Design I of Comstock and Robinson in the Emergence and Vigor of Sour Passion Fruit Seedlings

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ABSTRACT
The objective of this study was to verify the genetic influence of male parents in the expression of emergence and vigor traits of the sour passion fruit seedling, and to select the ones that contributed with the highest selection results. Controlled hybridizations were performed between genotypes of the Sour Passion Fruit Breeding Program of the Universidade Federal de Viçosa (UFV) and commercial cultivars of the Empresa Brasileira de Pesquisa e Agropecuária (Embrapa) according to Design I of Comstock and Robinson. The experiment was conducted in a completely randomized design with 24 treatments and four replications (50 seeds). The number of emerged seedlings was calculated daily to calculate emergence percentage and emergence speed index, total length of seedling (cm), length of shoot (cm) and length of main root (cm), seedling dry mass and weight of 100 seeds. The analysis of variance was performed using the Hierarchical Design Model I of Comstock and Robinson, and the combined selection for estimation of genetic gains using GENES statistical program. The Design I of Comstock and Robinson was appropriate to predict the genetic values of seedling emergence and vigor traits. Selection gains were high for the traits such as, weight of 100 seeds and dry mass per seedling. Male parents 2 and 6 were selected for most combinations.

KEYWORDS
Passiflora edulis Sims; genetic prediction; seeds; genetic design

Introduction

The sour passion fruit has various forms of propagation, it can be propagated per seeds or vegetative, through cutting or grafting (Meletti et al., 2012). The main commercially used method of propagation is for seeds, given the easiness and practicality of the process, as well as presenting lower production cost when compared to other methods (Leonel and Pedroso, 2005; Silva et al., 2015).

However, the seminiferous method has some limitations, which are mainly related to the physiological quality of the seeds, causing uneven germination, which leads to the formation of heterogeneous and less vigorous seedlings, compromising the quality of these (Negreiros et al., 2006). Part of this variation in germination may be due to genetic composition, due to the genotypes (parents) that crossed will originate the seeds (Alexandre et al., 2004).

Therefore, use of improved genotypes for germination traits and initial seedling vigor is crucial for homogeneous and vigorous seedling formation. Thus, it is important to perform evaluations and selections, aiming to properly choose male and female parents, giving a higher chance of genetic gains. In selection programs, genetic diversity can be assessed early by seed physiological quality, with germination and vigor tests (Rosado et al., 2017).
According to Viana et al. (2007), the choice of parents is of fundamental importance for the development of breeding programs for sour passion fruit, in order to obtain heterotic and superior hybrids. The right choice of parents and proper crossover planning can optimize the use of favorable genes or explore heterosis through crosses between individuals with good agronomic characteristics and with some degree of genetic divergence, making it possible to obtain superior cultivars (Negreiros et al., 2008).

To estimate genetic gains, it is necessary to use population genetic structures in order to allow the estimation of genetic variance components through inheritance studies, aiming at enhancing genetic gains. The population structure formed by Comstock and Robinson’s outline used in corn cultivation (Eyherabide and Hallauer, 1991), has skills that can optimize the selection of promising parents in sour passion fruit (Santos et al., 2011), because the structure of the crossings by this genetic design allows the use of a wider range of parents when compared to other methods, reducing the problems generated by the genetic incompatibility of the sour passion fruit.

Because the need for hybrids that have uniform germination and rapid development, and the importance of parents in the expression of these traits in progenies of sour passion fruit, this study aimed to verify the genetic influence of male parents by the Design I of Comstock and Robinson, in the expression of emergence traits and seedling vigor, and select the ones that contributed with the greatest selection gains.

**Methods and Materials**

*Plant Material and Obtaining the Seeds*

The experiment was carried out in the Department of Agronomy of the Universidade Federal de Viçosa (UFV), in the city of Viçosa, Minas Gerais, Brazil. Controlled hybridization was carried out among genotypes of the sour passion fruit breeding program of the UFV and commercial cultivars of the Empresa Brasileira de Pesquisa Agropecuária (Embrapa).

Hybridizations were structured according to the Design I proposed by Comstock and Robinson (1948). Twenty-four hybrids were formed, crossing eight pollen donor plants (male parent) and 24 recipient plants (female parent), according to Table 1, obtaining two fruits per plant. Fruits were harvested 60–90 days after pollination, and the criterion for harvesting was to change the color of the fruit from green to yellow, with at least 5% yellow color (Negreiros et al., 2006).

Extraction of the seeds was carried out by immersion in a solution containing 10% lime, by rubbing the seeds through a sieve to remove aryl, then washing the seeds in water. The seeds were placed on

| Hybrids | Male code | Male code | Male code | Male code | Male code |
|---------|-----------|-----------|-----------|-----------|-----------|
| 1       | 1         | UFV 23 (2) | UFV 16 (2) | 13        | UFV 16 (4) | UFV 22 (4) |
| 2       | 1         | UFV 23 (2) | UFV 16 (4) | 14        | UFV 16 (4) | UFV 23 (2) |
| 3       | 1         | UFV 23 (2) | UFV 1 (2)  | 15        | UFV 16 (4) | GA (4)    |
| 4       | 2         | UFV 20 (3) | UFV 22 (2) | 16        | GA (1)    | UFV 17 (1) |
| 5       | 2         | UFV 20 (3) | GA (2)    | 17        | GA (1)    | UFV 24 (3) |
| 6       | 2         | UFV 20 (3) | UFV 1 (4)  | 18        | GA (1)    | UFV 21 (1) |
| 7       | 3         | UFV 15 (3) | UFV 16 (3) | 19        | UFV 15 (1) | UFV 17 (2) |
| 8       | 3         | UFV 15 (3) | UFV 22 (3) | 20        | UFV 15 (1) | GA (3)    |
| 9       | 3         | UFV 15 (3) | RC (4)    | 21        | UFV 15 (1) | UFV 15 (3) |
| 10      | 4         | GA (2)    | UFV 20 (3) | 22        | UFV 15 (2) | UFV 24 (4) |
| 11      | 4         | GA (2)    | UFV 1 (2)  | 23        | UFV 15 (2) | UFV 17 (2) |
| 12      | 4         | GA (2)    | UFV 20 (4) | 24        | UFV 15 (2) | UFV 24 (1) |

*Derived of the genetic breeding program of sour passion fruit of UFV.
*Derived of Embrapa, GA – BRS GA1 and RC – BRS RC1.
*Number in parentheses refers to the code of half-sibling plants in progeny.
paper towels at room temperature (± 25°C) to remove excess of humidity for two days, and following drying, aryl residues still adhering to the seed were removed and the seeds were manually counted.

The seeds were placed in plastic trays containing washed inert sand and distributed in grooves at a depth of 2 cm, maintained in greenhouse for 28 days. Irrigation was performed daily with distilled water. Maximum, minimum and average temperatures recorded inside the greenhouse during the experiment were 34.16°C, 15.64°C and 24.90°C.

Traits Evaluated

The traits evaluated on seeds and seedlings were: weight of 100 seeds (WS) in grams (g), emergence (EME) in percentage (%), emergence speed index (ESI); total length of seedlings (TLS), length of main root (LR) and shoot (LS) in centimeters (cm); number of normal seedlings (NNS) and dry mass per seedling (DMS) in milligrams (mg).

The weight of 100 seeds was counted by weighing four samples of 100 seeds for each hybrid on an analytical scale (0.001 g) before sowing. The emergence and emergence speed index was evaluated daily by visual method, according to the Rules for Seed Analysis – RAS (BRASIL, 2009), considering seedlings when it was possible to visualize the emission of the cotyledons loop. Finally, the percentage of emergence was calculated using the count of emerged seedlings on the 28th day of evaluation.

The emergency speed index was determined according to Maguire (1962): 

\[
\text{ESI} = \frac{\text{N1} \times \text{E1} + \text{N2} \times \text{E2} + \ldots + \text{Nn} \times \text{En}}{\text{E1} + \text{E2} + \ldots + \text{En}} \]

where: N1 = number of days for the first count; E1 = number of emerged seedlings on the first count; N2 = number of days for the second count; E2 = number of emerged seedlings in the second count; Nn = number of days for the last count; En = number of emerged seedlings at the last count. The total length of seedlings (length of the main root and the shoot) was measured with the aid of a ruler graduated in millimeters at the end of the 28th day after assembly of the experiment. All normal seedlings of each treatment and replicate were measured separately in order to obtain an average. Normal seedlings number was counted in a simple count on the 28th day of the experiment, selecting seedlings that emerged completely and did not present any type of anomaly.

The dry mass per seedlings was obtained as follows: the normal seedlings on the 28th day were packed in paper bags and placed in a drying oven at 65°C for 72 hours. After the total dry mass of the seedlings was weighed on a semi-analytical scale, which was divided by the number of normal seedlings, obtaining the dry mass per seedling.

Statistical analysis

The experiment was conducted in a completely randomized design, with 24 treatments (Table 1) and four replications. Each plot consisted of 50 seeds.

The analysis of variance (ANOVA) was performed by the Hierarchical Model of the Genetic Structure in hybrid and reciprocal. The model was established with all fixed effects, as follows:

\[
Y_{ij} = \mu + M_i + F/M_{ij} + E_{ijk} \]

where, \(Y_{ij}\) is the observation regarding the crossing between parents; \(\mu\) is the general mean; \(M_i\) is the effect of the I order male progenitor (male); \(F/M_{ij}\) is the effect of the female j progenitor (female) hierarchized within male i; and \(E_{ijk}\) is the experimental error. After ANOVA processing, the combined selection was performed by estimating the genetic gains.

The analysis was performed using the GENES software application (Cruz, 2013).

Results and discussion

There was a statistical difference between hybrids for all traits evaluated, demonstrating the existence of variability and the importance of selecting hybrids regarding these traits (Table 2). According to Santos et al. (2008), the occurrence of variability is paramount when it is desired to establish a breeding program to promote selection for traits under evaluation. Alexandre et al. (2004) selecting passion
**Table 2.** Summary of analysis of variance in 24 sour passion fruit hybrids established by design I of Comstock and Robinson.

| Factors | DF | WS   | EME  | ESI  | TLS  | LR   | LS   | NNS  | DMS   |
|---------|----|------|------|------|------|------|------|------|-------|
| HIB     | 23 | 0.503** | 448.862** | 0.782** | 2.177** | 1.593** | 0.169** | 114.375** | 69.387** |
| M       | 7  | 0.665** | 445.785** | 0.797** | 0.447** | 0.613** | 0.093** | 102.541** | 65.989** |
| F/M     | 16 | 0.432** | 450.208** | 0.776** | 2.934** | 2.021** | 0.203** | 119.552** | 70.874** |
| F/M 1   | 2  | 0.471** | 794.333** | 0.577** | 3.624** | 2.718** | 0.122** | 198.583** | 63.270** |
| F/M 2   | 2  | 0.083** | 81.333**  | 0.420** | 0.739** | 0.726** | 0.278** | 22.7500** | 41.754** |
| F/M 3   | 2  | 1.045** | 1497.0**  | 0.987** | 1.487** | 1.022** | 0.047** | 404.333** | 25.618** |
| F/M 4   | 2  | 0.247** | 148.0**   | 0.099** | 0.935** | 1.276** | 0.032** | 54.75**  | 22.800** |
| F/M 5   | 2  | 0.284** | 37.333**  | 0.742** | 4.515** | 3.186** | 0.140** | 12.25**  | 152.335** |
| F/M 6   | 2  | 0.145** | 70.333**  | 0.098** | 0.133** | 0.154** | 0.088** | 16.75**  | 24.162** |
| F/M 7   | 2  | 0.419** | 787.0**   | 2.067** | 6.445** | 3.411** | 0.620** | 204.75** | 101.448** |
| F/M 8   | 2  | 0.765** | 186.333** | 1.218** | 5.591** | 3.680** | 0.382** | 42.25**  | 135.604** |
| Residual | 72 | 0.0004 | 32.833 | 0.041 | 0.257 | 0.220 | 0.0644 | 9.375 | 2.870 |
| Mean    |   | 2.044 | 88.041 | 2.488 | 10.371 | 7.776 | 2.597 | 43.562 | 16.767 |
| CV(%)   |   | 1.05  | 6.50  | 8.22  | 4.89  | 6.03  | 9.77  | 7.02  | 10.10 |

**F** significant at the level of 1% probability, respectively. *F* not significant at 5% level. *DF: degrees of freedom, CV(%): Coefficient of variation, WS: weight of 100 seeds (g), EME: emergence (%), ESI: emergence speed index, TLS: total length of seedlings (cm), LR: length of main root (cm), LS: length of the shoot (cm), NNS: number of normal seedlings and DMS: dry mass per seedling (mg) at different hybrids (HIB), male parent (M) and female parents inside male parent (F/M).
fruit genotypes for germination, obtained significant difference among genotypes for traits germination percentage and emergence speed index; however, for the traits shoot height, length of main root and total length of seedling, there was no effect significant by the F test.

Male parents differed in the traits such as, weight of 100 seeds, emergence, speed of emergence index, length of main root, number of normal seedlings and dry mass per seedling, not differing only in total length of seedling and length of the shoot. The results make it possible to infer that there is potential genetic variability among male parents for heritable traits, so it is possible to select those that transmitted favorable genetic traits to hybrids.

The significant difference between male parents (Table 2) shows that males have an effect on the expression of traits in the formed hybrids, being subject to selection. The selection of male parents favors heterotic effects, since Galicia-Juárez et al. (2017) emphasize that the heterotic effects are expected to be maximized in single crosses depending on the genetic diversity of the parental. Evaluating female parents for all male parents (F/M – Table 2), it is concluded that female parents differed for all traits in at least one of the male parents. These results demonstrate the genetic variability that exists among female parents.

Only the traits weight of 100 seeds and dry mass per seedling present differences among the female parents for each male parent (F/Mi), in all 8 male parents. In the traits such as, emergence, emergence speed index, total length of seedling, length of main root and number of normal seedlings, most comparisons among female parents in each male parent showed significant differences, only the shoot length that the females differed in three male parents (Table 2).

The overall emergence mean was 88.04%, demonstrating the potential of the genotypes used for this trait. The coefficient of variation presented its highest value (10.10%) for seedling dry mass and the smallest (1.05%) for 100 seeds weight, evidencing the experimental precision.

There was a significant selection gain for the weight of 100 seeds (Table 3), totaling 15.62% gain, regarding the selected male parents 2 and 6, where hybrids had the highest means for this trait, as shown in Table 4. According to Foster (1986), seed size may be related to the amount of reserve, and in larger seeds faster synthesis of important secondary compounds occurs in the germination process.

It can be inferred that there is a cytoplasmic effect of the female parent influencing these traits, higher seed weight may be related to higher seed reserve, and most of the genetic makeup of the endosperm is maternally, therefore the highest weight of 100 seeds observed for hybrids coming from male parents 2 and 6 is actually due to the female parents used in these crosses. Rosado et al. (2017) postulate the existence of cytoplasmic effects on the control of seed emergence and vigor traits in sour passion fruit. Therefore, important to target the paternal and maternal genitor in order to obtain seeds with high germination potential in Sour Passion Fruit, according to Rosado et al. (2020).

Another feature that also stood out regarding the selection gain was the dry mass per seedling, which showed a gain of 15.69%, the highest one recorded, where was selected male parents 2 and 6, indicating that these parents transmit this trait, resulting in greater vigor (Table 3). The heritability

Table 3. Means of 24 hybrids in the Design I of Comstock and Robinson for emergence and vigor traits of Sour Passion Fruit.

| Factors | \( \bar{x} \_S \) | \( \bar{x} \_M \) | DS \(^e\) | \( h^2 \) | SG | MPS |
|---------|-----------------|-----------------|--------|--------|----|-----|
| WS      | 2.04            | 2.36            | 0.32   | 0.99   | 15.62 | 2 e 6 |
| EME     | 88.04           | 93.50           | 5.45   | 0.32  | 2.02 | 5 e 6 |
| ESI     | 2.48            | 2.73            | 0.24   | 0.77  | 7.67 | 8 e 6 |
| TLS     | 10.37           | 10.57           | 0.20   | 0.00  | 0.00 | 2 e 6 |
| LR      | 7.77            | 8.06            | 0.28   | 0.74  | 2.70 | 2 e 3 |
| LS      | 2.59            | 2.68            | 0.09   | 0.00  | 0.00 | 6 e 1 |
| NNS     | 43.56           | 46.37           | 2.81   | 0.33  | 2.17 | 6 e 5 |
| DMS     | 16.76           | 19.67           | 2.90   | 0.90  | 15.69 | 2 e 6 |

\( \bar{x} \_S \): mean of all male parents, \( \bar{x} \_M \): mean of selected male parents, \( ^e \) DS: selection differential, \( h^2 \): heritability, SG: selection gain and MPS: male parents selected for WS: weight of 100 seeds (g), EME: emergence (%), ESI: emergence speed index, TLS: total length of seedlings (cm), LR: length of main root (cm), LS: length of shoot (cm), NNS: number of normal seedlings and DMS: dry mass per seedling (mg).
regarding male parents 2 and 6 for this trait was 0.90, being, therefore, very influenced by the parents and little influenced by the environment, standing out for presenting means values for these traits corresponding to the one sought by breeding programs.

These genetic gains results are similar to those observed by Gonçalves et al. (2007) working on predicting genetic gains in a population of sour passion fruit structured in design I of Comstock and Robinson, where they did get considerable selection gains. The highest gain obtained by the authors was 18.55% for the number of fruits per plant. Similarly, Neves et al. (2011) used design I to estimate genetic parameters in a sour passion fruit population for gain prediction purposes, obtaining gains of up to 34.73% for number of fruits harvested.

The selection gain for length of main root was positive among the traits that involve seedling length, selecting male parents 2 and 3. As for the number of normal seedling, the selection gain was 2.17%, with a heritability of 0.33, indicating that the mean obtained by hybrids resulting from crosses involving the selected male parents 6 and 5 is due to high influence of the environment.

Table 1 shows that hybrids H3 with H11 and H19 with H23 are half-sib, same female parent, verifying in Table 4 differences between them for all traits, proving the effect of the male in the formation of the hybrid.

According to the results found in this study, it is verified that there are genetic gains obtained by the Design I of Comstock and Robinson in the emergence and vigor of sour passion fruit seedlings. The Design I allows the prediction of genetic values in a study population structured in hybrids, which in many cases is not possible to perform by diallels, due to the self-incompatibility presented by passion fruit, making some combinations impossible to obtain.

**Conclusions**

The Design I of Comstock and Robinson was appropriate for predicting the genetic values of emergence and vigor traits of sour passion fruit seedlings. Selection gains were high for the weight
of 100 seeds and dry mass per seedling traits. There is effect of the male parent in the formation of the hybrid, and the male parents 2 and 6 selected for most combinations.

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