

Selection for Papaya Resistance to Multiple Diseases in a Base Population of Recurrent Selection

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25 **ABSTRACT:** Papaya has a narrow genetic base concerning disease resistance, with few genetically distinct cultivars
26 for planting in the world. Losses in crop production caused by fungal and viral diseases, added to the absence of
27 resistant cultivars available to producers, have reduced the competitiveness of crops. Therefore, this study aimed to
28 estimate the direct and combined genetic gains in the base population of the recurrent papaya selection and to indicate
29 promising individuals for resistance to phoma spot and black spot. The population used in this study originated from
30 the crossing of dioecious, female individuals and holders of a gene pool for resistance to phoma spot and black spot.
31 These were crossed with elite individuals with a gene pool for production traits and fruit quality. The experiment was
32 carried out without an experimental design, containing one plant per plot. Three measurements were taken on 255
33 individuals for five traits of disease resistance, being: four associated with black spot (incidence and severity on the
34 leaf and fruit) and one associated with phoma spot (severity on the leaf). With the observations obtained, the

35 REML/BLUP procedure was performed to estimate the temporary and permanent environmental effects. Such values
36 were used to know the genetic parameters of the population and to elaborate a combined selection index, as well as to
37 compare the gains with the direct selection. The results indicate low repeatability for the traits and that the gains
38 obtained from the use of the combined selection index better rank the genotypes in comparison to the gains obtained
39 by direct selection.

40 **Key word:** *Asperisporium caricae*, *Stagonosporopsis caricae*, direct selection, combined selection, selection index.

41 INTRODUCTION

42 Papaya (*Carica papaya* L.) is considered one of the most cultivated and consumed fruit trees in the
43 subtropical and tropical regions of the globe (Serrano and Cattaneo 2010; Carvalho et al., 2020). The expansion of
44 new cultivation areas faces challenges such as crop susceptibility to diseases of a viral, fungal, and bacterial nature.
45 Among the main fungal diseases that affect papaya at various stages of its development, the phoma spot
46 (*Stagonosporopsis caricae* (Sydow., P. Sydow)) stands out, which has the potential to reduce the leaf area and is
47 considered the second major disease of post-harvest fruits; and, the black spot (*Asperisporium caricae* (Speg.)
48 Maubl.) that by reducing the photosynthetic area of the leaves and commercially depreciating the fruits, can cause
49 severe losses of production (Liberato et al., 2004; Rezende and Fancelli, 2016).

50 The appearance of the first symptoms of phoma spot and black spot recommend the beginning of disease
51 control in the field (Ritzinger et al., 2000; Rezende et al., 2016). However, for the time being, for *S. caricae* there is
52 no product available capable of efficiently controlling this disease, for this purpose, integrated disease management
53 has been used, for example: avoiding injury to plant and fruit tissues. To the black spot control, sprays are made from
54 restricted organic chemical groups such as dithiocarbamates and triazoles, or inorganic as copper oxychloride and
55 cuprous oxide (Agrofit, 2020). The majority use of sprays of these chemical groups can result in 'selection pressure'
56 resulting in isolates of phytopathogens resistant to fungicides, which would further aggravate the crop's phytosanitary
57 barriers (Lucas et al., 2015; Dorigan et al., 2019).

58 Thus, the genetic resistance to diseases, the adaptation and the rusticity of papaya appear as sustainable
59 alternatives for the control of this phytosanitary problem (Oliveira et al. 2010). Different experimental techniques,
60 genetic designs and sophisticated tools are being explored by breeding programs to expand the genetic base and/or

61 explore the existing variability in the genus *Carica* (Serrano and Cattaneo 2010; Moreira et al., 2018; Pereira et al.,
62 2019a).

63 Regarding the improvement for resistance to phoma spot and black spot, the study is concentrated on two
64 types of studies: a) introduction, evaluation, and disclosure of resistant accesses (Dianese et al., 2007; Vivas et al. ,
65 2010a; 2012b; Poltronieri et al., 2019); and b) evaluation of combinatorial capacity and selection of families or
66 superior hybrids (Vivas et al., 2011; 2012a; 2013a, b, c; 2014a, b, c; 2015; 2016; 2017; Poltronieri et al., 2017; 2019;
67 2020; Moreira et al., 2018; Pirovani et al., 2018; Moraes et al., 2019).

68 Despite the work carried out, there is no commercial record of a genotype that has or is widely resistant to
69 such diseases. The main cause is the narrow genetic base. To circumvent this problem, dioecious papaya has been
70 explored for having a broad genetic basis and rusticity, which may confer resistance to phoma spot and black spot
71 (Vivas et al., 2010a; 2012b; 2013c; 2014 b; Moraes et al., 2019). It is believed that the correct characterization of the
72 gene pool for phytosanitary resistance traits belonging to dioic plants, can contribute to the genetic improvement of
73 the species.

74 Based on this premise, a group of dioic plants was used to compose a population of recurrent selection,
75 together with a group of elite parents - parents of hybrids registered at the Ministry of Agriculture, Livestock and
76 Supply - MAPA, (Moreira et al., 2019; Santa -Catarina et al., 2020a, b). This population has shown itself promising
77 to select genotypes with agronomic characteristics such as fruit yield, fruit firmness, total soluble solids, and
78 commercial fruits. The composition of this population was obtained by crossing these two gene pools, which generated
79 heterozygous individuals, making it impossible for individuals to repeat in an experimental design. Such a format
80 must have more precise forms of analysis, such as the mixed model methodology.

81 The mixed model can be used, both for the versatility of the non-mandatory experimental design, and the
82 precise and impartial predictive power over genetic responses from population samples. Even so, the REML/BLUP
83 procedure is incipient in the improvement of papaya (Pinto et al., 2013; Ramos et al., 2014; Cardoso et al., 2017;
84 Moreira et al., 2018; Moreira et al., 2019; Cortes et al., 2019; Santa-Catarina et al. 2020a, b) mainly in terms of disease
85 resistance characteristics (Vivas et al., 2014b; Moreira et al., 2018).

86 Given the above, it is assumed that the identification and selection of papaya genotypes resistant to black spot and
87 phoma spot may be affected by different selection strategies. In this context, the present study was carried out to study
88 the effect of direct and combined selection, and, consequently, selecting genotypes resistant to black spot and phoma
89 spot from the base population of the S0 recurrent family selection program (UCP -C0). For this, genetic parameters,
90 repeatability coefficient, and genetic selection gains in different alternatives for breeding purposes were estimated.

91 **MATERIALS AND METHODS**

92 **Obtaining the study population**

93 The material studied came from the base population of the recurrent papaya selection program called UCP-
94 C0 (UENF Caliman Population Cycle 0) (Santa-Catarina et al., 2020a). To obtain the UCP-C0, five half-sib progenies
95 were used as female parents, considered to be resistant to black spot and phoma spot diseases: STA-22 (3), STA-05
96 (5), STA -17 (6), STA-02 (6), and STA-04 (5), (Vivas et al., 2012, 2013a, 2014). Five andromonoecious progenies
97 (hermaphrodites) were used as pollen donors for being parents of hybrids already registered with MAPA - Ministry
98 of Agriculture, Livestock and Supply, named: SS-72/12, JS-12, Sekati, UC-36/7, and UC-41/7 (Pereira et al., 2019 a,
99 b, c).

100 To pollinate female plants, a mixture of pollen from andromonoecious plants was prepared from flowers near
101 the anthesis phase. Fifty flowers of hermaphroditic plants were collected from each elite parent to compose the "mix",
102 totaling 250 flowers. The pollen was removed from the flowers and placed in a 2 ml Eppendorf tube to prepare the
103 mixture and form the pollen mix. After obtaining the pollen mix, the flower buds of the female plants were pollinated
104 using a brush. After pollination, the flowers were protected with a paper bag 13.5 cm wide x 19.5 cm long to ensure
105 control of the crossing. As only female plants from the dioecious population were used and received pollen from
106 hermaphroditic plants, at this moment, there was sexual conversion through the replacement of the Y chromosome
107 (male) by the sex chromosome Y^h (hermaphrodite) (Ming et al., 2007).

108 In the end, pollinated fruits were collected, and from these fruits, equal amounts of seeds were collected, only
109 then were they grouped to form the base papaya population UCP-C0. In the greenhouse, the grouped seeds were sown,
110 and, after germination, the seedlings were acclimatized for approximately 30 days. After acclimatization, the seedlings
111 were transplanted in the experimental area.

112 **Experimental conditions**

113 The seedlings were transplanted to the experimental area, with 1.5m x 3.6m spacing, using three seedlings
114 per hole. The transplant took place in September 2016. The sexing of the plants occurred approximately 90 days after
115 the transplant, where the female plants were eliminated, leaving only one hermaphrodite plant per hole, which was
116 considered the measurement plot. The evaluations took place in June, September, and December 2017. Crop
117 management was carried out according to the recommendation for crop and guided by the company CALIMAN
118 agricultural SA

119 The experimental area was located at coordinates 19°23' S, 40°04' W, and 33 m altitude above sea level, in
120 Linhares, ES, in the Santa Terezinha farm, in the Caliman Agrícola SA company. During the experiment, there was a
121 rainfall volume of 1366 mm and the daily averages of wind speed, air temperature, and relative air humidity of 2.94
122 m.s⁻¹, 24.4 °C, 73.15 % respectively, data obtained by the weather station of Linhares - ES, INMET (Figure 1). The
123 region's climate is classified as Awi-type (humid tropical), with rainy summer and dry winter (Alvares et al., 2013).

124 It is noteworthy that it was not possible to use the experimental design for the evaluation since each plant
125 corresponds to a different individual. Thus, each plant was evaluated individually.

126 **Traits evaluated**

127 The traits evaluated were: Incidence of black spot on the leaf (IBSLF), calculated by the ratio between the
128 number of leaves with symptoms of black spot and the total number of leaves of the plant, according to the formula:

129 $IBSLF = \left(\frac{\text{No. of leaves with black spot}}{\text{Total number of leaves}} \right) * 100$. Incidence of black spot on fruit (IBSFT), calculated by the ratio between

130 the number of fruits with symptoms of black spot and the total number of fruits of the plant, according to the formula:

131 $IBSFT = \left(\frac{\text{No. of fruits with black spot}}{\text{Total number of fruits}} \right) * 100$. The severity of phoma spot on the leaf (SPSLF): observed on the leaf

132 posterior to the leaf whose petiole held the first newly opened inflorescence. The severity of black spot on the leaf

133 (SBSLF): observed on the leaf whose petiole held the first newly opened inflorescence; Severity of black spot on fruit

134 (SBSFT): observed in stage 0 fruits (harvest point).

135 The values of black spot severity on the leaf were estimated with the aid of a diagrammatic scale adopted by
136 Vivas et al. (2011) with the injured area values of 0.2; 1.6; 3.5; 5.4; 7.6 and 12.8%. The severity of black spot on the
137 fruit was estimated with the fruits at stage 0 of maturation, with the aid of a diagrammatic scale proposed by Vivas et

138 al. (2010), with percentages of the injured surface area of 0.1, 0.3, 0.6, 1.2, 2.5, 5.0, 10.0, and 20.0%. The phoma spot
139 severity values were estimated using a diagrammatic scale described by Terra et al. (2008), with the injured area values
140 of 1, 2, 4, 8, 16, and 32%.

141 **Methodology of mixed models**

142 The following components of variance (individual REML) and genetic parameters associated with
143 repeatability were estimated: σ^2_{fp} : permanent phenotypic variation between plants (genotypic variation + permanent
144 environmental variation between measurements); σ^2_{et} : temporary environmental variation; σ^2_f : individual phenotypic
145 variation ($\sigma^2_{fp} + \sigma^2_{et}$); r : individual repeatability $\left(\frac{\sigma^2_{fp}}{\sigma^2_{fp} + \sigma^2_{et}}\right)$; rm : repeatability of the mean of m repeated measurements
146 $\left(\frac{\sigma^2_p}{\sigma^2_{fp} + \frac{\sigma^2_{et}}{3}}\right)$; A_{cm} : accuracy based on the mean of m repeated measurements (\sqrt{rm}) , and OM : general mean of the
147 experiment. To estimate the Blup's, the following model was used: $Y = Xm + Wp + e$. Where: y is the observation
148 vector; m is the measurement effect vector (assumed to be fixed) added to the overall average; p is the plant's
149 permanent effect vector (genotypic effects + permanent and temporary environmental effects supposed at random);
150 and e the residual (random) vector (Resende, 2016). This is the basic model of repeatability used for experiments
151 without experimental design. The application of the model is justified since in the UCP-C0, each plant is a different
152 individual, and it is not possible to use an experimental design.

153 **Selection criteria adopted**

154 **Direct selection**

155 In the direct selection, 30 superior individuals (11.7%) (with lower values) were classified, and the selection
156 gain was estimated by $G_s = (D_s * rm)$, where D_s is the selection differential express by $D_s = (\underline{X}_s - \underline{X}_0)$, which
157 was calculated through the difference between the mean of the population X_0 , estimated through the general mean of
158 the experiment and the average value of the selected individuals X_s , and, rm : average repeatability, which provides
159 the upper limit of the heritability coefficients (Viana and Resende, 2014).

160 To assist the visualization of individuals in the direct selection, the Venn diagram was used as a tool for grouping
161 the genotypes. The top 30 individuals for each of the evaluated traits were plotted on the Venn diagram with the aid
162 of the “vennDiagram” package available in the statistical software R.

163 **Simultaneous selection based on the selection index**

164 To select the individuals simultaneously considering several traits in combination, a combined selection (ISC)
165 is used. The combined selection was carried out using the index that associates weights with standardized averages of
166 the selected traits. The index can be estimated using the following equation: $\sum_{i=1}^n (Fp_i * p_i)$. Where, Fp_i is the
167 standardized permanent phenotypic value of trait i , and p is the economic weight attributed to trait i .

168 The weights assigned to the five traits were: SPSLF (200), SBSFT (200), SBSLF (100), IBSFT (200), and
169 IBSLF (100). These weights were established based on the importance of the genetic resistance of each trait, according
170 to the knowledge of the breeders of the UENF/CALIMAN papaya breeding program. As in the studied population,
171 each plant is a different individual, higher weights (200) were attributed to the most important characteristics related
172 to the phytosanitary quality of the fruit (SBSFT, IBSFT), and the weight (200) for the SPSLF trait was by trial and
173 error aiming to maximize the gains for all traits. To build the index, Selegen REML/BLUP and Microsoft Office Excel
174 2016 software were used.

175 For each trait, the permanent phenotypic values were obtained for each individual, and these were standardized
176 using the following equations: $Fp_i = \left(\frac{X_i - \bar{x}}{\sigma} \right)$, where Fp_i is the standardized phenotypic value; X_i is the value for the
177 individual; \bar{x} is the general average of all individuals; σ is the standard deviation of the distribution. After
178 standardization, the permanent phenotypic values were multiplied by the weights taken from the index mentioned
179 above. The individuals with the lowest total sum of all characteristics (final ISC value) were the highest.

180 **RESULTS**

181 **Components of variance and genetic parameters**

182 The chi-square test demonstrated significant differences in the effects of genotypes, thus evidencing the
183 existence of significant differences between individuals. Thus, the existence of genetic variability among individuals
184 allows the selection of superior genotypes based on these evaluated traits (Table 1).

185 The temporary environmental variance (σ^2_{et}) represented the highest percentage of the individual phenotypic
186 variance (σ^2_t) for all traits analyzed (Table 2). These values indicate that the environmental variance for these traits
187 was relatively high, compared with the genotypic variance between plants. Such a fact may have occurred due to the
188 complexity in the interaction involved between plant x pathogen x environment and the nature of the trait.

189 It was observed that in the region of the experiment, an atypical climatic condition occurred with a
190 concentration of rain in short periods, with an accumulated volume of approximately 1366 mm (August/2016 ~
191 December/2017). Also, the maximum relative humidity in this period did not exceed 81%. A low wind speed (<3.0 m
192 s⁻¹) was also observed¹) during the experiment (Figure 1). The atypicality of climatic conditions may have influenced
193 the genetic response of individuals.

194 The repeatability coefficient based on the mean (rm) of three measures and the mean accuracy showed an
195 intermediate magnitude ($r > 0.3$) for the trait IBSLF, and the other traits had a low magnitude (Table 2). For the average
196 accuracy parameter, there was a variation from 0.19 (SBSFT) to 0.61 (IBSLF). In general, the use of selection
197 procedures based on mixed models is justified, because even with low repeatability characteristics, favorable genetic
198 gains were predicted, and the genotypes have the potential for selection (Tables 1 and 2). The low repeatability
199 indicates the strategy of selection gain in the medium to long term (Viana and Resende, 2014).

200 **Direct selection**

201 The genetic gain estimates were favorable when practicing direct selection (SD) for the traits SPSLF, SBSLF,
202 and IBSFT, as negative gains are observed for all other traits (Table 3). Carrying out direct selection for the SBSFT
203 and IBSLF traits capitalize gains only for themselves, which in terms of the resistance of a genotype can be considered
204 insufficient (Table 3).

205 It is observed in the Venn diagram the existence of a triple intersection (\cap) (Figure 2). Within each set formed
206 by the triple intersection are three genotypes, which are holders of genetic resistance to each trait that makes up a set,
207 and thus, indicating the importance of using these individuals within the breeding program.

208 In the Venn diagram, considering the traits SPSLF, SBSLF, or IBSLF, the individuals UCPC015-170, UCPC015-
209 159, and UCPC015-031 had these traits in common ($\text{SPSLF} \cap \text{SBSLF} \cap \text{IBSLF}$), highlights these genotypes are
210 promising holders of resistance to phoma spot and black spot on the leaf. Considering the traits SBSFT, IBSFT, or
211 IPSLF, three other genotypes stands out: UCPC015-042, UCPC015-178, and UCPC015-167. They had these traits in
212 common ($\text{SBSFT} \cap \text{IBSFT} \cap \text{IPSLF}$), these genotypes present a greater level of resistance to the black spot on fruits
213 and low incidence of black spot on leaves, standing out as promising holders of alleles of resistance to black spots.
214 They are indicated in the use of advance of generation and/or crossing to explore the combinatorial capacity and
215 heterosis.

216 When practicing selection for the SPSLF trait, a genotype (UCPC015-152) had the SBSFT trait in common,
217 SPSLF \cap SBSFT; two genotypes (UCPC015-047 and UCPC015-049), had the IBSFT trait in common, SPSLF \cap
218 IBSFT; seven other genotypes (UCPC015-023, UCPC015-035, UCPC015-124, UCPC015-148, UCPC015-149,
219 UCPC015-162 and UCPC015-163), had the SBSLF trait in common, SPSLF \cap SBSLF; two genotypes (UCPC015-
220 172 and UCPC015-062), had the IBSLF trait in common, SPSLF \cap IBSLF.

221 When practicing the selection for the SBSFT trait, six genotypes (UCPC015-115, UCPC015-117, UCPC015-
222 128, UCPC015-144, UCPC015-147, UCPC015-167), had the IBSFT trait in common, SBSFT \cap IBSFT; a genotype
223 (UCPC015-055), had the IBSLF trait in common, SBSFT \cap IBSLF.

224 For the selection based on the SBSLF trait, two genotypes (UCPC015-164 and UCPC015-197), had the
225 IBSFT trait in common, SBSLF \cap IBSFT.

226 And finally, when practicing selection for the IBSFT trait, three genotypes (UCPC015-087, UCPC015-091 and
227 UCPC015-166), had the IBSLF trait in common, IBSFT \cap IBSLF.

228 **Combined selection**

229 The Selection considering the index (ISC), presented favorable gains for all evaluated characteristics (Table
230 3). The gains obtained by the ISC were greater than or equal to the indirect gains observed by the direct selection.
231 This fact indicates that there is no loss in gain when practicing selection using the ISC. Also, there was a 93.3%
232 coincidence between individuals selected by ISC and those selected by direct selection.

233 It is observed that the ISC, indicates the selection of five of the six genotypes highlighted in the triple
234 intercession in the Venn diagram, the exception was for the “UCPC015-167” genotype. A situation that indicates a
235 good accuracy obtained by the index.

236 The permanent phenotypic values were below the average for the genotypes selected by the index. The UCPC015-
237 115 genotype stood out from the others for having the lowest permanent phenotypic value of 33.71 for IBSFT, while
238 the population average was 41.91. This genotype also stands out for SBSLF, where there is one of the lowest responses
239 to the development of the disease concerning the other genotypes. The material UCPC015-116 was able to provide
240 lower permanent phenotypic value for IBSLF (78.17) while the general average was 87.47. The excellent response of
241 the genotype also occurred in the SBSFT trait with a permanent phenotypic value of 1.14.

242 **DISCUSSION**

243 The variance of the temporary environmental effect (σ^2_{et}) showed a higher percentage of individual phenotypic
244 variance (σ^2_{τ}) for all traits. According to Viana and Resende (2014), σ^2_{et} is responsible for the temporary variation
245 associated with the momentary environmental effects manifested in each measurement, such as climatic fluctuations
246 of the years and their interactions with the effects verified in the plant. The year 2016 was considered irregular, with
247 a long period of drought (Figure 1), which may have modified the responses regarding resistance to the diseases under
248 study or reduced the potential of the inoculum (Moreira et al., 2020).

249 Climatic factors influence the response in plant development. These environmental conditions counteract the
250 severity and incidence of diseases that affect the crop, especially those of a fungal nature (Oliveira et al., 2011; Moreira
251 et al., 2020). Also, the severity and incidence values were low in general, pointing out that the climatic factors may
252 not have contributed so favorably to the occurrence of diseases (Figure 1). Temperatures between 27°C and 23°C plus
253 high rainfall and strong winds favor the occurrence of black spot. In contrast, the phoma spot occurs with greater
254 intensity in regions with high relative humidity and when rains occur which facilitate the penetration of the fungus
255 (Oliveira et al., 2011; Moreira et al. 2020).

256 Repeatability estimates are an important parameter in choosing a genotype, according to what is reported by
257 Cruz et al. (2014), as they can predict the stability of the response of a trait. Therefore, repeatability measures the
258 average correlation between two or more measurements from the same individual. Repeatability is influenced by its
259 nature and the environmental conditions to which the population is subjected (Cruz et al., 2014). Regarding the
260 repeatability of the characteristics evaluated in this work, they were considered of low repeatability, according to
261 Resende (2002). This fact indicates that a single measurement of character in the individual does not represent his real
262 capacity and, therefore, more than one measurement is necessary to decide on his use.

263 As it is a disease trait, it is expected that low repeatability occurs, of which, the environment exerts a great
264 influence on the host, on the pathogen, and the interaction of pathogen x host. Studies by Liberato et al. (2004) found
265 similar results for traits of resistance to anthracnose in papaya fruit.

266 In terms of selection, favorable results were found to reduce the magnitude of all traits if analyzed
267 simultaneously by the combined selection via index (ISC) or if analyzed individually by direct selection (DS) for the
268 traits SPSLF, SBSLF, and IBSFT. The possibility of selecting 30 superior genotypes shows that the initial population
269 of recurrent selection has the potential to advance the stages using the recurrent selection (RS) method and/or the

270 development of new papaya lines. In this case, different strategies can be adopted by breeders based on the objective
271 to be achieved. The classification of genotypes based on the ISC is considered satisfactory, since, in terms of genetic
272 gain, the lowest averages for different diseases were achieved based on the genotypes classified by the index.

273 ISC is more appropriate in the situation where the objective is to develop a population per se, in which
274 individuals are selected from the combination of all the traits being studied, in which the selected individuals have the
275 desired traits, both in terms of resistance the disease in the plant, and in terms of disease resistance in the fruits.

276 When it comes to the fact that individuals who are directly superior in terms of a particular trait are left out
277 of the combined selection, it must be considered that these individuals are sources of favorable alleles and can be used
278 during the recombination phase of superior individuals (Santa-Catarina et al., 2020a).

279 The DS of the SBSFT, IBSFT, and IBSLF traits indicated the individual “UCPC015-167”, which did not
280 occur in the ISC, however, in the case of RS, it should be selected as a source of resistance to severity and incidence
281 in the fruit and still incidence on the leaf for black spot. Thus, in the recombination stage of the S1 families, it is
282 indicated to select 30 superior individuals, considering both DS and ISC.

283 In recent studies for agronomic traits of interest such as production and fruit quality carried out by Santa-Catarina
284 et al. (2020a), the selection of 30 individuals in the UCPC0 population was indicated, from these, the DS or ISC also
285 indicated 19 proposed here. Highlighting the genotype “UCPC015-166”, which in the classification by Santa-Catarina
286 et al. (2020a) ranked second and which was also classified by the ISC proposed in this study. The genotypes:
287 "UCPC015-148", "UCPC015-152", "UCPC015-159", "UCPC015-162", "UCPC015-167", and "UCPC015-178" form
288 a group of individuals from the Formosa standard; the “UCPC015-144” and “UCPC015-197” genotypes form a small
289 group from the standard Solo. The genotypes above have favorable alleles for multiple diseases and agronomic traits
290 of production and quality, being recommended the selection of genotypes not only for the recombination stage but
291 also for advancing generation.

292 **CONCLUSION**

293 The genetic parameters revealed a high environmental effect on the estimates of traits related to resistance to
294 black spot and phoma spot.

295 The combined selection proved to be consistent for providing better selection gains and precision in the
296 selection process.

297 Thirty individuals were indicated for selection through the combined selection index, pointing out in these
298 genotypes a high genetic potential for the line development *per se* and/or hybrids with resistance to black spot and
299 phoma-spot.

300 The genotypes: "UCPC015-052", "UCPC015-166", "UCPC015-064", "UCPC015-202", "UCPC015-061",
301 "UCPC015-108", "UCPC015-144", "UCPC015-240" , "UCPC015-041", "UCPC015-003", "UCPC015-189",
302 "UCPC015-186", "UCPC015-152", "UCPC015-148", "UCPC015-197", "UCPC015-167", " UCPC015-178 ","
303 UCPC015-162 "," UCPC015-159 ", combine attributes of resistance to black spot and/or phoma spot, as well as traits
304 of agronomic interest, implying that the composition of the base population is satisfactory for the purpose for which
305 it was created.

306 **CONFLICT OF INTEREST STATEMENT**

307 The authors declare that there is no conflict of interest.

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Figures

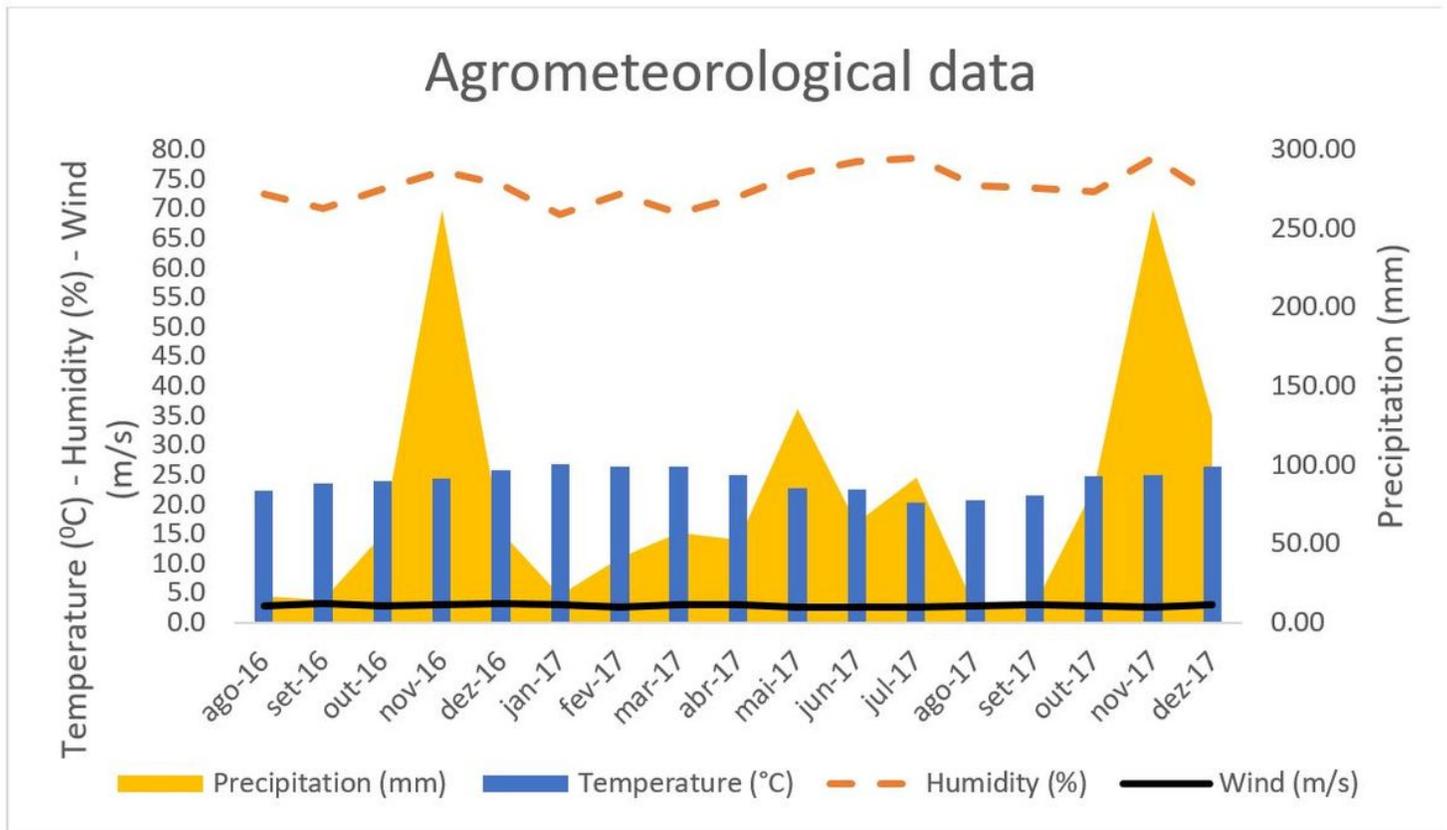


Figure 1

Climatic data from the Linhares-ES agrometeorology station from August 2016 to December 2017.

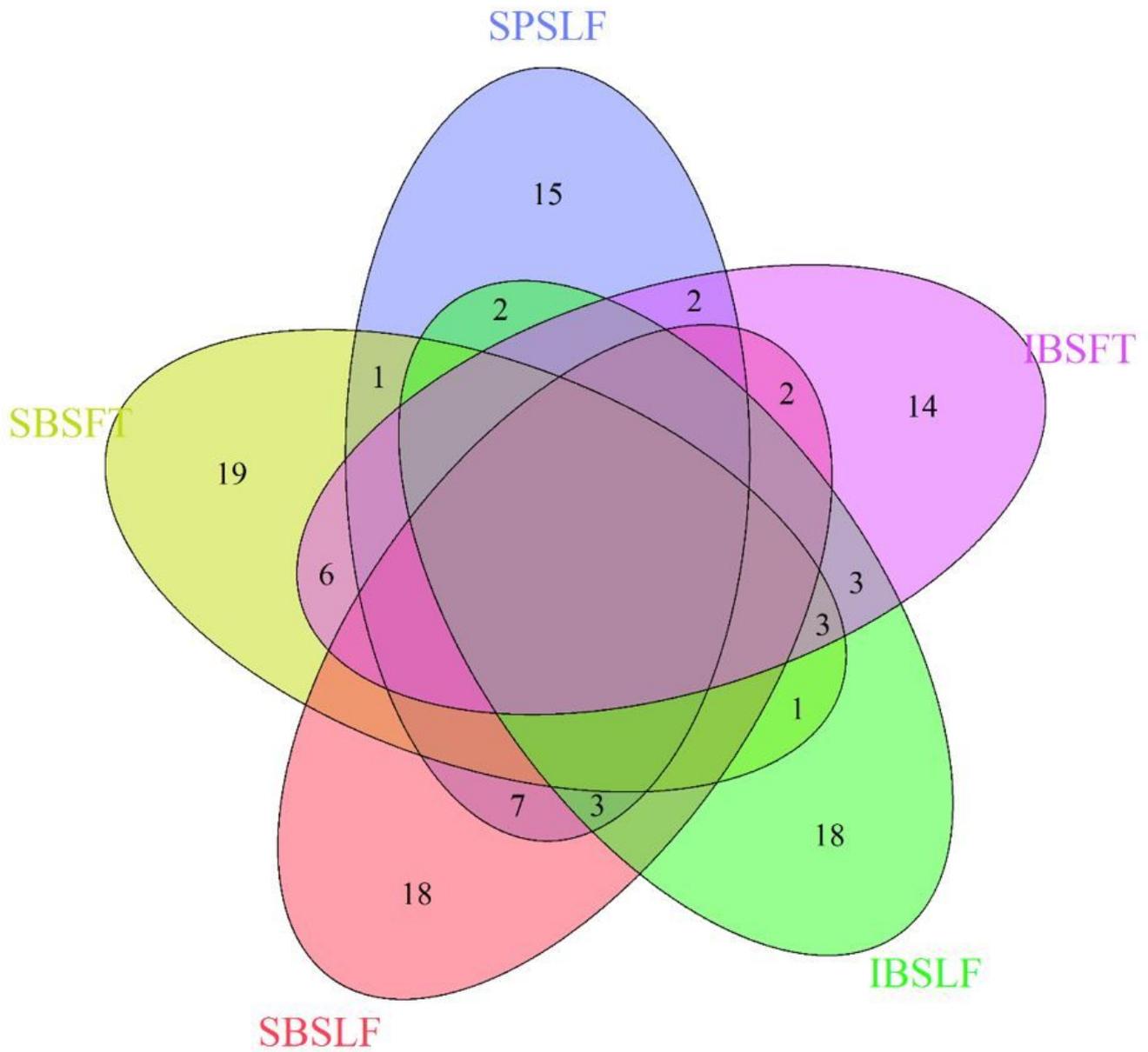


Figure 2

Venn diagram for 30 individuals selected by direct selection. SPSLF: Severity of phoma spot on the leaf. SBSFT: Severity of black spot on the fruit. SBSLF: Severity of black spot on the leaf. IBSLF: Incidence of black spot on the leaf. IBSFT: Incidence of black spot on the fruit.