four best genotypes of *Artemisia annua*: 2612_4-1 (A), 1315_2-2 (B), 1005_2-2 (E), and 2713_3-3 (F), selected by the restricted maximum likelihood/best linear unbiased predictor methodology and the control 'Artemis' (C and D).

There are few published articles on phenotypic characterization of *A. annua*. Paul et al. (2014) classified the canopy shape of *A. annua* plants in a plant breeding program for four years, using oval, pyramidal, and spread/rectangular shapes. Using open-cross-pollinated plants, the authors obtained a high variability among half-sibs in canopy shape, with the oval shape canopy plants having descendants with oval and pyramidal dossel shapes. The authors highlighted that the plants with the highest artemisinin content presented an oval-shaped canopy.

The positive correlation between LDM and PH (Table 4) corroborates the findings of Naghavi et al. (2019). Artemisinin positively correlates with plant height, primary stem diameter, internode distance, leaf length, and width (Fu et al., 2016). In the field, it was possible to select plants with higher biomass and canopy diameter or average stem diameter to obtain better leaf dry matter correlated with artemisinin. LDM also had significative correlation with canopy openness. PH was positively correlated with MBD and NB, similar to the results by Kumar et al. (2019) between plant height and number of branches in mustard (*Brassica juncea* L.). RLB had a significant negative correlation with CSM, MBD, and NB, which demonstrates that plants prioritizing leaf production over branches tend to have reduced volume, indicating that greater branch biomass production increases plant volume.

Quantitative and qualitative descriptors support the breeding selection of superior genotypes, as shown in studies with sweet potato (Palumbo et al., 2019) and field pea (Azam et al., 2024). Bolina et al. (2014) observed that the canopy diameter of *A. annua* plants was the trait that contributed the least to the genetic dissimilarity of the species. Naghavi et al. (2019) evaluated 60 *A. annua* accessions from different provinces of Iran and observed differences only in ART content, with no significant differences in plant height or biomass. Additionally, these two traits showed no significant correlation with ART. Therefore, the selection focus should prioritize phytochemical traits over agronomic characteristics.

Paul et al. (2011) observed positive correlations among artemisinin content, plant height, and number of branches. Fu et al. (2016) found a positive correlation between artemisinin content and yield with plant height. Although the artemisinin content was not evaluated, selecting higher leaf dry mass can increase plant height, which may indirectly increase artemisinin content, a commercially valuable product, or yield.

The phenotypic traits: canopy shape (COPA), type of ramification (RAMI), and intensity of branching (INTE) did not show a significative correlation with any agronomic trait. Only ABER had a negative

Table 4. Pearson linear correlation among the agronomic traits: branch dry mass (BDM), leaf dry mass (LDM), ratio leaf/branch (RLB), total plant biomass (BIOT), plant height (PH), diameter of the middle portion of the canopy (CSM), diameter of the middle portion of the main branch (MBD), total number of branches (NB) and phenotypic traits canopy shape (COPA), type of ramification (RAMI), intensity of branching (INTE), and canopy openness (ABER) of *Artemisia annua* genotypes in 2021.

	BDM	LDM	RLB	BIOT	PH	CSM	MBD	NB	COPA	RAMI	INTE
LDM	0.71**										
RLB	-0.70**	-0.40**									
BIOT	0.99**	0.81**	-0.67**								
PH	0.32*	0.46*	-0.25	0.37*							
CSM	0.46**	0.25	-0.43**	0.44**	0.08						
MBD	0.14	0.17	-0.33*	0.16	0.40**	0.25					
NB	0.31*	0.22	-0.31*	0.31*	0.42**	0.20	0.15				
COPA	0.28	0.09	-0.30	0.25	-0.03	-0.09	0.00	0.10			
RAMI	0.19	-0.05	-0.18	0.14	-0.18	-0.09	-0.11	-0.01	0.52**		
INTE	0.23	0.06	-0.18	0.20	-0.14	-0.14	-0.29	0.04	0.45**	0.47**	
ABER	-0.22	-0.45**	0.09	-0.28	-0.09	-0.26	-0.37*	0.11	-0.09	0.27	0.18

* and ** Significant at 5% and 1%, respectively.

correlation with LDM and MBD, indicating that materials with higher canopy openness tend to present lower leaf growth, resulting in lower plant mass.

Conclusions

1. The half-sibs of *Artemisia annua* present high phenotypic variability in agronomic traits.
2. The classification of the ten half-sibs with the greatest results based on the estimated mean of best linear unbiased predictor for dry mass of the leaves is as follows: 1005_2-1; 2713_3-2; 2612_4-1; 1315_2-2; 1107_3-1; 213_4-3; 2612_3-1; 213_1-1; 2612_4-2; and 2417_4-2.
3. The variation of genotypes within half-sib families in leaf dry mass, diameter of the middle portion of the canopy, and the total number of branches enables the selection of specific plant architectures.
4. The agronomic traits branch and leaf dry mass, diameter of the middle portion of the canopy, and total branches show higher heritability in *Artemisia annua* and are suitable for field selection.

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