Australian Journal of Crop Science
AJCS
AJCS 10(1):7-15 (2016) ISSN:1835-2707

Germination performance of papaya genotypes subjected to salinity, water restriction and high irradiance stress

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Abstract

The objective of this study was to characterize the stresses induced by salinity, water restriction, and irradiance during germination, and to evaluate the germination performance of four papaya (*Carica papaya*) genotypes under the different stresses. The seeds were subjected to osmotic potentials of zero, -0.2, -0.4, -0.6, and -0.8 MPa, induced by sodium chloride (NaCl) for salinity, and mannitol for drought, as well as to different levels of irradiation (60, 130, 580 and 1200 μmol m^{-2} s^{-1}) during germination. The salinity and water restriction at osmotic potentials below -0.4 MPa and irradiance of 1200 μmol m^{-2} s^{-1} (full sun) imposed stress conditions during germination of papaya seeds. Four papaya genotypes were then evaluated for germination performance under the different stresses. To induce salt and water restriction stress, -0.6 MPa of NaCl and mannitol were used, respectively; for elevated irradiance stress, full sunlight was used (1200 μmol m^{-2} s^{-1}). The hybrids Caliman 01 and JS12 x Waimanalo present the highest germination potential. The hybrid Caliman 01 presents greater germination performance under conditions of salt and water stress, while the hybrid JS12 x Waimanalo presents greater performance under elevated irradiance.

Keywords: *Carica papaya* L.; luminosity; osmotic stress; seeds; vigour.
Abbreviations: cv._cultivar; CV (%)_ coefficient of variation of variance analysis; DM_ dry mass of seedlings; F_{cal}_ F value calculated of variance analysis; FCG_ first count of germination; G_ germination percentage; G1_ genotype 1 (Caliman 01); G2 _ genotype 2 (Golden THB); G3_ genotype 3 (Triple hybrid); G4_ genotype 4 (Hybrid JS12 x Waimanalo); MGT_ mean germination time; NaCl_ sodium chloride; Ψs_osmotic potential; SCG_ second count of germination; SGI_ speed of germination index; TCG_ third count of germination.

Introduction

The papaya (*Carica papaya* L., Caricaceae) is a fruit specie originating in tropical climates, and has physiological responses strongly linked to environmental conditions (Campostrini; Glenn, 2007). In general, the papaya is considered a full sunlight plant, and a moderately tolerant specie to salinity and water restriction during field development. During the initial growth stage, the plants are sensitive to salinity and to high irradiance (Campostrini et al. 2001; Campostrini; Glenn, 2007; Fontes et al., 2008; Reis; Campostrini, 2008; Cavalcante et al., 2010; Mengarda et al., 2014). Papaya is sensitive to the stresses of extreme temperature, drought, winds, injuries, and viral diseases, especially during the reproductive phase. In papaya, the photosynthetic processes are sensitive to reducing humidity and light conditions (Carr, 2014). The high luminosity is generally associated with variations in temperature, water restriction, and limitations of CO_2 diffusion (Taiz; Zeiger, 2013). These environmental factors affect the photosynthetic processes of papaya (Campostrini; Glenn, 2007). The high irradiance affect the emergence, while the low irradiance affects the development of seedlings (Mengarda et al., 2014). There is a genetic variation of papaya response in relation to the light conditions, with adaptations on the leaf chlorophyll content, avoiding the photosynthesis photoinhibition (Campostrini; Glenn, 2007). In response to water stress, papaya accumulates abscisic acid, jasmonic acid, proline, sodium, potassium, and chloride, suggesting osmotic adjustment. Under water limitation is observed reduction in photosynthetic rate, in transpiration, in stomatal conductance, and in the leaf growth (Mahouachi et al, 2006; 2007; 2012). Some varieties of papaya present osmotic adjustment as a factor contributing to acclimation to drought; others showed a reduction in chlorophyll content, stomatal conductance, and photochemical efficiency when grown under moderate or severe water stress (Campostrini et al., 2001; Campostrini ; Glenn, 2007). The study of the papaya responses to environmental stresses seeks to minimize the unwanted effects these stresses have on physiological processes (Campostrini; Glenn, 2007), enabling new management strategies in seedling production, plant breeding, and in promoting higher productivity. The identification of genotypes with higher germination rates under abiotic stresses can provide relevant information for the propagation, crop management, and breeding of papaya. Thus, the production of seedlings depends on identification of the ideal environmental conditions for germination and seedling establishment.

The differentiated response of genotypes based on stress conditions during germination may be validated by the seeds' vigour tests (Marcos Filho, 2005). The characterization of seeds physicochemical and physiological quality associated with the diversity analysis are effective in identifying the highest performance genotypes (Mengarda et al., 2015).

There is genetic variation in the physiological responses of papaya plants in relation to temperature, irradiance, water restriction, and salinity (Campostrini; Glenn, 2007; Cavalcante et al., 2010; Fontes et al., 2008; Mengarda et al., 2014), which also impacts early development stages. Furthermore, it is known that different papaya genotypes have high genetic divergence for attributes related to the physiological quality of the seed (Cardoso et al., 2009; Macedo et al. 2013; Mengarda et al. 2015). However, responses of papaya genotypes to stressful conditions during germination have not been investigated. The objective of this study was thus to characterize the stresses induced by salt, water restriction, and irradiance on germination, and to evaluate the germination performance of papaya genotypes based on vigour tests in the different stresses.

Results

Characterization of stress induced by salinity, water restriction, and irradiance during germination

Under salt and water stress, as the osmotic potential was reduced, there was a decrease in the germination and vigour of papaya seeds. The response was adjusted to the linear regression model for all characteristics evaluated (Fig. 1).

An expressive reduction was observed for G, SGC, and TGC at the potential of -0.6 MPa of NaCl and mannitol (Fig. 1A, C and D). Germination was delayed in less negative osmotic potentials, as a reduction was observed in the FGC at -0.2 MPa of NaCl and mannitol, and an increase in the MGT for -0.2 MPa of NaCl (Fig. 1B and F). There was also a reduction in the SGI at -0.2 MPa of NaCl, and at -0.4 MPa of mannitol (Fig. 1E). In general, the salinity stress (NaCl) results in more accentuated reductions in germination than the watrer restriction stress (mannitol) (Fig. 1A, C, D, E and F). The results suggest that salinity and water restriction at osmotic potentials below -0.4 MPa result in stress conditions during the germination of papaya seeds.

Under different light intensities, higher mean percentage and speed of germination were observed at low and intermediate light intensities (60, 130 and 580 μmol m^{-2} s^{-1}), compared to the averages observed under high irradiance (1200 μmol m^{-2} s^{-1}) (Fig. 2). A linear decrease was observed in G, TGC, and SGI with increasing irradiance (Fig. 2A, D and E). Regarding the FGC, a response adjusted to the quadratic regression model was observed: under a low irradiance condition (60 and 130 μmol m^{-2} s^{-1}), the germination percentage in the first count was low (≤ 16%), with a maximum point estimated at the irradiance of 655 μmol l m^{-2} s^{-1} (33%), and reaching an average of near zero under full sunlight (1200 μmol m^{-2} s^{-1}) (Fig. 2B). For the SGC, there was also a response adjusted to the quadratic regression model, with a maximum peak irradiance of 278.18 μmol m^{-2} s^{-1} (86%) (Fig. 2C). For MGT, the minimum estimated point was at the irradiance of 509.38 μmol m^{-2} s^{-1} (13 days) (Fig. 2D). Therefore, papaya germination was especially impaired by high irradiance. Thus, the results suggest that full sunlight (1200 μmol m^{-2} s^{-1}) represents a condition of stress for the germination and vigour of papaya seeds.

Germination performance of papaya genotypes under stress

In the seeds' physiological characterization, the hybrids Caliman 01 and JS12 x Waimanalo exhibited higher average G and SGI (Table 1), suggesting greater germination potential for these genotypes.

The higher germination potential identified by germination testing under ideal conditions for the hybrids Caliman 01 and JS12 x Waimanalo related to responses of the vigour tests, where these genotypes maintained higher germination and seed vigour when subjected to different stresses (Table 1). With evaluation of relative performance, it was possible to more clearly differentiate genotypes with respect to tolerance to each stress situation during germination (Table 2). Under salinity, the Triple hybrid exhibited lower performance, with an 89% reduction in G. In contrast, Caliman 01 showed the smallest decrease in G (-2%), and highest performance under salt stress. The Triple hybrid also showed lower performance when seeds were subjected to stress by water restriction (-62% germination) (Table 2). The hybrid Caliman 01 stood out with the highest average G, SCG, and SGI (Table 1). This genotype showed a lower average reduction for the physiological characteristics in drought stress, with greater vigour under water restriction stress (Table 2). The Triple hybrid also displayed lower performance when seeds were subjected to stress by water restriction (-62% germination) (Table 2). The hybrid Caliman 01 stood out with the highest averages of G, SGC and SGI (Table 1). This genotype showed the lowest reduction in average physiological characteristics when submitted to drought stress, presenting greater vigour under this stress (Table 2). Considering G, all the genotypes used in this study (with the exception of hybrid Caliman 01) showed expressive reduction in relative performance when subjected to salt stress induced by NaCl (≥ 15%), and when subjected to stress by mannitol-induced water restriction (≥ 19%) (Table 2).

When subjected to the condition of high irradiance, seeds of the hybrids Caliman 01 and JS12 x Waimanlo presented greater average vigour, where hybrid JS12 x Waimanalo showed higher relative performance: no reduction in G and a 10% reduction in the SGI. Genotype Golden THB showed lower performance: 42% reduction in G, and 54% in SGI (Tables 1 and 2). Thus, it was found that high irradiance was most detrimental to germination of papaya cv. Golden THB.

From groupings based on the analysis of the dispersions graphs for principal components (PC), it was found that the hybrids Caliman 01 (1) and hybrid JS12 x Waimanlo (4), which presented higher initial seed quality, formed a separate group from cv. Golden THB (2) and the Triple hybrid (3), occupying a position far from the origin on the horizontal axis (corresponding to first principal components) (Fig. 3A).

It was also observed that the smaller values to the first and second principal components (PC1 and PC2), i.e., closer to the origin of the Cartesian plane, the lower the performance of genotypes with respect to germination characteristics. In salt stress, the Triple hybrid (3), which exhibited lower performance, was isolated from the other genotypes, occupying a position close to the origin on the horizontal axis (PC1) (Fig. 3B). Under water restriction, the genotypes did not form clusters. Caliman 01, the genotype with the best performance under this stress, was found in a position distant from the origin in relation to the horizontal axis (PC1) (Fig. 3C). The same was observed for stress by high irradiance, but

Table 1. Germination percentage (G), first (FGC) and second germination count (SGC), expressed as percentages (%), and speed germination index (SGI) of seeds of papaya genotypes in the physiological characterization, and under salinity, water restriction, and high irradiance stress.

	Physiological characterization			
Genotypes	G (%)[1]	FGC (%)	SGC (%)	SGI
Caliman 01	94 a[1]	6	94	2.30 a
Golden THB	72 b	0	32	1.20 c
Triple hybrid	72 b	0	33	1.27 c
JS12 x Waimanalo	96 a	10	82	1.76 b
F_{cal}	15.51**	-	-	66.76**
CV (%)	09	-	-	2.90
	Salinity			
Genotypes	G (%)	FGC (%)	SGC (%)	SGI
Caliman 01	87 a	6	94 a	2.15
Golden THB	69 a	0	62 b	1.62
Triple hybrid	10 b	0	04 c	0.21
JS12 x Waimanalo	82 a	2	65 ab	1.88
F_{cal}	48.44**	-	68.13**	-
CV (%)	13	-	13.07	-
	Water restriction			
Genotypes	G (%)	FGC (%)	SGC (%)	SGI
Caliman 01	92 a	12	92 a	2.67 a
Golden THB	52 c	4	49 c	1.36 c
Triple hybrid	34 c	0	26 d	0.72 d
JS12 x Waimanalo	78 b	10	70 b	1.97 b
F_{cal}	42.86**	-	59.66**	58.87**
CV (%)	10	-	9.37	5.22
	Elevated irradiance			
Genotypes	G (%)	FGC (%)	SGC (%)	SGI
Caliman 01	73 ab	0	23 bc	0.67 b
Golden THB	46 b	0	4 c	0.42 c
Triple hybrid	60 b	0	54 ab	0.63 b
JS12 x Waimanalo	89 a	1	78 a	0.94 a
F_{cal}	08**	-	11.98**	31.50**
CV (%)	15.63	-	40.68	3.30

[1]_ Means followed by the same letter (columns) are statistically equal by the Tukey test; **_ $p \leq 0.01$ (n=4); F_{cal}_ calculated F-value obtained from the analysis of variance; CV (%).

Table 2. Relative performance (%) of the papaya genotypes: hybrid Caliman 01, cv. Golden THB, Triple hybrid and hybrid JS12 x Waimanalo during germination under salt stress, water restriction, and elevated irradiance.

Genotypes	G	FGC	SGC	SGI
	Relative performance (%)			
	Salinity			
Caliman 01	-02	-95	-07	-30
Golden THB	-17	-100	-11	-35
Triple hybrid	-89	-100	-95	-91
JS12 x Waimanalo	-15	-97	-31	-43
	Water restriction			
Caliman 01	+03	-85	+03	-13
Golden THB	-37	-92	-30	-45
Triple hybrid	-62	-100	-67	-68
JS12 x Waimanalo	-19	-86	-26	-40
	Elevated irradiance			
Caliman 01	-28	-77	-100	-42
Golden THB	-42	-95	-100	-54
Triple hybrid	-23	-31	-100	-45
JS12 x Waimanalo	00	-13	-67	-10

(+) Relative increase and (-) decrease, using the absolute value of control as the reference (100%); FCG_ first count of germination; G_ germination percentage; SCG_ second count of germination; SGI_ speed of germination index.

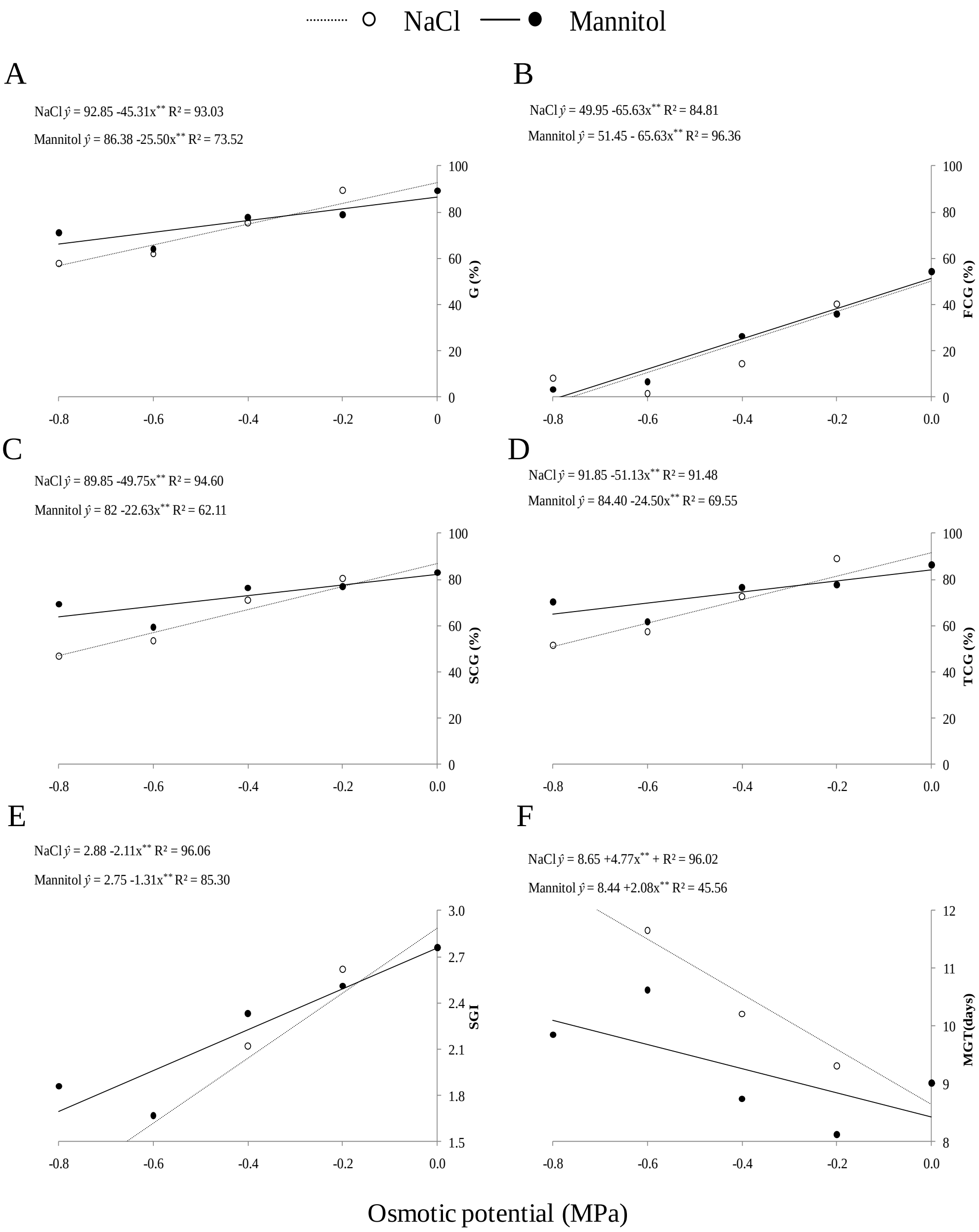

Fig 1. Physiological quality of papaya seeds subjected to different osmotic potentials induced by NaCl (salt stress) and mannitol (water restriction stress): A - germination percentage (G); B - first germination count (FGC); C - second germination count (SGC); D - third germination count (TGC), in percentage; E - speed germination index (SGI); F - mean germination time (MGT) in days. ** - $p \leq 0.01$.

in this case with the hybrid JS12 x Waimanalo occupying the distant position (Fig. 3D). Thus, using two-dimensional graphic dispersion, it was possible to distinguish the genotypes for seed vigour and tolerance of these different stresses during germination.

Discussion

Characterization of stress induced by salinity, water restriction and irradiance during germination

Under salt stress and water restriction, there was a decrease in the germination and vigour of papaya seeds. In general, the NaCl results in more accentuated reductions at osmotic potentials less reduced than mannitol. Seed germination is a complex process, and is dependent on various external factors, such as temperature, water, salinity, gas composition, and light (Marcos Filho, 2005). Osmotic potential reduction caused by water or salt stress reduces the water absorption gradient by the seed, interfering with respiratory and metabolic activities that release energy and nutrients to sustain embryo growth. Thus, osmotic stresses slow the speed and percentage of germination (Murillo-Amador et al., 2002; Patane et al. 2013). In response to osmotic stress, embryo cells and seed endosperm can accumulate solutes, such as sugars and proteins, which represent an adaptive strategy to avoid germination in stressful environments (Kosováa et al. 2011; Queiroz et al., 2011; Vardhini; Rao, 2003), resulting in delayed and reduced germination. In this work, the osmotic stress interfered more significantly with

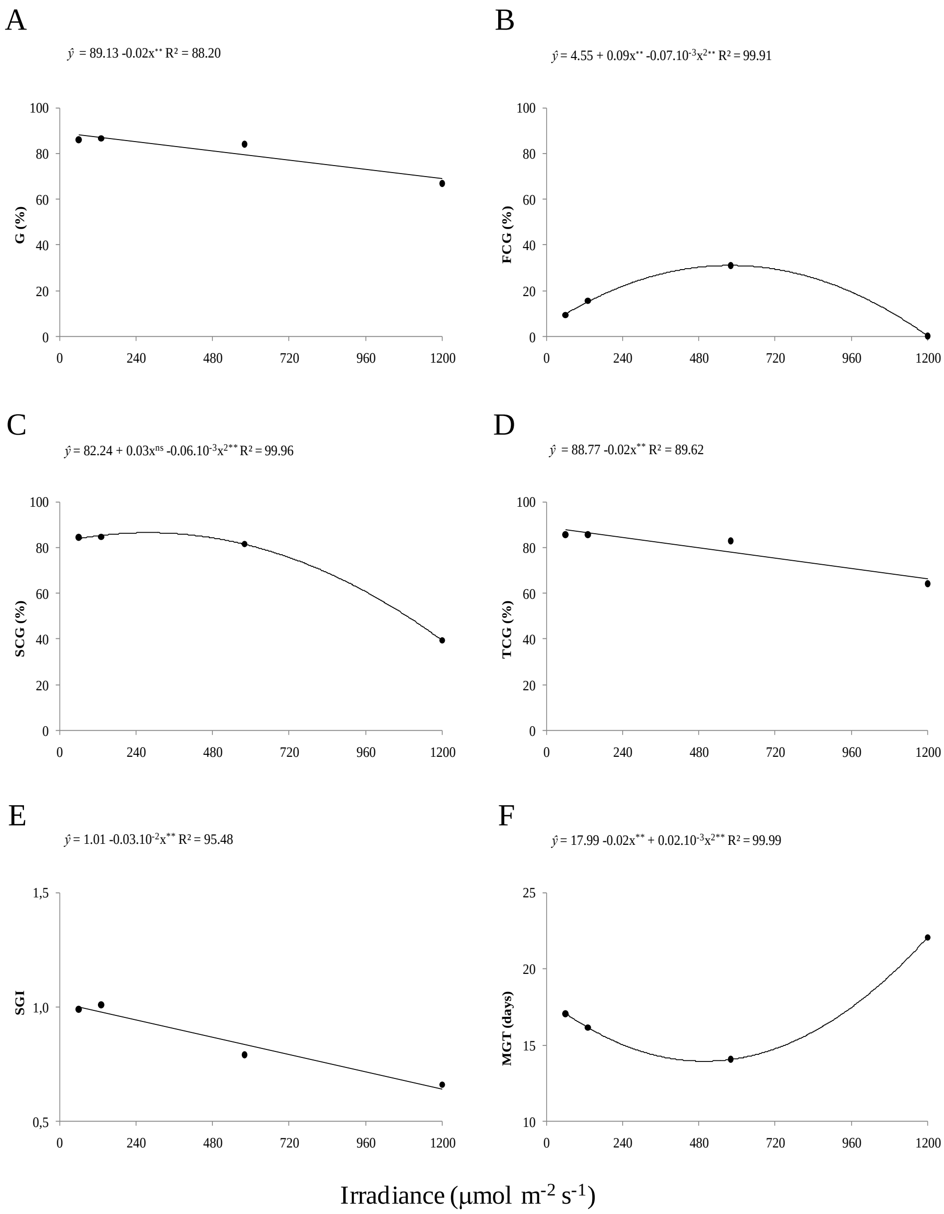

Fig 2. Physiological quality of papaya seeds subjected to different levels of irradiance: A - germination percentage (G); B - first germination count (FGC); C - second germination count (SGC); D - third germination count (TGC), in percentage; E - speed germination index (SGI); F - mean germination time (MGT) in days. ** - $p \leq 0.01$; ns – non-significant.

the speed of germination (MGT and FGC) than the total germination percentage (G) of papaya seeds. Under salt stress, while lower germination percentages in FGC and MGT were observed at -0.2 MPa, G presented a reduction at -0.6 MPa. The effect of water stress on seed viability and vigour of cotton cultivar seedlings was severe, at values equal to or lower than -0.4 MPa (Meneses et al., 2011). In sorghum cultivars, the percentage of seed germination was also reduced by water stress (induced by polyethylene glycol) at potentials inferior to -0.6 MPa; however, there was a delay in germination at -0.4 MPa (Patane et al., 2013), consistent with the results observed in this study.

In cultivars of cowpea, the germination and emergence also indicated a delay when seeds were subjected to salinity (NaCl) and water restriction (polyethylene glycol), and each cultivar responded differently (Murillo-Amador et al., 2002). However, in contrast to the results in this study with papaya, in sorghum and cowpea, the effect of salt stress (induced by NaCl) was less harmful than water restriction. In addition to losses induced by reduced water potential, salinity produces plant toxicity due to dissociation of ions that readily cross the membrane, and modify cell metabolism. Thus, NaCl has a double effect: toxicity, resulting from the release of Na^+ and Cl^- ions, and water restriction, caused by the osmotic effect of dilute solutes in the solution, which restrict water uptake by the seed during imbibition (Kosováa et al., 2011; Marcos Filho, 2005). This study observed that salt stress was more detrimental to the germination of papaya seeds than water restriction, which may be due to toxicity. During field development, the papaya is considered a moderately tolerant species to salinity and water restriction. The plants are more sensitive to the effect of salts during the growth stage than in emergence; in emergence, seeds tolerate between 4 and 6 dS m^{-3} (which approximately corresponds to the osmotic

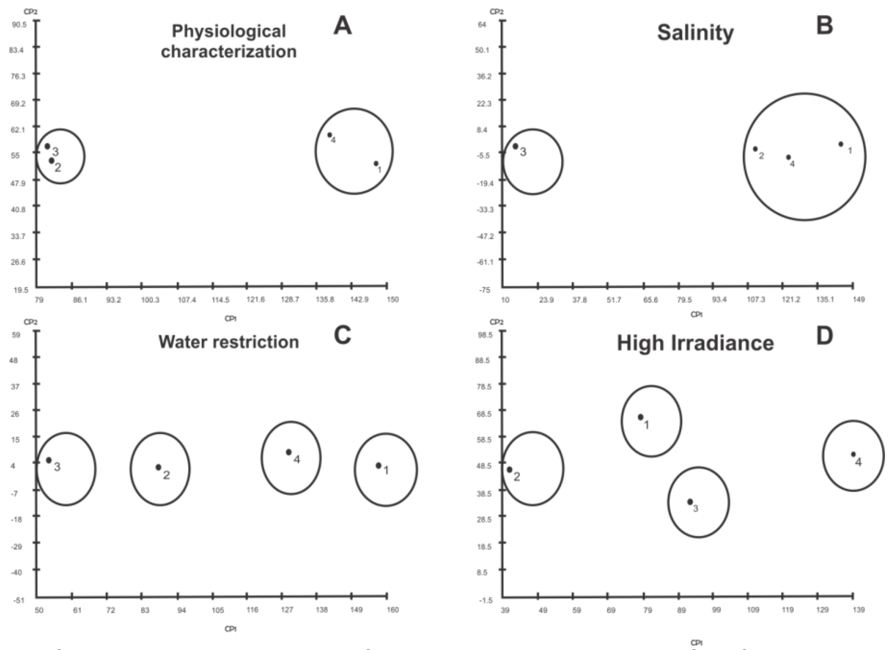

Fig 3. Dispersion graph of the two principal components for the characteristics and seeds of the four papaya genotypes: 1 – Caliman 01; 2 – Golden THB; 3 – Triple hybrid; 4 – hybrid JS12 x Waimanalo; under the conditions: A – physiological characterization; B – salt stress; C – water restriction stress; D – high irradiance stress. The cumulative percentage of the two principal components accounts for more than 99% of the total variation.

potential between -0.2 and -0.3 MPa induced by NaCl), while plant growth is hampered by salinity 10 times lower (0.4 dS m^{-3}) (Cavalcante et al., 2010). With respect to water potential, it is recommended to use at least 50% of field capacity in the cultivation of papaya in order to prevent losses in growth and productivity (Fontes et al., 2008). In this study, it was observed that salinity and water restriction at osmotic potentials below -0.4 MPa result in stress conditions during the germination of papaya seeds.With regard to stress by elevated irradiance, the results suggest that full sunlight (1200 μmol m^{-2} s^{-1}) represents a condition of stress, while shading with a screen (580 μmol m^{-2} s^{-1} irradiance) provides more suitable light conditions for germination and vigour of papaya seeds.

Intermediate irradiance is suitable for the development of the seedlings until 120 days after sowing (Mengarda et al., 2014). However, when adult, the papaya is considered a full sunlight plant. Stress caused by high irradiance can cause oxidative damage, and the formation of reactive oxygen species. Changes in luminosity are associated with variations in temperature, water availability, and gas exchange. These factors profoundly affect photosynthesis, and thus the growth and development of papaya plants (Campostrini; Glenn, 2007; Reis; Campostrini 2008). This study investigated the impact of stresses during germination, when seedling growth is dependent on seed reserves, and not the production of photoassimilates; although the species is considered a full sunlight plant, it was observed that high irradiance (and associated factors) negatively affected the germination of papaya seeds. This result confirms that to optimize papaya seedling formation, the use of protected environments with shadowing is necessary (Mengarda et al., 2014).

Germination performance of papaya genotypes under stress

The hybrids Caliman 01 and JS12 x Waimanalo showed greater germination potential, as well as exhibited higher average G and SGI. Analysis of SGI inferred the ability of the seed to germinate faster, and the higher the SGI, the smaller the chance that seeds and seedlings suffer biotic and abiotic stresses, and the higher the possibility of formation of an ideal plant stand. It is an index of vigour that also predicts change in metabolic events and efficiency in the synthesis of compounds essential for germination (Marcos Filho, 2005). Thus, the joint evaluation of G and SGI translates into high physiological quality of papaya seeds. All the genotypes used in this study, with the exception of hybrid Caliman 01, showed expressive germination reduction in relative performance when subjected to salinity and to stress by mannitol-induced water restriction. Although the papaya plant is considered moderately tolerant to salinity, above tolerance levels, salinity inhibits growth in height, stem diameter, leaf area, and biomass accumulation. In the cv. Sunrise Solo, increasing the salinity of irrigation water inhibited germination and plant growth (Cavalcante et al., 2010). The hybrid Caliman 01 showed the best performance under osmotic stresses. In investigating the responses of papaya to water stress, it was found that certain papaya varieties exhibit osmotic adjustment as a factor that contributes to adaptation to drought (Campostrini et al. 2001; Campostrini; Glenn, 2007), a fact that may have contributed to hybrid Caliman 01 presenting greater tolerance to high salinity and water stress during germination. In a study of the ionic effects of salinity in soybeans, it was found that the restriction capacity for Cl^- in above-ground tissues, such as leaves, may represent an important factor in salinity tolerance (Zhang et al., 2011). Also, in soybeans, salinity reduces the percentage of pectin, and increases the percentage of cellulose in the cell wall of root cells, suggesting that salinity increases rigidity of the cell wall, and thereby inhibits root growth. It was observed that cultivars able to maintain their cell wall formation have an apparent advantage with regard to salt tolerance. Thus, there appears to be a relationship between polysaccharides making up the cell wall with soybean tolerance to salinity (An et al., 2014). Thus, cell wall characteristics, and their functionality with respect to ion transport, may represent an important role in stress tolerance.

These characteristics show specificity with respect to the papaya genotypes responses. Genetic differences observed among cultivars in germination performance under salinity and water stress can be used to better understand physiological responses to stress tolerance (Murillo-Amador et al., 2002), as well as to improve the cultivated species.

Genetic differences in tolerance to abiotic stresses have been reported in sorghum, generating information that may aid in identifying genotypes adapted to semi-arid regions, which commonly present saline soils and/or water restriction (Patane et al. 2013; Yu et al. 2004). The study of the growth, biomass allocation, and biochemical alterations in sugarcane under conditions of water stress, based on univariate and multivariate analyses, allowed for inferences to be drawn regarding greater tolerance to drought conditions among cultivars (Queiroz et al., 2011). Moreover, the study of germinative responses (germination and vigour) to water stress (polyethylene glycol) enabled testing of the differentiation of cotton cultivars with respect to sensitivity to osmotic potentials (Meneses et al., 2011).

When subjected to high irradiance conditions, seeds of the hybrids Caliman 01 and JS12 x Waimanlo presented greater average vigour. The hybrid JS12 x Waimanlo showed the highest germination performance, while cv. Golden THB demonstrated a lower performance. Thus, genotype-specific responses to stresses were also observed in papaya.

There is a genetic variation in the response of papaya to light conditions during its early development and vegetative and reproductive phases. The cv. Golden has low leaf chlorophyll content compared to other cultivars, such as JS12, Solo7212, Tainung 01, and Caliman 01. This can substantially increase light reflection, and reduce leaf temperature under high light conditions. During emergence and early development, the cv. Golden THB is more sensitive to high irradiance, while hybrids Caliman 01 and JS12 x Waimanalo tolerate this condition (Campostrini; Glenn, 2007; Mengarda et al., 2014).

The papaya shows high genetic divergence for attributes related to the physiological seed quality, which can be used in the selection of genotypes based on germination characteristics (Cardoso et al., 2009; Macedo et al., 2013; Mengarda et al., 2015). In this study, a genotype-specific response was observed for the physiological characteristics of seeds for each stress condition.

From groupings based on analysis of the dispersions graphs for principal components (PC), it was possible to distinguish genotypes for seed vigour, and tolerance to these different stresses: in salt stress and under water restriction, the Caliman 01 displayed the best performance; under high irradiance, the hybrid JS12 x Waimanalo showed greater performance. According to Cardoso et al. (2009), diversity and clustering analysis were effective in discriminating among papaya genotypes, providing accurate estimations of genetic variability for seed quality. According to Mengarda et al. (2015), the diversity analysis and the genetic parameters based on the assessment of the physicochemical and physiological characteristics of papaya seeds can indicate the highest-performing genotypes. In this study, the dispersion analysis of PC, together with the mean test and the evaluation of relative performance, enabled the identification of the genotypes with the highest germination performance in environmental stress conditions imposed during germination. Due to the scarcity of studies related to seed vigour under stress conditions, studies such as this one provide relevant information from the perspective of propagation and improvement of papaya, as they aid in the selection of genotypes (or parents) of greater germination performance under specific environmental stress conditions.

Materials and Methods

Plant materials

The present study was developed in the Laboratory of Seed Analysis (Laboratório de Análise de Sementes – LAS), and the greenhouse at the Center for Agricultural Sciences, Federal University of Espírito Santo (Centro de Ciências Agrárias da Universidade Federal do Espírito Santo, CCA-UFES), located at coordinates 20º36’13” S and 41º11'05" W, elevation of 271 m, with Cwa climate (dry winter and wet summer, with an average annual rainfall of 1,200 mm). Seeds from four genotypes of papaya (*Carica papaya* L.) were used: hybrid Caliman 01 (G1), cv. Golden THB (G2), Triple hybrid (G3), and hybrid JS12 x Waimanalo (G4) described by Mengarda et al. (2015). The seeds were provided by Caliman Agrícola S/A. The following procedures and evaluations were performed:

Salt stress and water restriction induction

In the laboratory, papaya seeds were subjected to different osmotic potential levels during germination: zero, -0.2, -0.4, -0.6 and -0.8 MPa, induced by solutions of sodium chloride (NaCl) and mannitol (Vetec®). The osmotic potential was calculated according to the equation $\Psi s = - RTCi$ (Salisbury; Ross, 1991). Sowing was performed on germitest paper rolls, moistened with the solutions at a volume of 2.5 times the mass of the dry paper, and placed in a germination chamber with alternating temperatures (20-30 ºC).

Irradiance stress induction

In the greenhouse, the papaya seeds were subjected to different levels of irradiance during emergence: 1200 (full sun), 580, 130, and 60 $\mu mol\ m^{-2}\ s^{-1}$. The irradiance factor was achieved by overlapping of the black shade screens (polyolefin), and the irradiance was determined using a radiometer (Light meter LI -250A, LI-COR, USA). Seeds were sown in plastic tubes with capacities of 50 mL, containing commercial substrate (HS vegetables, Holambra substrates®), at 1.5 cm deep. Irrigation was conducted as needed.

Germination test

The measurements evaluated were: *germination* (G), assessed daily until stabilization, and counting the number of seedlings with primary root protrusion in the laboratory, or whose cotyledons were encountered above the substrate in the individual shaded greenhouses. Stabilization occurred after 28 days in the laboratory, and 35 days after sowing in the greenhouse; *first, second, and third germination counts* (FGC, SGC, and TGC) were determined together with the germination test. In the laboratory, the percentage of seedlings germinated after 7, 14, and 21 days after sowing were evaluated. In shaded greenhouses, we evaluated the percentage of emerged seedlings after 14, 21, and 28 days after sowing; *speed germination index* (SGI) was calculated according to Maguire (1962); *mean germination time* (MGT) was calculated according to Labouriau (1983).

Experimental design

We adopted a completely randomized design, with four replicates of 25 seeds per treatment. Data analysis was performed independently for each stress condition, performing a regression analysis and adopting regression models with significance ($p \leq 0.05$) and higher-order terms (R^2), using the statistical program R (The R Foundation for Statistical Computing, version 3.1.1).

Germination performance of papaya genotypes

To characterize the physiological potential of the four genotypes [Caliman 01 (G1), cv. Golden THB (G2), Triple hybrid (G3), and hybrid JS12 x Waimanalo (G4)] in the laboratory, seeds were subjected to germination tests in the absence of stress. We used four replicates of 25 seeds distributed in paper rolls, moistened with distilled water at a proportion equivalent to 2.5 times the mass of dry paper, and maintained in a growth chamber with alternating temperatures (20-30 ºC). Assessments of G, FGC, SGC, TGC, SGI and MGT were performed as described above.

Germination performance of papaya genotypes under salt stress and water restriction

A NaCl solution was used for the induction of salt stress, and a mannitol solution for water stress, both at the osmotic potential of -0.6 MPa. Seeds were distributed in paper rolls, moistened with distilled water (no stress) or solutions of NaCl and mannitol, at the proportion of 2.5 times the dry paper mass, and maintained in a growth chamber with alternating temperatures (20-30 ºC). Assessments of G, FGC, SGC, TGC, SGI and MGT were performed as described above.

Germination performance of papaya genotypes under high irradiance

The seeds were submitted to the conditions of no stress (control) = 580 $\mu mol\ m^{-2}\ s^{-1}$ and high irradiance stress = 1200 $\mu mol\ m^{-2}\ s^{-1}$ (full sunlight). Sowing in plastic tubes with a capacity of 50 mL containing a commercial substrate (HS vegetables, Holambra substrates®) was performed at a 1.5 cm depth, and the tubes were maintained under ambient temperature and humidity. Irrigation was performed as required. Assessments of G, FGC, SGC, TGC, SGI and MGT were performed as described above.

Statistical analysis

The experiment was conducted in a completely randomized design, with four replications of 25 seeds per treatment. Characteristics of the TGC and MGT were eliminated from the analysis in order to fit the multivariate data analysis to the diagnosis of multicollinearity. The results were submitted to analysis of variance and Tukey's test at 1% probability, using the statistical program R (The R Foundation for Statistical Computing, version 3.1.1).

With respect to the genotype with the highest performance under each stress, its relative importance was calculated, represented by the increase or reduction in relation to the control, considering the control as an absolute value (100%). The major components were estimated, and the dispersion graphic in the formation of groups was analysed using the statistical software Genes (Genes Computational Application in Statistics and Genetics, version 2013.5.1).

Conclusion

Salinity (NaCl) and water restriction (mannitol) at osmotic potentials below -0.4 MPa, and elevated irradiance (1200 $\mu mol\ m^{-2}\ s^{-1}$), result in stress conditions during the germination of papaya seeds.
The hybrid Caliman 01 shows higher germination performance under conditions of salinity and water restriction. The hybrid JS12 x Waimanalo shows higher germination performance under elevated irradiance.

Acknowledgements

To Caliman Agrícola S/A for providing the plant material, CAPES for providing the graduate student scholarships, and CNPq for the productivity scholarship.

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