Australian Journal of Crop Science AJCS
AJCS 9(3):223-231 (2015) ISSN:1835-2707

Diversity analysis of papaya (*Carica papaya* L.) genotypes related to seed quality

Liana Hilda Golin Mengarda[1], José Carlos Lopes[2*], Rafael Fonseca Zanotti[1], Rodrigo Sobreira Alexandre[3]

[1]Postgraduate Program in Plant Production, Federal University of Espírito Santo (Universidade Federal do Espírito Santo), Alegre, Espírito Santo, Brazil
[2]Department of Plant Production, Federal University of Espírito Santo (Universidade Federal do Espírito Santo), Alegre, Espírito Santo, Brazil
[3]Department of Forest Sciences, Federal University of Espírito Santo (Universidade Federal do Espírito Santo), Alegre, Espírito Santo, Brazil

***Corresponding author: jcufes@bol.com.br**

Abstract

The objective of this study was to identify the genotype of papaya (*Carica papaya* L.) with the highest initial performance through the analysis of diversity and genetic parameters related to the physicochemical and physiological characteristics of the seeds. Seed quality was assessed through the following parameters: moisture, size, weight, levels of reserves compounds, percentage and speed of germination, percentages of normal and abnormal seedlings, and seedlings length and weight. Clustering methods and the sum of rank index were applied to identify the genotype with the highest initial performance. The characteristics of greatest importance in the discrimination of the genotypes and the genetic parameters for each characteristic were estimated. The genotype Caliman 01 (G4) seeds had the highest mean length (0.55 mm), germination percentage (94%), speed germination index (2.30), and normal seedlings percentage (90%). Based on the physicochemical and physiological characteristics of the seeds, the clustering using unweighted pair group method with arithmetic mean (UPGMA) and principal components analysis showed the formation of four groups: group 1 included the genotypes G1, G2, and G3; group 2 included the genotype G4; group 3 included the genotype G8; and group 4 included the genotypes G5, G6, and G7. Comparing each group, it was found that G4 (group 2) had higher means for 50% of the characteristics. The second count of germination (SCG), a characteristic relationship with germination seed speed, was most important for the analysis of diversity among the genotypes of papaya. Results showed that the Caliman 01 genotype had the highest initial performance.

Keywords: *Carica papaya* L.; clustering; germination; plant breeding; vigor.
Abbreviations: AS_ percentage of abnormal seedlings; cv._cultivar; CV_ coefficient of variation of variance analysis; D_ diameter; DM_ dry mass of seedlings; F_ F value calculated of variance analysis; FCG_ first count of germination; FM_ fresh mass of seedlings; G_ germination percentage; G1_ genotype 1 (Caliman 01 generation F2); G2_ genotype 2 (Golden); G3_ genotype 3 (Tainung 01 generation F2); G4_ genotype 4 (Caliman 01); G5_ genotype 5 (Sunrise Solo); G6_ genotype 6 (Golden THB); G7_ genotype 7 (Triple Hybrid); G8_ genotype 8 (Hybrid JS12 × Waimanalo); L_ seed length; Lp_ lipid levels; M_ moisture; MGT_ mean germination time; NS_ percentage of normal seedlings; P_ protein levels; RL_ root length; S_ levels of soluble sugars; SCG_ second count of germination; SGI_ speed of germination index; SL_ shoot length; St_ starch levels; TCG_ third count of germination; TSW_ thousand-seed weight; UPGMA_ Unweighted pair group method with arithmetic mean; Σ = sum.

Introduction

Papaya (*Carica papaya* L.) is a fruit originating from tropical America, and its cultivation is an important economic activity for American and Asian countries. The commercial varieties of papaya are divided in Solo group, represented by pure inbred lines, and Formosa group represented by hybrids (Dantas; Lima, 2001). The agronomic potential evaluation of different pure inbred lines and hybrids of papaya has been gaining attention. However, there are few studies investigating the genetic diversity related with germination and seed vigor characteristics (Cardoso et al., 2009; Macedo et al., 2013). The studies on genetic diversity of cultivated species increase the genetic basis to support breeding programs. The papaya germplasm has considerable phenotypic variation for characteristics of agronomic importance such as the size and shape of fruit, color pulp, taste and soluble solids content, duration of the juvenile period, and plant height (Kim et al., 2002). Quantification of the genetic diversity level allows, among other advantages, the genotypic selection for biologically oriented crossings (Vieira et al., 2007; Bertan et al., 2009; Macedo et al., 2013).
Commercial breeding of papaya (*Carica papaya* L.) is performed through seeds, which play an important role in the procurement and dissemination of genetic characteristics that are incorporated into improved varieties and are co-responsible for productivity. Assessment of seed quality can reveal relevant information for obtaining vigorous and healthy seedlings and more productive plants (Popinigs, 1985; Marcos Filho, 2005; Ruggiero et al., 2011). To promote the development of the papaya crop, it is necessary to develop and identify varieties that are environmentally,

socially, and economically adapted to the producing regions, in addition to providing producers with seeds of excellent quality, with an impact on productivity (Ruggiero et al., 2011). Quality seeds possess a set of "gold standard" characteristics that determine their performance potential after sowing in the field. The seed physiological quality is primarily characterized by its germination and vigor. The physicochemical quality of seeds involves morphological and physical characteristics such as size and moisture in addition to the biochemical composition and its association with vigor (Popinigs, 1985; Marcos Filho, 2005). Genetic factors are primarily responsible for seed physiological quality (Popinigs, 1985). Some studies indicate that genetic divergence acts on the germination and vigor of seeds of different papaya genotypes. Many morphological and physiological characteristics related to the quality of the seeds show high heritability, indicating that they can be exploited in the breeding of this species (Cardoso et al., 2009; Macedo et al., 2013). The analysis of multiple characteristics and the relationships among them can lead to informative and efficient assessment of individuals in several aspects, providing a holistic view of a genotype (Cruz et al., 2011). Studies conducted on genetic variability, the relationships among the characteristics, and the estimation of their heritability provides essential information for the selection of papaya genotypes (Marin et al., 2006; Silva et al., 2007; 2008; Oliveira et al., 2010; 2012; Dias et al., 2011; Ferreira et al., 2012), thus allowing the clustering of individuals with similar genetic material and the identification of genotypes relevant for certain traits of agronomic interest (Cruz et al., 2011) such as germination and vigor. Such information can support the selection of progenitors to obtain genotypes with the highest germination performance and the evaluation of new cultivars. Thus, the objective of this work was to identify papaya (*C. papaya* L.) genotypes with a higher initial performance through the analysis of diversity and genetic parameters related to the physicochemical and physiological characteristics of the seeds.

Results and Discussion

Physicochemical characterization

According to the physicochemical characterization of the seeds, the average moisture ranged from 5.01% (G7) to 7.90% (G2) for different genotypes (Table 1). These water levels are considered adequate for the maintenance of seed viability during storage (Marcos Filho, 2005; Berbert et al., 2008). Berbert et al. (2008) found that drying papaya seeds cv. Caliman 01 to water levels of 7.7% had a positive effect on their vigor, and that value is similar to that observed in this study for the G1, G2, and G4 genotypes. However, very low water levels, such as those observed for G7 (5.01%), can harm imbibition because the difference in water potential between the environment and the intracellular space leads to very rapid water uptake, thus placing the germination process at risk (Marcos Filho, 2005). The G4 seeds had the highest mean length. Although they also had the highest mean diameter, it was not significantly different from the G5, G6, G7, and G8 seeds. The genotypes G1, G4, and G7 had the highest thousand-seed weights (TSW) (Table 1). According to Martins et al. (2005), papaya seeds of greater weight (TSW between 17 and 19 g) have higher vigor, which was found in this study for G4 (18.18 g). The G8 seeds had the highest levels of soluble sugars (6.38%) and starch (0.62%), which were not statistically different from those of G4 (0.58%). However, G8 had reduced lipid levels (4.33%), whereas G4 had one of the highest mean lipid levels (11.58%) (Table 1). Soluble sugars and starch represent more readily available forms of energy, although they are less efficient than lipids (Marcos Filho, 2005). Thus, the G8 and G4 genotypes have greater energy reserves, which may be reflected in greater performance. The greatest levels of lipids (≥ 10.21%) were observed in G2, G4, G5, G6, and G7. The protein levels did not differ among the genotypes (Table 1).

Physiological quality

Seeds of the G4 and G8 genotypes had higher germination percentages (94% and 96%, respectively) and higher mean counts after 14 days (94% and 82%, respectively) (Table 2). The G4 seeds showed faster germination: they had a higher mean SGI (2.30), and by the second count, they had already reached the maximum germination percentage (94%). On the first count, G4, G5, and G8 were the only genotypes that had any germination. In addition, the G4 genotype is also distinguished by its percentage of normal seedlings: G4 and G3 had more than 90% normal seedlings, whereas G2 and G5 had a greater percentage of abnormal seedlings (≥ 25%).

The G4 had the highest seed quality related to physicochemical (size, starch, and lipid levels) and physiological (percentage and speed of germination and percentage of normal seedlings) characteristics. However, the seedling development shows a wide variation among the different genotypes (Table 2). The G6 seedlings had the highest mean shoot and root lengths, whereas G1 and G2 had the lowest mean lengths. In contrast, G1 had a higher mean dry mass, whereas G4 had a lower mean dry mass. The contrast between the germination and the seedling characteristics suggests an inverse relationship with the speed of germination. G6 and G1 are among the genotypes with the lowest SGI mean, i.e., their germination occurred more slowly. However, these genotypes have greater seedling length and dry mass, respectively. In contrast, G4 had higher SGI but lower seedling development. The mean germination time for G4 was approximately 9 days, whereas it was 15 days for G6 and G1. Thus, although the G4 seeds are of higher quality (and germinate more quickly), the seedlings deteriorated in quality, possibly because of the time of residence in the substrate after germination.

Correlation analysis

The correlation analysis showed that the characteristic second count of germination (SCG) was negatively correlated with DM (Table 3), suggesting that rapid germination favors the deterioration and loss of dry mass of the seedlings. A significant correlation was also observed between SGI and MGT; SGI and FM; NS and AS; NS and FM; AS and FM; and SL and RL (Table 3). A significant positive correlation was observed in the germination values of G and TCG, with an R^2 value of 0.99 (Table 3); the mean values of these characteristics were very similar (Table 2). This suggests that there was stability in the germination of nearly all genotypes after 21 days. Thus, the assessment of the total germination and seedling development could be completed earlier, at 21 days, thus avoiding the seedling deterioration (and DM reduction) observed in the G4 genotype. In addition, G and TCG are positively correlated with SGI and negatively correlated with MGT. Thus, there is an association between the percentage and speed of germination, which represents an important test of vigor. The speed of germination index and the mean germination time are an important indication of

Table 1. Physicochemical characteristics of seeds of the papaya genotypes: moisture (M), length (L), diameter (D), thousand-seed weight (TSW), and levels of soluble sugars (S), starch (St), lipids (Lp), and total protein (P).

Genotype	M (%)	L (cm)	D (cm)	TSW (g)
G1	$7.60^{a} \pm 0.21$	$0.42^{c} \pm 0.016$	$0.27^{b} \pm 0.008$	$16.90^{a} \pm 0.70$
G2	$7.90^{a} \pm 0.11$	$0.42^{c} \pm 0.008$	$0.26^{b} \pm 0.006$	$12.65^{b} \pm 1.17$
G3	$5.58^{e} \pm 0.19$	$0.36^{d} \pm 0.012$	$0.22^{c} \pm 0.004$	$14.40^{b} \pm 0.74$
G4	$7.00^{b} \pm 0.14$	$0.55^{a} \pm 0.017$	$0.30^{a} \pm 0.010$	$18.18^{a} \pm 0.62$
G5	$6.41^{c} \pm 0.28$	$0.45^{c} \pm 0.012$	$0.30^{a} \pm 0.005$	$14.20^{b} \pm 0.49$
G6	$5.97^{d} \pm 0.04$	$0.44^{c} \pm 0.011$	$0.31^{a} \pm 0.007$	$12.88^{b} \pm 0.72$
G7	$5.01^{f} \pm 0.12$	$0.50^{b} \pm 0.009$	$0.29^{a} \pm 0.004$	$15.80^{a} \pm 0.23$
G8	$5.81^{d} \pm 0.10$	$0.51^{b} \pm 0.004$	$0.32^{a} \pm 0.011$	$12.75^{b} \pm 0.09$
F	37.36^{**}	25.89^{**}	16.24^{**}	9.37^{**}
CV (%)	5.17	5.15	8.83	9.16
	S (%)	St (%)	Lp (%)	P (%)
G1	$2.93^{c} \pm 0.29$	$0.46^{b} \pm 0.002$	$7.55^{b} \pm 1.09$	$28.23^{a} \pm 0.13$
G2	$3.35^{c} \pm 0.12$	$0.48^{b} \pm 0.014$	$12.71^{a} \pm 0.81$	$28.13^{a} \pm 0.07$
G3	$3.47^{c} \pm 0.23$	$0.43^{b} \pm 0.006$	$7.78^{b} \pm 0.89$	$28.50^{a} \pm 0.02$
G4	$5.13^{b} \pm 0.25$	$0.58^{a} \pm 0.067$	$11.58^{a} \pm 0.42$	$28.56^{a} \pm 0.13$
G5	$2.68^{c} \pm 0.18$	$0.35^{b} \pm 0.030$	$10.21^{a} \pm 1.10$	$27.50^{a} \pm 0.08$
G6	$5.46^{b} \pm 0.33$	$0.50^{b} \pm 0.035$	$12.04^{a} \pm 0.65$	$27.81^{a} \pm 0.03$
G7	$4.49^{b} \pm 0.63$	$0.41^{b} \pm 0.039$	$12.07^{a} \pm 0.76$	$28.00^{a} \pm 0.09$
G8	$6.38^{a} \pm 0.47$	$0.62^{a} \pm 0.044$	$4.33^{c} \pm 0.36$	$29.13^{a} \pm 0.05$
F	14.43^{**}	6.00^{**}	13.50^{**}	0.90^{ns}
CV (%)	16.57	14.99	16.43	3.67

Means followed by the same letters (in the column) are grouped by the Scott-Knott test at 1% (**) probability. ^{ns} = not differ statistically (n = 4). F = the F value calculated using analysis of variance. CV (%) = coefficient of variation.

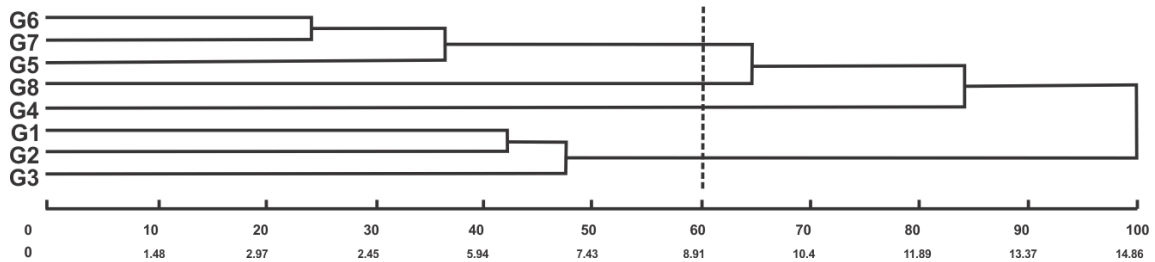

Fig. 1. UPGMA dendrogram for the physical, biochemical, and physiological data of seeds from eight papaya genotypes (cophenetic coefficient = 0.6). Group 1 = G1 (Caliman 01 generation F2), G2 (Golden), and G3 (Tainung 01 generation F2); group 2 = G4 (Caliman 01); group 3 = G8 (Hybrid JS12 × Waimanalo); group 4 = G6 (THB), G7 (Triple hybrid), and G5 (Sunrise Solo).

vigorous plants (Aosa, 2002; Marcos Filho, 2005). A significant positive correlation between the length of the seeds (L) and the percentage and speed of germination (G, SCG, TCG, and SGI) was observed. There were also correlations between seed diameter and both FCG and SCG (Table 3). Thus, according to the analysis of this study, larger seeds had superior and faster germination. The G4 genotype had a higher mean seed size, percentage germination, and SGI. A simple phenotypic correlation measures the degree of association between two variables and is determined by genetic and environmental factors. A genetic correlation (Silva et al., 2007; Cruz et al., 2011) is distinguished by the exclusion of environmental factors. In this study, genetic correlations were significant only between G and TCG and between NS and AS. For the remaining correlations (phenotypic), the associations do not necessarily occur because of genetic and heritable characteristics.

Genetic diversity

Among diversity analyses, clustering methods represent an efficient technique for evaluating individuals based on numerous characteristics, thus providing a holistic view of each genotype and identifying progenitors with interesting genetic characteristics for breeding (Cruz et al., 2011). Based on the 20 physicochemical and physiological characteristics of the seeds, the clustering using UPGMA (cophenetic coefficient = 0.6) showed the formation of four groups: the first group included the genotypes G1 (Caliman 01 generation F2), G2 (Golden), and G3 (Tainung 01 generation F2); the second included G4 (Caliman 01); the third included G8 (Hybrid JS12 × Waimanalo); and the fourth group included the genotypes G5 (Sunrise Solo), G6 (THB), and G7 (Triple hybrid) (Fig. 1). Principal components analysis enables the interpretation of multiple variables and is effective in predicting the divergence among genotypes, thus allowing them to be grouped so that there is homogeneity within the groups and heterogeneity among groups (Cruz et al., 2011). In the present study, only two principal components were needed to estimate 87.03% of the total variance. In graphical analysis of the first two principal components, the genotype groupings were similar to those obtained by UPGMA: G1, G2, and G3 were grouped together; G6, G7, and G5 were grouped together; and G4 and G8 remained isolated from each other and from other groups (Fig. 2). Comparing the means of the characteristics of each group, G4 (group 2) had higher means for 50% of the characteristics, and the sum of the means was much greater

Table 2. Physiological characteristics of seeds of papaya genotypes: germination percentage (G), first count of germination (FCG), second count of germination (SCG), third count of germination (TCG), speed of germination index (SGI), mean germination time (MGT), in days, percentages of normal seedlings (NS) and abnormal seedlings (AS), shoot length (SL), root length (RL), fresh mass (FM), and dry mass (DM) of normal seedlings.

Genotype	G (%)	FCG (%)[1]	SCG (%)	TCG (%)
G1	$86^{b} \pm 2.58$	0 ± 0.00	$24^{c} \pm 5.89$	$85^{b} \pm 3.00$
G2	$74^{c} \pm 5.29$	0 ± 0.00	$21^{c} \pm 5.00$	$70^{c} \pm 5.29$
G3	$66^{c} \pm 4.76$	0 ± 0.00	$28^{c} \pm 4.90$	$65^{c} \pm 4.43$
G4	$94^{a} \pm 1.14$	6 ± 1.15	$94^{a} \pm 1.15$	$94^{a} \pm 1.15$
G5	$80^{b} \pm 1.63$	5 ± 2.52	$59^{b} \pm 4.43$	$77^{b} \pm 3.42$
G6	$72^{c} \pm 3.27$	0 ± 0.00	$35^{b} \pm 3.42$	$69^{c} \pm 2.52$
G7	$72^{c} \pm 4.32$	0 ± 0.00	$33^{b} \pm 8.06$	$72^{c} \pm 4.32$
G8	$96^{a} \pm 2.83$	10 ± 3.83	$82^{a} \pm 7.02$	$96^{a} \pm 2.83$
F	11.24^{**}	-	45.44^{**}	12.44^{**}
CV (%)	8.83	-	12.11	9.01
	SGI	MGT (days) [1]	NS (%)	AS (%)
G1	$1.41^{c} \pm 0.06$	15.33 ± 0.33	$81.79^{b} \pm 0.34$	$18.21^{b} \pm 0.34$
G2	$1.21^{d} \pm 0.09$	15.57 ± 0.29	$74.71^{c} \pm 5.17$	$25.29^{a} \pm 5.17$
G3	$1.11^{d} \pm 0.08$	15.08 ± 0.20	$92.06^{a} \pm 2.14$	$7.94^{c} \pm 2.14$
G4	$2.30^{a} \pm 0.04$	9.15 ± 0.24	$98.08^{a} \pm 1.92$	$1.92^{c} \pm 1.92$
G5	$1.40^{c} \pm 0.04$	12.97 ± 0.83	$73.12^{c} \pm 2.78$	$26.88^{a} \pm 2.78$
G6	$1.20^{d} \pm 0.06$	15.36 ± 0.18	$86.53^{b} \pm 2.67$	$13.47^{b} \pm 2.67$
G7	$1.27^{d} \pm 0.08$	14.56 ± 0.17	$85.39^{b} \pm 1.81$	$14.61^{b} \pm 1.81$
G8	$1.76^{b} \pm 0.04$	11.26 ± 0.64	$83.69^{b} \pm 1.95$	$16.31^{b} \pm 1.95$
F	30.80^{**}	-	9.61^{**}	9.61^{*}
CV (%)	3.50	-	6.33	34.28
	SL (cm)	RL (cm)	FM (mg)[1]	DM (mg)
G1	$2.79^{c} \pm 0.23$	$0.74^{e} \pm 0.09$	61.18 ± 5.98	$5.35^{a} \pm 0.11$
G2	$2.87^{c} \pm 0.14$	$0.54^{e} \pm 0.05$	45.19 ± 2.19	$4.25^{b} \pm 0.15$
G3	$4.86^{b} \pm 0.31$	$1.98^{b} \pm 0.11$	57.97 ± 3.42	$3.47^{c} \pm 0.32$
G4	$4.79^{b} \pm 0.16$	$1.61^{c} \pm 0.16$	98.81 ± 2.83	$2.49^{d} \pm 0.21$
G5	$4.72^{b} \pm 0.39$	$1.49^{c} \pm 0.13$	59.80 ± 4.41	$4.05^{b} \pm 0.16$
G6	$6.15^{a} \pm 0.15$	$3.44^{a} \pm 0.29$	69.71 ± 4.89	$3.62^{c} \pm 0.20$
G7	$4.82^{b} \pm 0.14$	$1.47^{c} \pm 0.08$	54.81 ± 4.13	$3.46^{c} \pm 0.21$
G8	$4.35^{b} \pm 0.25$	$1.08^{d} \pm 0.07$	56.47 ± 2.03	$3.08^{c} \pm 0.15$
F	21.78^{**}	39.84^{**}	-	18.54^{**}
CV (%)	10.76	18.45	-	10.65

Means followed by the same letters (in the column) are grouped by the Scott-Knott test at 5% (*) and 1% (**) probability (n = 4). F = the F value calculated using analysis of variance. CV (%) = coefficient of variation. [1]Data were not normal, and therefore, analysis of variance could not be performed.

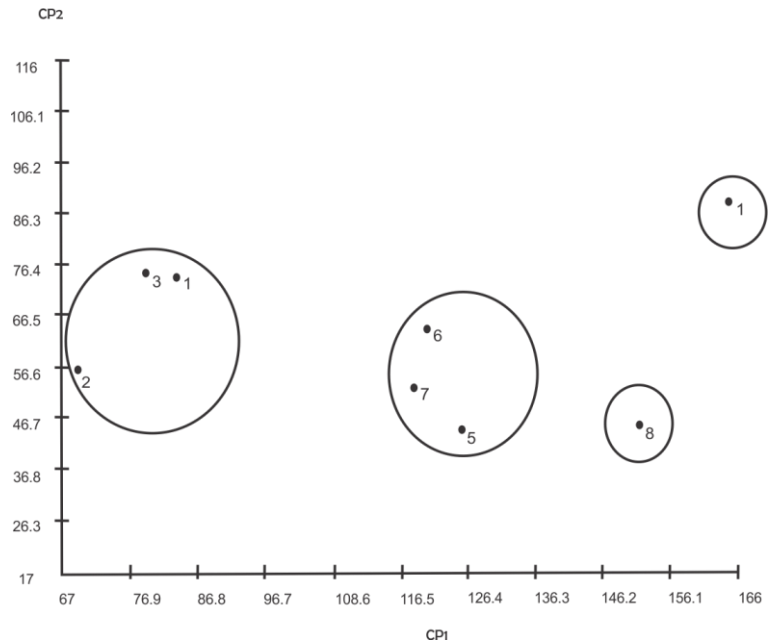

Fig. 2. Graphical analysis of the germination and development characteristics of the seedlings from eight papaya genotypes based on the first two principal components (87.03% of total variance, with CP1 = 72.01% and CP2 = 15.02%).

than that for the other groups (Table 4). This was reflected in the assignment of this genotype to an isolated group, both by the UPGMA clustering method (Fig. 1) and by the graphical analysis of the principal components (Fig. 2). The sum of the means of the characteristics for G4 (Caliman 01) was greater than the other groups. The sum for the group of G1 (Caliman 01 generation F2), G2 (Golden), and G3 (Tainung 01 generation F2) was 38.4% less for G4; the sum for the group of G5 (Sunrise Solo), G6 (THB), and G7 (Triple hybrid) was 32.7% less for G4; and the sum for the group of G8 (Hybrid JS12 × Waimanalo) alone was 17% less for G4. Cardoso et al. (2009) observed that the clustering based on graphical analysis of the canonical variables was more efficient in discriminating genotypes than the UPGMA method, with a better estimate of genetic variability regarding the quality of papaya seeds. In the present study, both the UPGMA and the graphical analysis of the principal components enabled the determination of the genotype with a higher initial performance based on the physicochemical and physiological characteristics of the seeds. Finally, the genotypes were ranked based on the sum of ranks index (Mulamba and Mock, 1978), and the genotype G4 (Caliman 01) was ranked the highest. The choice of superior genotypes should be based on their agronomic performance, which can be represented by germination and vigor in seeds. Thus, seeds of genotype Caliman 01 have a higher agronomic performance. Using the principal components analysis, the importance of each characteristic to the total available variation among the studied genotypes can be evaluated. The variables with greatest weight in the later eigenvectors are considered less important, and variables with more weight in earlier eigenvectors are considered more important (Cruz et al., 2011). In the present study, the characteristics with greater weight in the earlier eigenvectors were the second (SCG) and third counts of germination (TCG) and the fresh mass of seedlings (FM). Similar to that reported by Singh (1981), the SCG was also the characteristic with the greatest relative contribution (33.72%) to the analysis of genetic diversity among the genotypes under study (Table 5).

Genetic parameters

The estimates of the genetic parameters for each characteristic evaluated are shown in Table 5. The evaluation of protein levels indicated a lack of methodological adjustment in the estimates of the phenotypic and environmental variances, thus presenting negative estimates of genetic variance. Therefore, its genetic parameters were not described. The use of genetic parameters in plant breeding allows the identification of genetic variability to maximize the gains of selection. This depends on the superiority of the genetic causes attributed to phenotypic rather than environmental (or experimental) causes (Cruz et al., 2011). Among the components of variance, there was a greater contribution of the genotypic variance (σ^2_g) compared with that of the environmental (σ^2_e) variance for all of the characteristics studied (Table 5). The coefficient of genetic variation (CV_g), calculated as the ratio between the genetic standard deviation and the mean of the genotypes, was very high for FCG (133.79), RL (57.49), SCG (51.43), and AS (50.31). The CV_g is an important indicator of the relative extent of the changes that can be achieved by selection for each characteristic. The CV_g/CV_e ratio, which represents the ratio between the variations resulting from genetic causes and from experimental error, was greater than one for all characteristics, showing higher values for SCG (3.84%), RL (3.11%), M (3.01%), and SGI (3.00). This suggests that, in addition to greater experimental precision, there is an advantage for possible genetic gains during selection based on these characteristics. In addition, characteristics showing high coefficients of genetic variation and high heritability estimates represent the possibility of significant gains through selection based on the physiological quality of the papaya seeds (Cardoso et al., 2009). In general, the estimated heritability (h^2) was high (≥ 81.54%) for all characteristics evaluated in this study (Table 5). Silva et al. (2008) found heritability estimates greater than 80% for the morphological and agronomic characteristics of papaya such as plant height, stem diameter, height of insertion of the first fruits, number of flowers and fruits, mass, length, and diameter of fruits, productivity, and internal and external fruit firmness. Among the seed quality characteristics, Cardoso et al. (2009) observed high coefficients of genetic correlation and high heritability estimates (≥ 90%) for the thousand-seed weight, root length, and seedling mass. Macedo et al. (2013) found that most of the characteristics related to the quality of the seeds and fruits have a high coefficient of genotypic determination (H^2). Thus, the genetic variability related to parameters of physiological quality of the seeds can be exploited to obtain a higher germination performance in the breeding of cultivars. The heritability and the genotypic determination coefficient together determine the amount of phenotypic variation that can be attributed to genetic causes, and consequently, the possibility of changing a given characteristic through selection (Cruz et al., 2011). Thus, the diversity analysis of the characteristics, including the first count of germination, speed of germination index, root length, percentage of abnormal seedlings, and in particular, the second count of germination (which also had a higher relative contribution, as reported by Singh), may assist in the selection of genotypes with a higher initial performance.

Materials and Methods

Plant materials

The present study was developed in the Laboratory of Seed Analysis (Laboratório de Análise de Sementes - LAS) of the Center for Agricultural Sciences, Federal University of Espírito Santo (Centro de Ciências Agrárias da Universidade Federal do Espírito Santo, CCA-UFES). Seeds from eight genotypes of papaya (*Carica papaya* L.) were used: Caliman 01 generation F_2 (G1); Golden (G2); Tainung 01 generation F2 (G3); Caliman 01 (G4); Sunrise Solo (G5); THB (G6); Triple Hybrid (G7); and Hybrid JS12 × Waimanalo (G8) (Supplementary table). A F2 generation was used to compare the performance of F1 (maximum heterosis) with segregating materials F2 (possible reduction of the hybrid vigor). The seeds were provided by Caliman Agrícola S/A. The following procedures and evaluations were performed:

Physicochemical characterization

The initial moisture (M) of the seeds was determined using the oven method, drying them at 105 ± 3ºC for 24 h (adapted from Brasil, 2009), with four subsamples of 0.18 g for each genotype; the results are expressed as a percentage (wet basis). The seed size was measured using a digital caliper; the length (L) was characterized as the distance between the base and the apex, and the diameter (D) was the measure of the middle portion of the seed (cm). The thousand-seed weight (TSW) was calculated according to the procedure reported by Brasil (2009). Seed samples of approximately 0.1 g dry mass

Table 3. Pearson correlations between the physical, biochemical, and physiological characteristics of the seeds of papaya genotypes: moisture (M), length (L), diameter (D), thousand-seed weight (TSW), levels of soluble sugars (S), starch (St), lipids (Lp), and total protein (P), germination percentage (G), first count of germination (FCG), second count of germination (SCG), third count of germination (TCG), speed of germination index (SGI), mean germination time (MGT), percentages of normal seedlings (NS) and abnormal seedlings (AS), shoot length (SL), root length (RL), fresh mass (FM), and dry mass (DM) of normal seedlings.

	M	L	D	TSW	S	St	Lp	P	G	FCG	SCG	TCG	SGI	MGT	NS	AS	SL	RL	FM	DM
M		-0.14	-0.04	0.15	-0.41	0.14	0.17	0.05	0.29	-0.76	-0.44	0.21	0.18	0.04	-0.31	0.31	-0.72*	-0.51	0.07	0.50
L			0.81*	0.34	0.64	0.54	0.06	0.26	0.71*	0.34	0.86**	0.73*	0.81*	-0.81*	0.29	-0.29	0.22	-0.05	0.55	-0.61
D				-0.01	0.59	0.42	0.38	-0.02	0.64	1.00*	0.81*	0.61	0.55	-0.56	-0.09	0.09	0.29	0.14	0.37	-0.30
TSW					-0.16	-0.01	0.11	0.04	0.33	-0.56	0.10	0.39	0.55	-0.40	0.53	-0.53	-0.12	-0.16	0.66	-0.06
S						0.80*	-0.20	0.56	0.42	0.87	0.69	0.45	0.45	-0.49	-0.48	0.48	0.49	0.36	0.34	-0.70
St							-0.33	0.80*	0.68	0.75	0.41	0.68	0.66	-0.57	-0.45	0.45	0.01	-0.03	0.42	-0.49
Lp								-0.67	-0.45	-0.93	-0.08	-0.48	-0.13	0,19	-0.04	0,04	0.18	0.26	0.17	-0.06
P									0.54	0.88	0.17	0.58	0.47	-0.46	0.47	-0.47	-0.18	-0.29	0.13	-0.40
G										0.74	0.56	0.99**	0.86**	-0.79*	0.14	-0.14	-0.23	-0.37	0.46	-0.22
FCG											0.72	0.72	0.71	-0.13	0.10	-0.10	-0.95	-0.92	0.39	-0,32
SCG												0.58	0.66	-0.79	0.28	-0.28	0.58	0.28	0.52	-0.72*
TCG													0.87**	-0.81*	0.22	-0.22	-0.20	-0.37	0.49	-0.26
SGI														-0.94**	0.48	-0.48	0.02	-0.17	0.78*	-0.55
MGT															-0.44	0.44	-0.19	0.09	-0.70	0.70
NS																1.00**	0.44	0.40	0.72*	-0.64
AS																	-0.44	0.40	-0.72*	0.64
SL																		0.91**	0.40	-0.63
RL																			0.36	-0.36
FM																				-0.53
DM																				

Significance at 5 or 1% based on a t-test is represented by * or **, respectively.

Table 4. Means of each characteristic for the groups of papaya genotypes generated by the UPGMA method and based on graphical analysis of the first two principal components. G1 (Caliman 01 generation F2), G2 (Golden), and G3 (Tainung 01 generation F2); G4 (Caliman 01); G5 (Sunrise Solo), G6 (THB), and G7 (Triple hybrid); and G8 (Hybrid JS12 × Waimanalo).

Characteristic	G1+G2+G3	G4	G5+G6+G7	G8
Moisture (M)	7.03	7.00	5.80	5.81
Length (L)	0.40	0.55	0.46	0.51
Diameter (D)	0.25	0.30	0.30	0.32
Thousand-seed weight (TSW)	14.65	18.18	14.29	12.75
Soluble sugars (S)	3.25	5.13	4.21	6.38
Starch levels (St)	0.46	0.58	0.42	0.62
Lipid levels (Lp)	9.35	11.58	11.44	4.33
Protein levels (P)	4.53	4.57	4.44	4.66
Germination (G)	75.33	94.00	74.67	96.00
First count of germination (FCG)	0.00	6.00	1.67	10.00
Second count of germination (SCG)	24.33	94.00	42.33	82.00
Third count of germination (TCG)	73.33	94.00	72.67	96.00
Speed of germination index (SGI)	1.24	2.30	1.29	1.76
Mean germination time (MGT)*	15.33	9.15	14.30	11.26
Normal seedlings (NS)	82.85	98.08	81.68	83.69
Abnormal seedlings (AS)*	17.15	1.92	15.07	16.01
Shoot length (SL)	3.51	4.79	5.23	4.35
Root length (RL)	1.09	1.61	2.13	1.08
Fresh mass (FM)	54.78	98.81	61.44	56.47
Dry mass (DM)	4.36	2.49	3.71	3.08
Σ	328.26	532.90	358.81	442.24

* negative values assigned to the sum. Σ = sum.

Table 5. Relative contributions of the characteristics (% cr) as reported by Singh (1981) and estimates of phenotypic (σ^2_p), genotypic (σ^2_g), and environmental (σ^2_e) variances of the coefficient of genetic variation (CV_g), index of variation (Iv), heritability (h^2), and intraclass correlation (IC) for 20 variables related to the physical, physiological, and biochemical quality of seeds of the eight papaya genotypes.

Characteristic	Cr%	σ^2_p	σ^2_g	σ^2_e	CV_g	CV_g/CV_e	h^2	IC
Moisture (M)	9.31	1.0239	0.9965	0.0274	15.58	3.01	97.32	90.09
Length (L)	0.03	0.0036	0.0035	0.0001	12.86	2.54	96.28	86.62
Diameter (D)	0.06	0.0009	0.0008	0.0001	10.09	2.03	94.30	80.54
Thousand-seed weight (TSW)	4.28	4.2569	3.8026	0.4543	13.25	1.45	89.32	67.66
Soluble sugars (S)	2.40	1.7781	1.6549	0.1231	30.37	1.83	93.07	77.05
Starch levels (St)	0.06	0.0077	0.0064	0.0013	16.76	1.12	83.34	55.58
Lipid levels (Lp)	0.46	8.5172	8.0695	0.6558	29.04	1.77	92.59	75.75
Protein levels (P)	0.03	0.0062	0.0000	0.0068	-	-	00.00	-
Germination (G)	1.41	121.14	108.81	12.333	13.04	1.49	89.82	68.80
First count of germination (FCG)	0.47	15.125	12.333	2.792	133.79	1.05	81.54	52.48
Second count of germination (SCG)	33.72	972.41	956.20	16.21	51.43	3.84	98.30	93.65
Third count of germination (TCG)	2.11	139.71	126.88	12.83	14.35	1.57	90.81	71.20
Speed of germination index (SGI)	0.20	0.1563	0.1521	0.0042	26.76	3.00	97.30	90.02
Mean germination time (MGT)	1.13	5.5289	5.3486	0.1803	16.94	2.72	96.74	88.12
Normal seedlings (NS)	0.71	68.522	61.392	7.1303	9.28	1.47	89.59	68.30
Abnormal seedlings (AS)	30.52	68.536	61.405	7.1303	50.31	1.47	89.59	68.28
Shoot length (SL)	0.67	1.2322	1.1757	0.5659	24.53	2.28	95.41	83.86
Root length (RL)	0.03	0.8092	0.7889	0.0203	57.49	3.11	97.49	90.66
Fresh mass (FM)	12.27	256.19	240.60	15.5834	24.62	1.97	93.92	79.42
Dry mass (DM)	0.11	0.7285	0.6891	0.0392	22.30	2.09	94.61	81.43

Table 6. Supplementary table.

Genotype	Name	Tracking number	Country	Parental	Group	Observation	Reference
G1	Caliman 01 generation F2	12359 (RNC/Brazil)[1]	Brazil	JS12 and Sunrise Solo	Formosa	First generation segregating (F2) of the hybrid. Higher agronomic characteristics, and phenotype predominantly Formosa group.	[2]
G2	Golden	26485 (RNC/Brazil)	Brazil	-	Solo	Inbred lines. In Brazil most of the crop is exported.	[3]
G3	Tainung 01 generation F2	-	Taiwan	not be published	Formosa	First segregating generation (F2) of the hybrid. Genotype much cultivated in many countries. Most of the crop for domestic consumption in Brazil.	-
G4	Caliman 01	12359 (RNC/Brazil)	Brazil	JS12 and Sunrise Solo	Formosa	Hybrid. Features superior agronomic, and phenotype predominantly Formosa group.	[2]
G5	Sunrise Solo	05028 (RNC/Brazil)	Hawai	-	Solo	Inbred lines. Genotype much cultivated in many countries.	[4]
G6	Golden THB	26459 (RNC/Brazil)	Brazil	Golden	Solo	Inbred lines. Material selected from the 'Golden', the crop mainly for export.	-
G7	Triple Hybrid	-	Brazil	-	-	Not registered material (UENF e Caliman Agrícola S/A).	-
G8	Hybrid JS12 x Waimanalo	-	Brazil	JS12 and Waimanalo	-	Not registered material UENF e Caliman Agrícola S/A). Parental Waimanalo is tolerant to *Phythophthora* sp.	[5]

[1] Registro Nacional de Cultivares (RNC), Ministério da Agricultura, Pecuária e Abastecimento (MAPA), Brasil [*National Cultivars Record* (RNC), *Ministry of Agriculture* (MAPA), *Brazil*]. Acesso em 10 de dezembro de 2014. Disponível em: http://www.agricultura.gov.br/vegetal/registros-autorizacoes/registro/registro-nacional-cultivares.

[2] Ferreguetti GA (2003). Caliman 01 – o primeiro híbrido de mamão formosa brasileiro. In: Simpósio do papaia brasileiro, Papaya Brasil - qualidade do mamão para o mercado interno. Analls... Incaper, Vitória, ES, Brazil

[3] Marin SLD, Gomes JÁ (2000) Técnicas do cultivo do mamão. In: Semana Internacional de Fruticultura e Agroindústria. Annals... Sindifruta – Frutal, Fortaleza, CE, Brazil

[4] Litz RE (1984) Papaya. In: Sharp RW, Evans DA, Ammirato PV, Yamada Y (Eds.) Handbook of plant cell culture. MacMillan, New York, p. 349-368

[5] Manshardt R (2014) History and future of the Solo papaya. In: Ming R, Moore PH (Eds.) Genetics and genomics of papaya. New York, Springer, 10: 95-113

were macerated and centrifuged in an Eppendorf tube and then the two liquid phases were separated using the methanol, chloroform, and water method (MCW, 1:1:1). The upper, hydrosoluble phase (methanol + water) was used to quantify the soluble sugars, and the lower, liposoluble phase (chloroform) was used for the quantification of lipids. The solid phase (pellet) was used for starch quantification. *Quantification of lipids* (Lp) - The lower phase solution was placed in 2 mL Eppendorf tubes and weighed on an analytical balance (0.0001 g). Next the samples were dried in an oven at 60ºC and weighed again (Bligh and Dyer, 1959, modified) for lipid quantification. *Quantification of soluble sugars* (S) - The carbohydrates in the upper phase solution were hydrolyzed and dehydrated using concentrated sulphuric acid, modifying the dehydrated simple sugars to furfural (Yemn and Willis, 1954). The products of this reaction were condensed with anthrone, forming a petroleum-blue colored substance that was analyzed for soluble sugars in a spectrophotometer (FEMTO, Cirrus 80ST) at 620 nm. *Quantification of starch* (St) - The solid phase (pellet) was digested in 3% HCl in a water bath (95–100ºC) for 3 h and then centrifuged (4000 rpm for 5 min.). The supernatant was collected and used for the anthrone method as described above. *Quantification of total proteins* (P) - This analysis was performed by inference from nitrogen levels (Silva, 1999; Galvani and Gaertner, 2006). Seed samples of 0.1 g dry mass were crushed and digested until the color changed to green and were subsequently distilled and titrated. The NH_4^+ produced in the digestion in sulphuric acid was distilled in a strongly alkaline medium. The nitrate was condensed in a boric acid solution and titrated with HCl. The crude protein (CP) was converted using the factor 6.25, considering that most proteins contain approximately 16% nitrogen in their molecules. The results of the quantification of lipids, soluble sugars, starch, and proteins were expressed as a percentage (% w/w) of the dry mass of the seeds.

Physiological quality

For the germination test, seeds were distributed on paper rolls (three sheets of germitest paper), impregnated with distilled water in the proportion equivalent to 2.5 times the mass of the dry paper, and placed in a BOD incubator set to the alternating temperature of 20–30ºC. The following assessments were made daily: percentage of germination (G), considering germination to be indicated by root protrusion of 0.2 cm, up to 28 days after sowing; first count of germination (FCG), performed at seven days, second count (SCG), performed at 14 days, and third count (TCG) at 21 days, with the numbers expressed as a percentage; speed of germination index (SGI) (Maguire, 1962); mean germination time (MGT) (Labouriau, 1983); and the percentage of normal (NS) and abnormal seedlings (AS), calculated in relation to the total number of germinated seedlings (G). Twenty-eight days after the initiation of the experiment, the shoot (SL) and root length (RL), expressed in cm seedling^{-1}, were evaluated, and measurements of fresh mass (FM) and dry mass (DM) of normal seedlings (dry mass was obtained in an oven of forced circulation at 70ºC for 72 h) were performed on an analytical balance (0.0001 g); the results were expressed in mg seedling^{-1}.

Study design and statistical analysis

The experiment was conducted in a completely randomized design (CRD) with four replicates of 25 seeds per treatment and the eight genotypes. The results obtained were subjected to analysis of variance and the Scott-Knott test at 5% or 1% probability (Assistat, 2012).

Correlation, genetic diversity, and genetic parameters

Estimates were made of the simple (Pearson) and genetic correlations between the characteristics of physical (H, L, D, and TSW), biochemical (S, St, Lp, and P), and physiological (G, FCG, SCG, TCG, SGI, MGT, NS, AS, SL, RL, FM, and DM) quality of the seeds. To identify the genotype with the greatest initial performance, the mean standardized Euclidean distance was obtained, and subsequently, the UPGMA was applied with the cophenetic coefficient of 0.6. From the means of the characteristics, the principal components were estimated and graphed to analyze the formation of groups; the sum of ranks index (Mulamba and Mock, 1978) was applied to select the higher performing genotypes. To determine the most important characteristics in discriminating the genotypes, the characteristics with higher weight in the first eigenvectors of the principal component analysis were identified; an analysis of the relative contribution was performed according to the method reported by Singh (1981), and the genetic parameters for each characteristic were estimated. Analyses were performed using the Genes program (Cruz, 2013).

Conclusions

The diversity analysis and the genetic parameters based on the assessment of the physicochemical and physiological characteristics of papaya seeds indicate that the genotype Caliman 01 has the highest performance. This genotype is an elite genetic material and can be used by breeders like progenitor to develop new cultivars.

Based on the analysis of the relative contribution, the principal components analysis, and the genetic parameters, the second count of germination is the most important characteristic in the diversity analysis for the papaya genotypes studied.

References

Assistat 7.6 Beta (2012) Assistat assistência estatística. DEAG-CTRN-UFCG, Campina Grande.

Association of Official Seed Analysts (2002) Seed vigor testing handbook. Contribution, 32. AOSA, Lincoln, 105p

Berbert PA, Carlesso VA, Silva RF, Araújo EF, Thiébaut JTL, Oliveira MTR (2008) Qualidade fisiológica de semente de mamão em função da secagem e do armazenamento. Rev Bras Sementes. 30 (1): 40-48.

Bertan I, Carvalho FIF, Oliveira AC, Benin G, Vieira EA, Valério IP (2009) Morphological, pedigree, and molecular distances and their association with hybrid wheat performance. Pesqui Agropecu Bras. 44 (2): 155-163.

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol. 37 (8): 911-917.

Brasil (2009) Regras para análise de sementes, Ministério da Agricultura, Pecuária e Abastecimento, MAPA/DAS/ACS, Brasília, 395p.

Cardoso DL, Silva RF, Pereira MG, Viana AP (2009) Diversidade genética e parâmetros genéticos relacionados à qualidade fisiológica de sementes em germoplasma de mamoeiro. Ceres. 56 (5): 572-579.

Cruz CD (2013) Genes- a software package for analysis in experimental statistics and quantitative genetics. Acta Sci Agron. 35 (3): 271-276.

Cruz CD, Ferreira FM, Pessoni LA (2011) Biometria aplicada ao estudo da diversidade genética. Suprema, Visconde do Rio Branco, 620p.

Dantas JLL, Lima LF (2001) Seleção e recomendação de variedades de mamoeiro – avaliação de linhagens e híbridos. Rev Bras Frutic. 23 (3): 617-621.

Dias NLP, Oliveira EJ, Dantas JLL (2011) Avaliação de genótipos de mamoeiro com uso de descritores agronômicos e estimação de parâmetros genéticos. Pesqui Agropecu Bras. 46 (11): 1471-1479.

Ferreira JP, Schmildt O, Schmildt ER, Piantavinha WC, Cattaneo LF (2012) Correlações entre características morfo-agronômicas de acessos de mamoeiro. Enciclop Biosfera. 8 (14): 246-257.

Galvani F, Gaertner E (2006) Adequação da Metodologia Kjeldahl para determinação de Nitrogênio Total e Proteína Bruta. Circular Técnica 63. Embrapa Pantanal, Corumbá, 9p.

Kim MS, Moore PH, Zee F, Fitch MM, Steiger M, Manshardt RM, Paull RE, Drew RA, Sekioka T, Ming R (2002) Genetic diversity of *Carica papaya* as revealed by AFLP markers. Genome. 45: 503-512.

Labouriau LFG (1983) A germinação das sementes. Secretaria Geral da Organização dos Estados Americanos, Washington, 174p.

Macedo CMP, Pereira MG, Cardoso DL, Silva RF (2013) Evaluation of seed physiological quality of papaya elite hybrids, their reciprocal crosses and parents. J Seed Sci. 35 (2): 190-197.

Maguire JD (1962) Speeds of germination-aid selection and evaluation for seedling emergence and vigor. Crop Sci. 2: 176-177.

Marcos Filho J (2005) Fisiologia de sementes de plantas cultivadas. FEALQ, Piracicaba, 495p.

Marin SLD, Pereira MG, Amaral Júnior AT, Martelleto LAP, Ide CD (2006) Partial diallel to evaluate the combining ability for economically important traits of papaya. Sci Agric. 63 (6): 540-546.

Martins GN, Silva RF, Araújo EF, Pereira MG, Vieira HD, Viana AP (2005) Influência do tipo de fruto, peso específico das sementes e períodos de armazenamento na qualidade fisiológica de sementes de mamão do grupo formosa. Rev Bras Sementes. 27 (2): 12-17.

Mulamba NN, Mock JJ (1978) Improvement of yield potential of the Eto Blanco maize (*Zea mays* L.) population by breeding for plant traits. Egypt J Genet Cytol. 7: 40-57.

Oliveira EJ, Lima DS, Lucena RS, Motta TBN, Dantas JLL (2010) Correlações genéticas e análise de trilha para número de frutos comerciais por planta em mamoeiro. Pesqui Agropecu Bras. 45 (8): 855-862.

Oliveira EJ, Dias NLP, Dantas JLL (2012) Selection of morpho-agronomic descriptors for characterisation of papaya cultivars. Euphytica. 185: 253–265.

Popinigs F (1985) Fisiologia da semente. 2. Ed. Ministério da Agricultura, Brasília, 289p.

Ruggiero C, Marin SLD, Durigan JF (2011) Mamão, uma história de sucesso. Rev Bras Frutic. special: 76-82.

Silva FC da (1999) Manual de análises químicas de solos, plantas e fertillizantes. Embrapa, Brasília, 370p.

Silva FF, Pereira MG, Ramos HCC, Damasceno Junior PC, Pereira TNS, Ide CD (2007) Genotypic correlations of morpho-agronomic traits in papaya and implications for genetic breeding. Crop Breed Appl Biotechnol. 7: 345-352.

Silva FF, Pereira MG, Ramos HCC, Damasceno Junior PC, Pereira NTS, Gabriel APC, Viana AP, Ferreguetti GA (2008) Selection and estimation of the genetic gain in segregating generations of papaya (*Carica papaya* L.). Crop Breed Appl Biotechnol. 8: 1-8.

Singh D (1981) The relative importance of characters affecting genetic divergence. Indian J Genet Pl Br. 36: 237-245.

Vieira EA, Carvalho FIF, Bertan I, Kopp MM, Zimmer PD, Benin G, Silva JAG, Hartwig I, Malone G, Oliveira AC (2007) Association between genetic distances in wheat (*Triticum aestivum* L.) as estimated by AFLP and morphological markers. Genet Mol Biol. 30 (2): 392-399.

Yemn EW, Willis AJ (1954) The estimation of carbohydrate in plant extracts by anthrone. Biochem J. 57: 508-514.