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PLANT BREEDING

# Tomato families possessing resistance to late blight also display high-quality fruit

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**ABSTRACT.** In recent years, several efforts have been made to develop tomato cultivars displaying both late blight resistance and good organoleptic fruit quality. Selection indexes are considered the best option to perform genotype selection when many different traits are being considered to select genotypes as close to the desired ideotype as possible. Therefore, this study aimed at selecting late blight-resistant tomato families based on their fruit quality attributes using factor analysis and ideotype-design / best linear unbiased predictor (FAI-BLUP) index. For this purpose, we assessed the fruit quality parameters of 81 $F_{3:5}$ tomato families previously selected as late blight resistant. The tomato cultivars Thaise, Argos, and Liberty were included in the trial as checks. The experimental arrangement consisted of complete randomized blocks with three replicates. Each plot was formed by five plants, three of which were used in the fruit quality assessment. The quality parameters assessed were fruit diameter, fruit length, fruit color (L, a*, C, and H), fruit firmness, titratable acidity, soluble solids content, hydrogen potential, and SS:TA ratio. Fruit quality data were analyzed using the mixed model methodology via REML/BLUP (restricted residual maximum likelihood / best linear unbiased prediction) to obtain BLUPs that were further subjected to the FAI-BLUP selection index. The FAI-BLUP was efficient in selecting late blight-resistant tomato genotypes based on their fruit quality attributes. Fourteen tomato families were classified as closest to the desirable ideotype for fruit quality. These genotypes should move on to the following stages of the tomato breeding program.

**Keywords:** quality parameters; FAI-BLUP index; *Phytophthora infestans*; *Solanum lycopersicum*.

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## Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely grown vegetables worldwide. In Brazil, tomatoes rank second in importance after potatoes (Foolad, 2007; Socaci et al., 2014; Instituto Brasileiro de Geografia e Estatística [IBGE], 2019). Belonging to the solanaceous family, this crop is widely known for its socioeconomic and nutritional benefits. In 2019, 180.8 million tons of tomatoes were grown throughout the world on about 5 million hectares of farmland (Food and Agriculture Organization of the United Nations [FAOSTAT], 2021).

Due to its importance, tomato has been the target of many studies, especially those regarding increased crop yield, improved water, and nutrient use efficiency, resistance to pests and diseases, and enhanced fruit quality (Copati et al., 2019; Dariva et al., 2021; Shibzukhov, Bagov, Shibzukhova, Khantsev, & Akbar, 2021; Wu et al., 2021; Oliveira Dias et al., 2023). Tomatoes are susceptible to approximately 200 different diseases caused by fungi, bacteria, or nematodes (Nick et al., 2013; Campos, Félix, Patanita, Materatski, & Varanda, 2021). Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is considered one of the most destructive because entire fields can be lost in just a few days if control measures are not applied (Hashemi et al., 2022)

Currently, late blight management in tomato production fields is performed through the application of both preventive and curative fungicides (Copati et al., 2019; Kilonzi, Mafurah, & Nyongesa, 2023). Such an approach substantially increases production costs and causes serious contamination problems for workers and the environment (Kilonzi et al., 2023). Therefore, the use of genetically resistant varieties to control late blight in tomato fields has been considered a promising strategy (Kumar et al., 2022).

In addition to disease resistance, a tomato variety must also display satisfactory agronomic performance and good fruit quality attributes to succeed in the seed market. In the last few years, several initiatives have been carried out to combine late blight resistance and good fruit quality in tomato genotypes.

Tomato fruit quality can vary depending on the growing season, the cultivar, and the crop management practices adopted (Maach et al., 2020). This affects sales, as only fruits that meet consumer expectations are purchased. To be considered high quality, tomato fruit must possess a combination of desirable traits, such as fruit size, shape, firmness, color, taste, and soluble solids content. In the process of releasing a variety that displays multiple desirable traits, such as disease resistance and fruit quality, in the same genotype, plant breeders must adopt selection strategies that contemplate all traits simultaneously.

Selection indexes are extremely useful when dealing with more than one trait of interest at a time as they allow genotype selection based on multiple traits simultaneously so that the selected genotypes are as close to the desirable ideotype as possible. The first selection indexes used for plant and animal breeding were proposed by Smith (1936) and Hazel (1943). Subsequently, several other selection indexes were proposed (Elston, 1963; Pešek & Baker, 1969; Mulamba & Mock, 1978). Although largely used, all indexes have their own limitations regarding reductions in selection precision, which lead to mistaken conclusions (Woyann et al., 2019).

The FAI-BLUP index (factor analysis and ideotype-design / best linear unbiased predictor) proposed by Rocha, Machado, and Carneiro (2018) combines factorial analyses with genotype–ideotype design for multi-trait selection. This index has been successfully used to select the genotypes of several food crops (Silva et al., 2018; Oliveira et al., 2019; Rocha et al., 2019; Woyann et al., 2019; Pessoa et al., 2022). The main advantages of using this index are that there is no need to assign economic weights for each trait and that this index is free from multicollinearity issues. As assigning economical weights for the fruit quality attributes would be an artificial and inaccurate metric, and multicollinearity is a possibility since not all these traits are orthogonal, the FAI-BLUP index stands as a good approach to successfully select late blight-resistant tomato genotypes displaying good fruit quality attributes.

Experimental trials to evaluate late blight resistance usually involve the artificial inoculation of the pathogen, and even genotypes possessing a level of resistance can have damaged fruit, making it difficult to select genotypes that are resistant and possess high-quality fruit in the same trial. A strategy to overcome this problem is to perform two separate trials: one to select disease-resistant genotypes and another where the fruit quality of these genotypes can be measured without the presence of the pathogen. In a previous work, Copati et al. (2021) uncovered a set of genotypes possessing resistance to late blight. In the current work, the main goal was to select late blight-resistant tomato families displaying enhanced fruit quality using the FAI-BLUP index.

## Material and methods

### Plant material

We assessed 81 $F_{3:5}$ tomato families (*Solanum lycopersicum*) obtained from successive self-pollination cycles of the cultivar Iron Lady ($F_1$), which was previously selected as late blight resistant by Copati et al. (2021). This cultivar is known for carrying the late blight resistance genes Ph2 and Ph3 (Ozores-Hampton & Roberts, 2014). The 81 $F_{3:5}$ tomato families were selected as late blight resistant in a previous field trial. We also included the cultivars Thaise, Argos, and Liberty in the trial as commercial checks. The checks were chosen because they currently stand as the commercial fruit quality standard. Tomato seeds were sown in polystyrene trays of 128 cells each containing commercial substrate Tropstrato®. Field transplanting occurred 45 days after sowing when seedlings had 4–5 true leaves.

### Site and field trial

The trial was carried out in the Research and Extension Farm Unit *Horta Velha* belonging to the Department of Agriculture at *Universidade Federal de Viçosa*, located in Viçosa, Minas Gerais State, Brazil (20°45'14" S; 42°52'53" W; 648 m altitude).

The soil texture of the 0–20 cm layer was classified as sandy clay (Lemos & Santos, 1996). The soil chemical and physical attributes were as follows: pH (water) = 6.0; P = 67.1 mg $dm^{-3}$; $K^+$ = 150.0 mg $dm^{-3}$; OM = 3.0 dag $kg^{-1}$; $Al^{+3}$ = 0.0 $cmol_c$ $L^{-1}$; $Ca^{+2}$ = 4.5 $cmol_c$ $L^{-1}$; $Mg^{+2}$ = 1.0 $cmol_c$ $L^{-1}$; CEC = 10.2 $cmol_c$ $L^{-1}$; BS (%) = 58; Al%ECEC (%) = 0.0; clay = 36%; sand = 46%; and silt = 18%.

The trial was carried out in a randomized complete block design with three replicates. Tomato plots consisted of five plants in a row. Three of the five plants (the central ones) were used for the fruit quality assessment; there were 1,260 plants in total, but only 756 were assessed.

Trellising consisted of weaving a twine in and out of each plant and in bamboo stakes, which were regularly spaced within the rows. Plants were pruned until the first flower cluster. In-row and between-row spacing was 0.5 × 1.0 m, respectively. Water was provided to plants via drip irrigation. Production practices were performed weekly according to needs and crop recommendations. Fertilization was carried out according to the soil fertility results and recommendations of Ribeiro, Guimarães, and Alvarez (1999) and Alvarenga (2013).

## Fruit quality attributes

Fruit quality was assessed in three plants per plot, and the mean values for the three plants were used in the statistical analysis. Five pink-to-red mature fruits were harvested from the medium portion of the plants. The fruit was then transported to the Genetic Resources Laboratory of the Department of Agriculture, where fruit quality assessments took place. The 11 traits assessed were fruit diameter (FD), length (FL), color (L*, a*, C, and H), and firmness (Firm), titratable acidity (TA), soluble solids content (SS), hydrogen potential (pH), and SS:TA ratio.

FD and FL measurements, expressed in millimeters (mm), were recorded using a digital caliper for more precise results.

Fruit color measurements, which consisted of the color numeric components L*, a*, and b*, from the L*a*b* CIELAB color space (Commission Internationale de l'Eclairage, 1978), were measured on two different spots of the fruit skin (180° apart from one another) of each fruit selected using a colorimeter (model CR-10, Konica Minolta, China). L* represents the lightness and darkness of color and ranges from 0 to 100 (0 = dark and 100 = white). a* represents color directions from green (-a = −60 to 0) to red (+a = 0 to +60), and b* represents color directions from yellow (-a = −60 to 0) to blue (+a = 0 to +60). The chromaticity index (C), which is a measure of saturation or vividness of color, was calculated using the formula $(a^{*2} + b^{*2})^{1/2}$, while the Hue angle (H), which represents the tint of color (0° = red; 90° = yellow; 180° = green, and 270° = blue), was calculated using the formula $\tan^{-1}(b^*/a^*)$.

FF, described as the mean maximum penetration force required for pericarp rupture and expressed in Newtons (N), was measured in the equatorial region of the fruit. Two measurements, located 180° apart from one another, were taken in the equatorial region of each fruit.

After color and firmness measurements, all five selected fruits were macerated together in a blender to produce the tomato juice used to determine total acidity (pH), TSS, and TA.

TA was determined by adding about 10 grams of tomato juice to a 50 mL volumetric flask and filling it to capacity with distilled water. An aliquot of 10 mL from this solution was then titrated with a 0.1 N NaOH solution, using 1% phenolphthalein as an indicator. The results were expressed in grams of citric acid per 100 grams of tomato juice.

SS, expressed in °Brix, was determined using a digital refractometer (model HI 96801, Hanna Instruments, Italy).

Hydrogen potential (pH) was determined using a benchtop pH meter (model pH 21, Hanna Instruments, Italy) periodically calibrated with buffer solutions of pH 4 and 7.

The SS:TA ratio was obtained by dividing the SS by the TA.

## Statistical analysis

Fruit quality data were analyzed via the mixed model methodology REML/BLUP (restricted residual maximum likelihood / best linear unbiased prediction) (Patterson & Thompson, 1971; Henderson, 1975), using the R software package lme4.

The statistical model was denoted as follows:

$$Y = Xr + Zg + Wp + \varepsilon$$

where y = data vector; r = vector of replication effects (assumed as fixed) and added to the overall mean; g = vector of genotype effects (assumed as random); p = vector of plot effects (assumed as random); ε = residue vector (random); and X, Z, and W are the incidence matrixes of the given effects.

For the random effects, the significance of the likelihood ratio test was tested using the chi-square statistic with one degree of freedom. Genetic values (BLUP means) were predicted for each of the 84 genotypes based on the 11 traits assessed in this study.

### Family ranking

Genetic values (BLUP means) were submitted to the selection index FAI-BLUP, based on factorial analyses and genotype–ideotype design, to rank the genotypes. Principal component analysis, factor analysis, ideotype determination, and genotype–ideotype distance were determined using the FAI-BLUP index routine developed by Rocha et al. (2018) in R software.

Principal component analysis was used to extract factorial loads from the correlation matrix between genetic values. The varimax criterion described by Kaiser (1958) was used for analytic rotation. As for the calculation of the factor scores, the weighted least squares method described by Bartlett (1978) was used.

The number of ideotypes was defined based on the combination of desirable and undesirable factors according to the objective of the selection. The number of ideotypes was given by the algorithm:

$$NI = 2^n$$

where NI = number of ideotypes and n = number of factors.

The ideotype for fruit quality was determined by considering the ideal values for each trait (minimum, mean, or maximum values of traits) shown in Table 1. The ideotype considered the maximum predicted genetic value for the traits FD, Firm, L, a, C, Firm, TA, SS, pH, SS/TA, and TA, and the minimum predicted genetic value for H. Desirable versus undesirable trait classification consisted of comparing our data with those available in the recent literature for each trait.

**Table 1.** Maximum, minimum, and mean values and desirable and undesirable ideotypes for each fruit quality trait assessed.

| | FL | FD | Firm | L | a | C | 1.1.1 H | SS | pH | SS/TA | TA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Maximum | 80.59 | 92.46 | 23.25 | 41.60 | 107.55 | 121.04 | 56.67 | 4.45 | 5.70 | 24.52 | 0.56 |
| Mean | 39.28 | 49.30 | 5.30 | 30.40 | 28.55 | 45.71 | 46.58 | 2.20 | 4.08 | 6.25 | 0.11 |
| Minimum | 57.67 | 69.73 | 13.46 | 35.63 | 41.79 | 60.92 | 31.59 | 3.28 | 4.43 | 10.46 | 0.32 |
| Desirable | med | max | max | min | max | max | min | max | min | med | max |
| Undesirable | min | min | min | max | min | min | max | min | max | max | min |

After ideotype determination, genotype-ideotype distances were estimated and converted into spatial probability, enabling genotype ranking. The following algorithm was used:

$$P_{ij} = \frac{\frac{1}{d_{ij}}}{\sum_{i=1;j=1}^{i=n;j=m} \frac{1}{d_{ij}}}$$

where $P_{ij}$ = probability of the $i_{th}$ genotype (i = 1, 2, ..., n) o is similar to the $j_{th}$ ideotype (j = 1, 2, ..., m); $d_{ij}$ = genotype–ideotype distance from the $i_{th}$ genotype to the $j_{th}$ ideotype based on standardized mean Euclidean distance.

## Results

Table 2 shows the eigenvalues and cumulative variances obtained from the principal component analysis using the correlation matrix between genetic values. The first five components had eigenvalues greater than 1, suggesting that the data were dimensionally reduced into five factors only (Kaiser, 1958). About 76% of the genetic variability present within the dataset was accumulated in the first five components.

Factorial loadings after varimax rotation for the four factors are shown in Figure 1. Colors indicate correlations among traits within the factor ($p < 0.05$). The bluer the square, the more negative the value. The redder the square, the more positive the value. High-magnitude correlations among the traits were observed for all factors. The more intense the color, the more the trait correlated within the factor.

Figure 2 shows trait clustering into factors. FD, FL, TA, and SS were grouped in the first factor. Fruit color parameters a* and chroma (C) were grouped in the second factor. L* and hue were grouped in the third factor. pH and the SS:TA ratio were grouped in the fourth factor, and Firm was assigned to the fifth factor. In this analysis, traits highly correlated with one another were grouped into the same factor. Genetic correlations between traits may occur in the same or opposite directions.

**Table 2.** Eigenvalue estimates from the principal component analysis and the proportion of the total variance explained by each.

| Principal component | Eigenvalue | Cumulative variance |
|---|---|---|
| PC1 | 2.55358006 | 23.21436 |
| PC2 | 2.06649323 | 42.00067 |
| PC3 | 1.45365486 | 55.21571 |
| PC4 | 1.24418716 | 66.52650 |
| PC5 | 1.07256214 | 76.27707 |
| PC6 | 0.71968365 | 82.81965 |
| PC7 | 0.68371233 | 89.03521 |
| PC8 | 0.46125121 | 93.22841 |
| PC9 | 0.38395639 | 96.71892 |
| PC10 | 0.31448092 | 99.57784 |
| PC11 | 0.04643806 | 100.00000 |

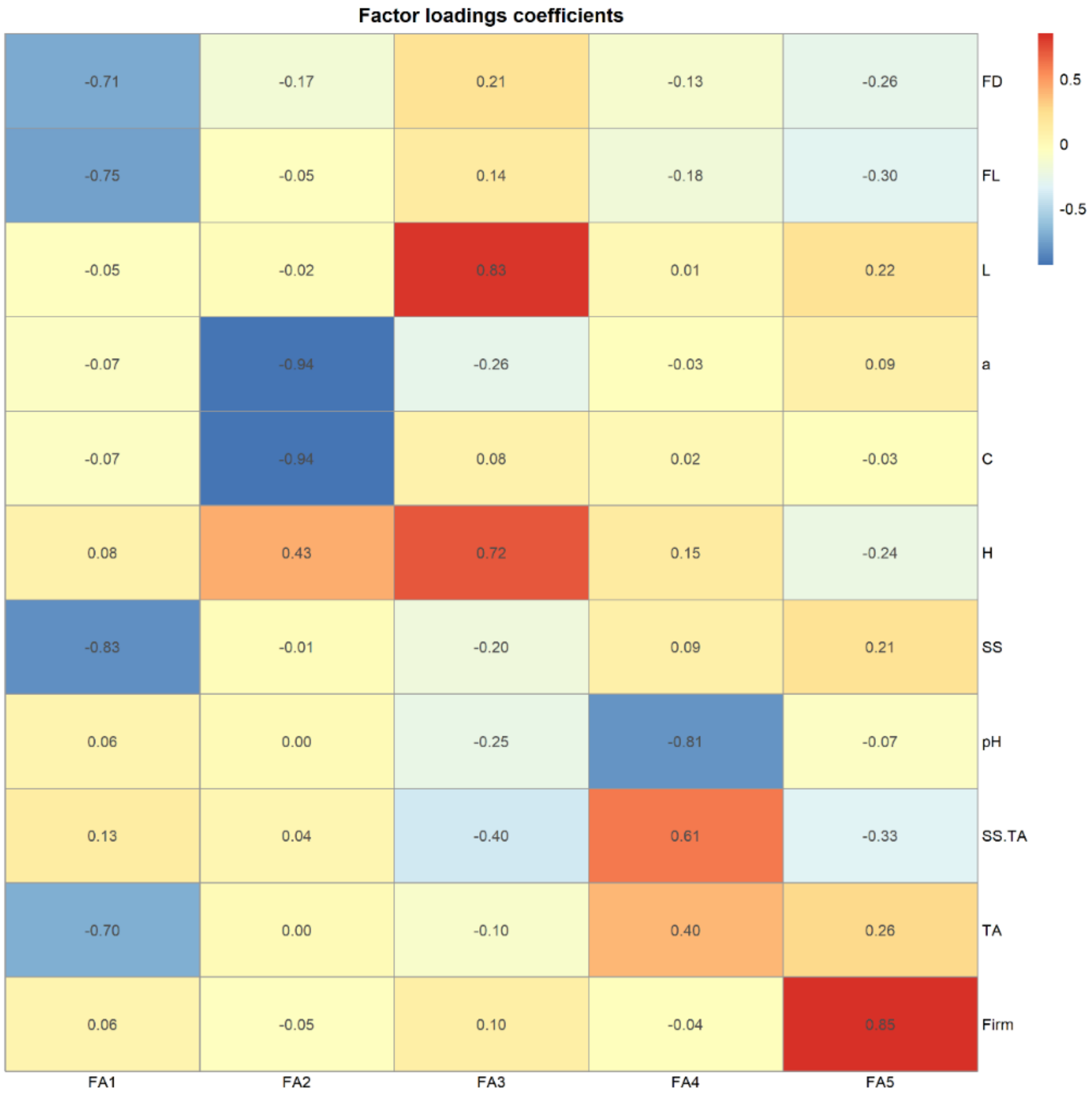


**Figure 1.** Heat map showing factorial loadings after varimax rotation for the factors. L = lightness/darkness of color; a = color directions from green to red; C = chromaticity index; H = Hue angle; FD = fruit diameter; FL = fruit length; SS = soluble solids content (°Brix); TA = titratable acidity; pH = total acidity; SS/TA = SS:AT ratio; Firm = fruit firmness.

The fruit quality ideotype was that with desirable traits for all factors. Figure 3 shows the family ranking according to the FAI-BLUP index and the probability of distance from the family to the desirable ideotype for fruit quality. The best families for fruit quality according to the selection index were 77, 8, 13, 58, 43, 33, 10, 9, 4, 83, 3, 54, 44, 49, 32, 20, and 65. The cultivar Argos ranked close to the desirable ideotype. Tomato families 72, 67, 80, 12, 74, 19, 62, 71, 5, and 23 ranked farthest from the desirable ideotype.

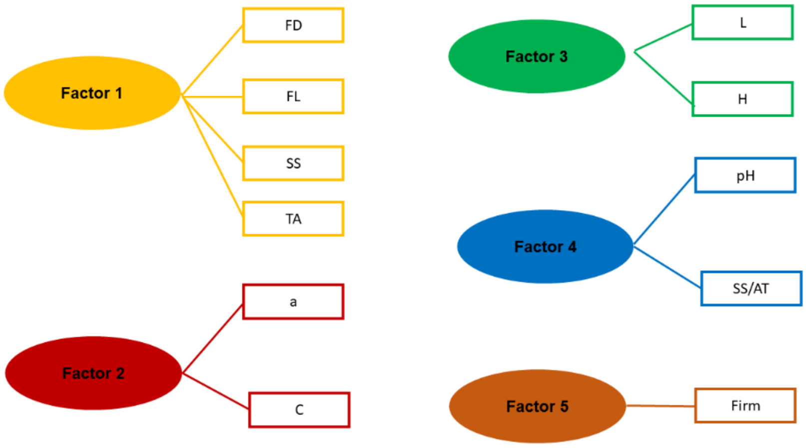


**Figure 2.** Fruit quality traits grouped into five factors. a* = color directions from green to red; C = chroma; H = Hue; FD = fruit diameter; SS = soluble solids content (°Brix); TA = titratable acidity; pH = hydrogen potential; SST/AT = SS/AT ratio; L = lightness and darkness of color; Firm = fruit firmness.

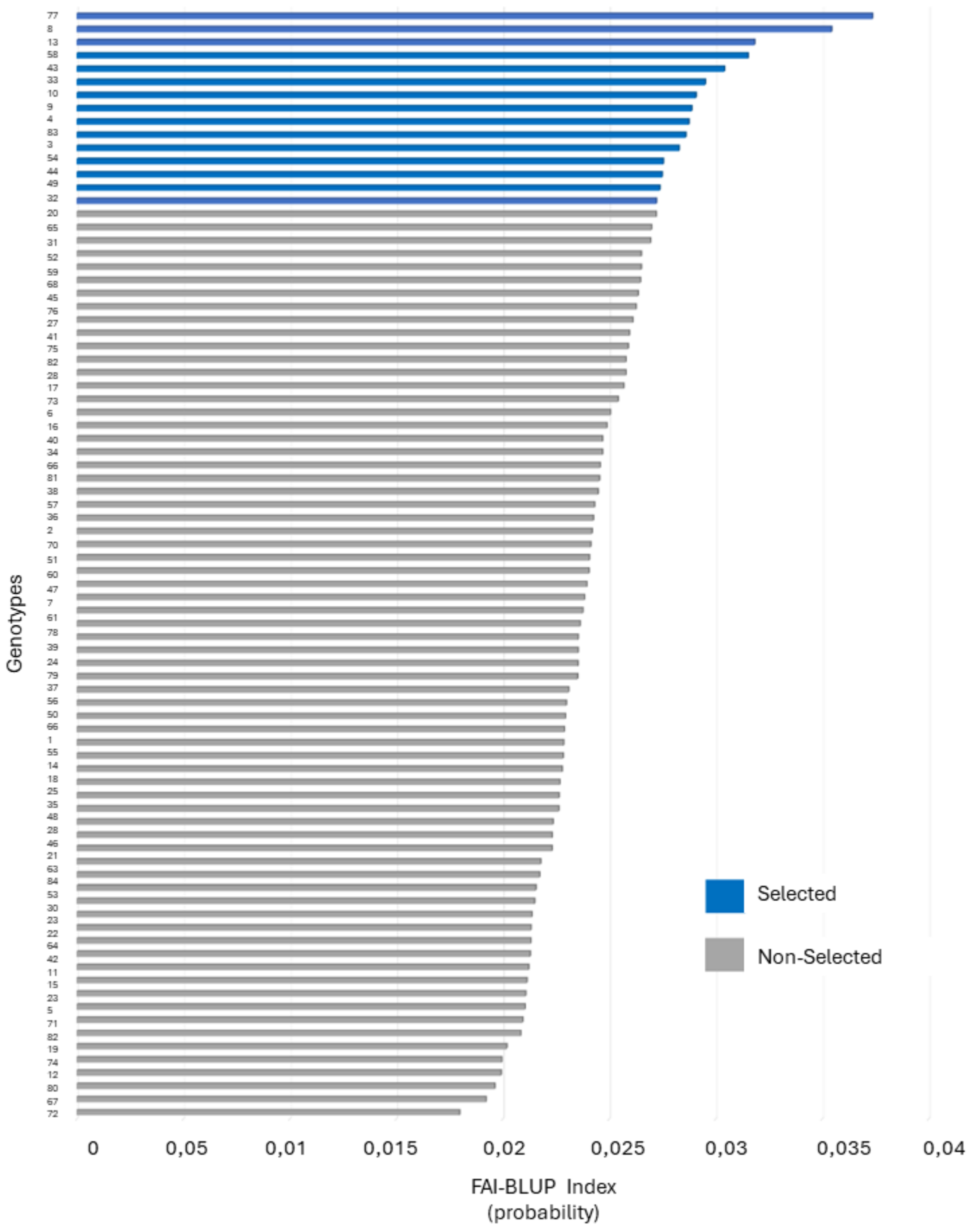


**Figure 3.** Family ranking using the FAI-BLUP selection index. Families in blue were selected due to their close similarity to the desirable ideotype for fruit quality. Commercial cultivars: genotype 82 is Thaise, 83 is Agro, and 84 is Liberty.

## Discussion

In addition to disease resistance, a tomato genotype should display good fruit quality to be released as a cultivar on the market. Fruit quality and market value are determined by fruit size, shape, firmness, color, taste, and SS, traits that vary depending on the growing season, cultivar, and crop management practices adopted (Maach et al., 2020). Therefore, the use of selection indexes is necessary in breeding programs of crop species, as they allow the combined selection of multiple traits. However, when using this type of methodology, genetic gains should be assessed together, as reductions in genetic gains can be observed for some variables when assessed alone (Zetouni, Henryon, Kargo, & Lassen, 2017).

The selection of tomato genotypes previously evaluated for late blight resistance displaying good fruit quality attributes can be done successfully using the FAI-BLUP index. This selection index was first proposed for use in elephant grass breeding for bioenergy (Rocha et al., 2018). This methodology consists of ranking genotypes based on genotype–ideotype distance, considering multiple traits. The FAI-BLUP index has already been used for the genotype ranking of several crop species (Silva et al., 2018; Oliveira et al., 2019; Rocha et al., 2019; Woyann et al., 2019).

Compared to the selection indexes commonly used, the FAI-BLUP index does not require economic weights to be assigned to each trait and is free from multicollinearity (Rocha et al., 2018). Multicollinearity is a common problem when working with several traits. The analysis of data containing multicollinearity issues can compromise the selection process due to inflated errors, leading to imprecise results in significance tests (Dormann et al., 2013; Prunier, Colyn, Legendre, Nimon, & Flamand, 2015).

The first step of the FAI-BLUP index methodology is to perform a principal component analysis and a factorial analysis to extract factorial loadings from the genetic correlation matrix. Then, based on the combination of desirable and undesirable factors, considering the breeding purpose, the ideotypes are determined. After ideotype determination, genotype–ideotype distances are estimated and converted into spatial probability, allowing genotype ranking (Rocha et al., 2018).

The principal component analysis here reduced the 11 variables into 5 components comprising 76% of the total genetic variability in the population. This result was even better than that found by Bojarian, Asadi-Gharneh, and Golabadi (2019) when assessing the fruit quality of tomato families using principal components and factor analysis. Bojarian et al. (2019) grouped 68.2% of genetic variability into five factors. Principal components and factor analyses are efficient methodologies for crop breeding when dealing with traits with low heritability, especially in the first generation of selection (Bojarian et al., 2019). This approach groups multiple traits into a few artificial ones that can be used for genotype ranking and selection so that it is especially advantageous when studying a large number of traits simultaneously (Golbashy, Ebrahimi, Khorasani, & Choukan, 2010; Beiragi, Ebrahimi, Mostafavi, Golbashy, & Saied, 2011).

In this study, the first factor grouped traits associated with fruit size and sweetness, and the second and third factors grouped traits associated with fruit color. The fourth factor grouped traits associated with fruit chemical attributes, and the fifth factor considered fruit firmness.

SS and TA, grouped in the first factor, were positively correlated with FL and FD. Fruit size is often affected by the dry matter content of fruit, which may also affect SS and TA (Beckles, 2012). SS in fruits is inversely correlated with fruit weight and plant yield (Dariva et al., 2021). We, therefore, expected a high and negative correlation between the fruit size traits, FD and FL, and SS within factor 2, which was not observed.

Selection for color is easily performed since all traits have correlations of the same magnitude within the second and third factors. Fruit color is one of the main attributes consumers consider when purchasing tomatoes. Additionally, it is indicative of sugar and acid content and fruit taste (Wan, Toudeshki, Tan, & Ehsani, 2018), and it is widely used to infer fruit ripening (Arivazhagan, Shebiah, Selva Nidhyanandhan, & Ganesan, 2010).

The color aspect has also been used by consumers to evaluate and determine the quality of apples and peaches (Li, Cao, & Guo, 2009; Wan et al., 2018). Numerical color components can also be used in indirect selection for increased lycopene content in tomato fruit. Correlation coefficients ranging from 0.75 to 0.93 between CIELAB color numeric components, a* and b*, and lycopene content, the main pigment of ripe tomato fruit, have been reported (Gómez et al., 2001; Weingerl & Unuk, 2015; Ilahy et al., 2018).

Factor 4 grouped traits related to fruit taste and consumer appreciation. Although SS is an important trait used to determine fruit taste, it was not grouped into factor 4. This may have happened due to inconsistencies in comparing SS from different genotypes, as SS content may change if the fruit accumulates very low (Gautier et al., 2008) or very high (Luengwilai, Fiehn, & Beckles, 2010) acid levels. Therefore, using the SS:TA ratio is more appropriate (Beckles, 2012).

Fruit firmness was assigned to factor 5 alone. Fruit firmness affects sales (Causse et al., 2010) and interferes with taste, aroma perception, and fruit shelf life (Seymour, 2002; Bertin & Génard, 2018). Firmer fruit tends to be more resistant to pathogen attack and long-distance transportation.

The tomato families 77 and 8 ranked closest to the desirable ideotype and were considered even better than the commercial checks. Only the commercial check Argos ranked close to the desirable ideotype for fruit quality. Nine families in this study, however, had performance superior to that of Argos (genotype 83), which highlights the great potential of our plant material in terms of fruit quality, especially if we consider that the commercial cultivars already have high fruit quality. The commercial cultivars Thaise and Liberty (genotypes 82 and 84) were ranked in positions 27 and 66 of the ranking, which demonstrates the fruit quality superiority of many evaluated families in comparison. With a 20% selection intensity, we selected the tomato families 77, 8, 13, 58, 43, 33, 10, 9, 4, 3, 54, 44, 49, and 32 as closest to the desirable ideotype. These families will move on to the next stages of our breeding program, as they combine late blight resistance with improved fruit quality. The tomato families ranked far from the desirable ideotype should not remain in our breeding program, as they will make it more difficult for us to achieve a cultivar with the high fruit quality standard expected by today's consumers.

## Conclusion

Fifteen tomato families were selected for this study by the FAI-BLUP index for combined late blight resistance and high fruit quality. The cultivar Argos ranked close to the desirable ideotype for fruit quality, demonstrating that the FAI-BLUP index can identify superior plant materials. Nine tomato families were closer to the desirable ideotype than the cultivar Argos and therefore displayed potential for tomato improvement. These families should move on to the next stages of our breeding program.

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