# SENSITIVITY TO ENVIRONMENTAL STRESS OF PRATA, JAPIRA AND VITÓRIA BANANA CULTIVARS PROVEN BY CHLOROPHYLL a FLUORESCENCE<sup>1</sup>

PRISCILA NOBRES DOS SANTOS<sup>2</sup>, DIOLINA MOURA SILVA<sup>3</sup>, CAMILLA ZANOTTI GALLON<sup>4</sup>, JOSÉ AIRES VENTURA<sup>5</sup>

**ABSTRACT**-This study aimed to evaluate the physiological responses to environmental stress during pre- and post-harvest of the following banana cultivars: Prata (AAB), Japira (AAAB) and Vitoria (AAAB). Analyses were carried out on young plants at vegetative stage (daughter-plant) and adult plants at reproductive stage (motherplant). The experimental design was completely randomized. In the *in vivo* pre-harvest analysis were used seven replications, in a factorial scheme (3x2x2), three cultivars and two stages (vegetative and reproductive) and two collection periods (March and June). For the analysis of post-harvest quality were used five replications in a factorial design (3x2x5), corresponding to three cultivars, two development stages and five periods of post-harvest analysis, carried out every two days from stage 4 of fruit ripening. The chlorophyll a fluorescence emission kinetics showed low photochemical performance of the three cultivars in June, a period characterized by lower temperatures and water deficit. Prata was the cultivar with the lowest tolerance to abiotic physiological behavior changes, which also reflected in fruit quality, because there was a change in physical and physicochemical parameters. Japira and Vitoria cultivars showed similar physiological responses in the pre- and post-harvest periods, according to their phylogenetic proximity. The total performance index, i.e., the conservation of energy absorbed by PSII up to the reduction of the final PSI acceptors (PI<sub>total</sub>) and the di-malonic aldehyde (MDA) content were significantly higher in Japira and Vitoria cultivars compared to Prata cultivar in the reproductive phase. There was no significant change in the potential quantum efficiency of PSII  $(F_V/F_M = \phi P_0)$  among the three cultivars. It was concluded that Japira and Vitoria cultivars showed greater plasticity to tolerate or even adapt to abiotic variations keeping higher fruit yield. PI total is the most sensitive parameter during the banana life cycle and important tool for distinguishing different cultivars yields.

**Index terms**– Musa spp., Chlorophyll fluorescence, pigments, lipid peroxidation, post-harvest quality.

# SENSIBILIDADE AO ESTRESSE AMBIENTAL EM BANANEIRA COMPROVADA PELA FLUORESCÊNCIA DA CLOROFILA *a*

RESUMO-Este trabalho objetivou avaliar as respostas fisiológicas ao estresse ambiental, durante a pré e a pós-colheita, das cultivares: Prata (AAB), Japira (AAAB) e Vitória (AAAB). As análises foram realizadas em plantas jovens, em estádio vegetativo (planta-filha) e em plantas adultas, em estádio reprodutivo (planta-mãe). O delineamento experimental foi inteiramente casualizado. Nas análises de pré-colheita, in vivo, utilizaram-se sete repetições, em esquema fatorial (3x2x2), três cultivares, dois estádios: vegetativo e reprodutivo e dois períodos de coleta (março e junho). Para a qualidade pós-colheita, utilizaram-se 5 repetições, em esquema fatorial (3x2x5), três cultivares, dois estádios de desenvolvimento e cinco períodos de análise, realizadas a cada dois dias a partir do estádio 4 de amadurecimento dos frutos. A cinética de emissão da fluorescência da clorofila a mostrou baixo desempenho fotoquímico das três cultivares no mês de junho, período caracterizado por temperaturas mais baixas e deficiência hídrica. A cv. Prata foi a que apresentou um comportamento fisiológico menos tolerante às alterações abióticas, o que refletiu também na qualidade dos frutos, pois houve alteração nos parâmetros físicos e físicoquímicos. As cultivares Japira e Vitória apresentaram respostas fisiológicas na pré e pós-colheita bem semelhantes, condizente com sua maior proximidade filogenética. O índice de desempenho total, isto é, a conservação da energia absorvida pelo PSII até a redução dos receptores finais (PI<sub>total</sub>) e o conteúdo do di-aldeído malônico (MDA) foram significativamente mais elevados nas cv. pira e Vitória em comparação com a cv. Prata na fase reprodutiva. Não houve mudança significativa na eficiência fotoquímica potencial máxima do FSII  $(F_V/F_M = \phi P_0)$  entre as três cultivares. Conclui-se que as cv. Japira e Vitória mostraram maior plasticidade para tolerar ou até mesmo se adequar às variações abióticas mantendo maior rendimento do fruto. PI<sub>total</sub> é o parâmetro mais sensível durante o ciclo de vida da bananeira e importante ferramenta para distinguir cultivares de diferentes rendimentos.

**Termos para indexação** – *Musa spp.*, Fluorescência da clorofila *a*, pigmentos, peroxidação lipídica, qualidade pós-colheita.

<sup>&</sup>lt;sup>1</sup>Received September 28, 2015. Accepted October 25, 2016.

<sup>&</sup>lt;sup>2</sup>Biologist. Graduate Program in Plant Biology, Federal University of Espírito Santo. Av. Fernando Ferrari, 514. Vitória-ES. 29075-900. E-mail: priscillanobres@gmail.com

<sup>&</sup>lt;sup>3</sup>Biologist. Full Professor. Department of Biological Sciences, UFES. E-mail: diolina.silva@ufes.br

<sup>&</sup>lt;sup>4</sup>Biologist. PNPD / PPGBV / UFES scholarship holder. E-mail: millazg@gmail.com

<sup>&</sup>lt;sup>5</sup>Agronomist, Capixaba Institute for Research, Technical Assistance and Rural Extension, Rua Afonso Sarlo 160, Bento Ferreira, Vitória-ES, 29052-010, Brasil. E-mail: ventura@incaper.es.gov.br

# INTRODUCTION

Banana culture is particularly important in the agribusiness of the state of Espírito Santo, being responsible for the generation of employment and income for producers, mostly family-based, who are involved in the production and commercialization process (COSTA et al., 2006). Planting is performed in geographically rough areas, which prevent the traditional chemical control of pests and diseases. Since 1976, the Capixaba Institute for Research, Technical Assistance and Rural Expansion (INCAPER) has been conducting research on the selection and evaluation of banana genotypes of the Prata subgroup using a germplasm bank in order to obtain high productivity bananas, tolerant to drought and cold and that have the preference of consumers (VENTURA; GOMES, 2005; DONATO et al., 2006). Among the results obtained, Japira and Vitória, tetraploid hybrids (AAAB) cultivars obtained from the crossing between female parental Pacovan, cv., triploid (AAB), and male parental M53, diploid (AA), showed good productivity and resistance to the main pests and diseases such as Panama disease, yellow sigatoka and black sigatoka that affect the Prata cultivar (VENTURA et al., 2007). These attributes have contributed to the preference of producers for Japira and Vitória cultivar to the detriment of Prata cultivar. As a result of this change process, they already represent a large part of what is produced in banana farming in the state of Espírito Santo.

The identification of new pest- and disease-resistant cultivars with quality characteristics traditionally accepted by the consumer has been made for Prata-Anã cultivar, but this variety is highly susceptible to Panama disease and yellow and black Sigatoka (CASTRICINI et al., 2015), thus not representing an alternative to ES producers that seek disease-resistant cultivars.

Banana (*Musa spp.*) is one of the most consumed fruits in the world due to its taste and aroma, in addition to being an attractive fruit in the diet for children and older adults because it presents carbohydrates of easy digestion (MARASCHIN, 2015). Information on the quality of fruits of new cultivars present in the market is important not only in terms of fruit shelf life but also in relation to flavor.

Banana productivity is dependent not only on biotic factors but also on abiotic factors, as they interfere with metabolic processes and photosynthetic activity (MELO et al., 2009). Strauss et al. (2011) observed inhibition of photosystem II (PSII) and inhibition of the CO<sub>2</sub> reduction cycle enzymes activity in cold sensitive soybean genotypes when exposed

to cold air and low soil temperature. Under these conditions, damage also occurs in roots and aerial part of the plant (production and export of nitrogen compounds and photoassimilates, respectively). Alteration of the nitrogen metabolism in roots limits the nitrogen supply to the rest of the plant and accelerates the progressive development of chlorosis. Lower water availability and temperature alternation can cause (1) membrane damage, that is, loss of membrane integrity and intracellular organelles leading to solute leakage and compartmentalization disturbance (JANSKA et al., 2010), (2) osmotic stress, that is, water deficiency due to the decrease of water absorption by roots and restriction by the closure of stomata (LUKATKIN et al., 2012) and (3), and oxidative stress due to the excess production of reactive oxygen species (ROS), which lead to membrane lipid peroxidation, protein oxidation, enzyme inactivation and DNA damage (YUSUF et al., 2010).

Currently, there is a great deal of knowledge about the use and physiological significance of various parameters of chlorophyll a fluorescence, both from fast fluorescence and modulated fluorescence (KALAJI et al., 2016), making it an interesting technique that can be used in research and field application, such as in ecosystem monitoring (MENGARDA et al., 2012), phenotyping (CHOUDHARY; AGRAWAL, 2015) and plant production (GAMA et al., 2013). Therefore, the analysis of the kinetic parameters of chlorophyll a fluorescence is an excellent tool in determining the functionality profile of photosystems in plants under stress in the field (SALVATORI et al., 2014).

Considering the great importance of banana cultivation in the Brazilian market and the demand for cultivars that serve as a commercial alternative for producers, the aim of this study was to evaluate the pre-harvest characteristics of Japira and Vitória cultivars resistant to Panama disease, yellow sigatoka and black sigatoka and post-harvest quality of fruits, comparing them to the Prata cultivar, which is widespread but more susceptible to biotic and abiotic stresses.

#### MATERIAL AND METHODS

Plant material and sampling conditions:

the plant material was obtained from a banana germplasm bank of the Institute for Research, Technical Assistance and Rural Extension of Espírito Santo (Incaper) located at the "Alfredo Chaves" Experimental Farm, municipality of Alfredo Chaves, Espírito Santo (20°37'S and 40°43'W - 35m

a.s.l.). According to the Köppen classification, the municipality has Aw-type climate, with average minimum temperature of 18°C and average maximum temperature of 22°C and minimum precipitation of less than 60 mm. The agroclimatic data presented were obtained from the meteorological station of the National Institute of Meteorology (INMET) / INCAPER installed in the farm (Figure 1). Banana trees were planted with seedlings, with a spacing of 3mx3m, and fertilization was based on soil analysis. Weeding was performed by mowing and cultural operations such as thinning of followers, defoliation and management of plants, according to technical recommendations for 'Prata' banana cultivar in the state of Espírito Santo (VENTURA; GOMES, 2005). Fruits were collected from plants identified as Daughter-Plant, which are young plants in the vegetative stage, and fruits from adult plants, in the reproductive stage, identified as Mother-Plant. Data collection was performed in uniform clumps in Prata (AAB), Japira (AAAB) and Vitória cultivars (AAAB), in the months of March and June, enough time for them to be collected in the second sampling, fruits at the same development stage obtained in the first collection. All destructive and non-destructive analyses were performed on the third leaf fully open from the apex (candle leaf) between 10 and 25 cm from the median inner part.

Chlorophyll a fluorescence emission kinetics: HandyPEA® portable fluorometer (Hanstech, King's Lynn, Norkfolk, UK) was used. Readings were performed in the morning, after adjusting samples to dark for 30 minutes, so that the primary electron acceptor of PSII - Quinone A (Q<sub>A</sub>) - was fully oxidized (at time in which all reaction centers are open). Chlorophyll fluorescence emission was induced in a leaf area of 4 mm in diameter by exposing the sample to a red light peak of 3,000 µmol m<sup>-2</sup> s<sup>-1</sup>. To normalize OJIP transients (curves), steps O ( $F_0 = 0.02$  ms) and P (maximum fluorescence,  $F_M$ =  $\sim$  300ms) were used. These curves are expressed as the relative variable fluorescence  $[W_{OP} = (F_T - F_0)/$  $(F_M-F_0)$ ]. After the normalization of curves, the kinetic difference was obtained using the "Prata" cultivar as control (= 0). To allow visualization of the L-band ( $\sim 0.15$ ms), variable relative fluorescence was normalized between steps 0 and K (0.3ms) ( $W_{OK}$ =  $[F_T - F_0]/[F_K - F_0]$ ) and the kinetic difference was obtained by the equation  $(\Delta W_{OK} = [W_{OK(cultivar assessed)}]$  $W_{OK(control)}$ ]). The K-band (~ 0.3 ms) was visualized when the kinetic difference between the variable fluorescence in steps O and J (2ms)  $(W_{OJ} = [F_T - F_0]/F_0$  $[F_J - F_0]$ ) and the kinetic difference ( $\Delta W_{OJ} = [W_{OJ(cultivar})]$ assessed - W<sub>O.I(control)</sub>]) were calculated. The biophysical

variables that quantify the energy flow through the electron transport chain were analyzed according to the JIP test proposed by Strasser and Strasser (1995). Among the several parameters used in this work, the photochemical performance index of PSII based on absorption (PI<sub>ABS</sub>) and the total photochemical performance index of a sample (PI<sub>total</sub>) stand out (GAMA et al., 2013, CHEN et al. 2016a).

Chlorophyll index: determined on the same leaves used for measurements of chlorophyll fluorescence kinetics using a portable SPAD-502® chlorophyll meter (Soil Plant Analysis Development, Minolta Camera Co. Ltd., Osaka, Japan).

**Lipid peroxidation:** Malonic di-aldehyde was determined as an indicator of lipid peroxidation. The methodology proposed by Heath and Packer (1968), with modifications (HODGES et al., 1999) was used. Quantification was performed from the malonic di-aldehyde (MDA) formed by the thiobarbituric acid reactive substances method (TBARS or TBA) (HALLIWELL; GUTTERIDGE, 1989). MDA content was expressed as ηmol of dry mass MDA g<sup>-1</sup>.

Fruit sampling: Fruits from Prata, Japira and Vitória cultivars obtained from the second bunch were harvested in Mother-Plants in which preharvest measures were carried out. The maturation scale used was based on the maturation scale of Von Loesecke, available in the banana classification standard prepared by "Companhia de Entrepostos e Armazéns Gerais de São Paulo, Ceagesp (PBMH and PIF, 2006). Fruits were harvested at stage 1, completely green in color, and kept in plastic trays at 22°C until reaching maturation stage 4, when the bark color was more yellow than green, representing the physiological maturity of fruits. Analyses were initiated in stage 4 (when fruits were physiologically mature), repeated every other day for 12 days (when fruits reached totally yellow bark with brown areas).

**Post-harvest analysis:** Five fruits were detached from the bunch with the pedicel at 1.0 cm from the cushion and evaluated for the physical characteristics of fresh mass (g), length and diameter (cm) and pulp / bark ratio. The pulp firmness was evaluated with a portable penetrometer (Mark EFFEGI, Mod. TEC-2, Trento, Italy) and expressed in Newton. Physicochemical analyses were performed after homogenization of the fruit pulp, in mixer, until creamy consistency was reached. pH was determined using a digital pH meter (TECNAL, TEC-2, São Paulo, Brazil) and titratable acidity (g.100g-1) by titration with 0.1N NaOH. Soluble solids content (SS) of the pulp juice filtrate was determined using a digital bench refractometer (Instrutherm, RTD-

45, São Paulo, Brazil). The results were expressed in Brix

**Statistical analysis:** For the pre-harvest in vivo analyses, a completely randomized design was used, with seven replications, in a factorial scheme (3x2x2), corresponding to three cultivars (Prata, Japira and Vitória), two development stages (vegetative and reproductive) and two collection periods (March and June). For the post-harvest quality analyses, a completely randomized design was used, with five replications, in a factorial scheme (3x2x5), corresponding to three cultivars (Prata, Japira and Vitória), two development stages (vegetative and reproductive) and five periods of post-harvest analysis, performed every two days from maturation stage 4. Data were analyzed using analysis of variance (ANOVA) and comparisons between means were performed using the Tukey test (p < 0.05).

### RESULTS AND DISCUSSION

From January to July 2010, the municipality of Alfredo Chaves went through a period of great thermal amplitude, with maximum temperature of 40.3°C in March and a minimum of 12.8°C in June (Figure 1). In the first quarter of 2010, there was a long period of scarce rainfall, and in January and February, no precipitation was observed, followed by an atypical rainfall season for the region. It is known that the ideal percentage of water available in the soil for banana crop should not be less than 75% (GONDIM et al., 2009). Considering a precipitation between 100 and 180 mm desirable for the crop, it is verified that, except for the month of March, the values found during this work were lower than expected. Thus, it can be suggested that plants were submitted to a water stress condition, that is, low water availability.

Typical fluorescence induction curves obtained from the leaves of Prata, Japira and Vitória banana cultivars are shown in Figure 2. Leaf measurements under normal conditions show a polyphase rise, called transient chlorophyll *a* fluorescence (OJIP), in which the O-J phase lasts about two milliseconds, the J-I phase lasts about 30 ms and the I-P phase lasts about 500 ms. In response to stresses, an additional K step can be observed at about 300 µs as well as an L step at 150 µs. These changes in the OJIP curve for an OKJIP or OLKJIP curve seem to be a specific response to thermal (KALAJI et al., 2016) and water stress (OUKARROUM et al., 2007).

It was observed that in the months of

March and June, plants presented very similar photochemical performance with low absolute  $F_0$ ,  $F_J$ ,  $F_I$  and  $F_V$  values (Table 1). According to Congming and Vonshak (2002), decreases in the initial fluorescence (F<sub>0</sub>) can be attributed to an inhibition of the flux of electrons to the oxidant site of photosystem II (PSII). Similar results were also found in desiccation-tolerant plants, Ramonda serbica and Ramonda nathaliae, where  $F_0$ ,  $F_M$  and F<sub>v</sub> values decreased during the dry season (GASHI et al., 2013). The authors point out the correlation between loss of PSII function with loss of power to generate potentially harmful reagents; therefore, it acts as a stress sensor. Therefore, the significant decrease in PSII activity in leaves under conditions of water deficit  $(F_0, F_1, F_1 \text{ and } F_v)$ , as observed in this work, may be a protective mechanism to maintain membrane integrity.

Also in March and June, after double normalization of values resulting from the subtraction of O and J steps, K step (K band) was observed in Prata, Japira and Vitória cultivars in the two development stages and after double normalization of the O-K values, a new step, known as L band was observed (Figure 3). However, changes in the transients of the OJIP curve, and especially the appearance of L and K bands, have a predictive value in relation to leaf vitality and tolerance of plant varieties to dry stress (OUKARROUM et al., 2007; CHEN et al., 2016a). The appearance of these new steps indicates that water availability and temperature affected the first phases of photosynthetic induction, with special reference to the inactivation of the oxygen evolution complex (positive K band) and the loss of connectivity between PSII subunits (positive L band). Positive K and L bands were clearly visible in March in both development stages in Vitoria cultivar, while for Japira cultivar, it was only detected in daughter plants. In June, considering the effect of water restriction and low temperatures, the results of this study clearly demonstrate that the presence of the K and L bands are adequate to detect PSII structure adjustment to environmental stress (water availability and temperature variations). Japira and Vitória cultivars presented negative and very similar K and L bands when compared to Prata cultivar, indicating better photochemical performance of both cultivars. The similarity of the results evidences the phylogenetic proximity: both are tetraploid hybrids (AAAB), obtained from the crossing between female parental Pacovan cv., triploid (AAB), and male parental M53, diploid (AA), while Prata is a triploid hybrid (AAB).

A multiparametric chart (radar) with the

average of all measurements of the two months in plants at the vegetative stage (Daughter plant) and in the reproductive stage (Mother plant), considering the Prata cultivar as reference, is presented in the Figure 4. It was observed that in the month of June, the parameters related to the specific energy flow per reaction center, i.e., those representing the "energy transduction cascade" (ABS/RC, TR<sub>0</sub>/RC, ET<sub>0</sub>/ RC and DI<sub>0</sub>/RC) showed a reduction compared to values obtained in March in all cultivars, in both development stages. This reduction is associated with damages in the oxygen evolution complex (OEC) already confirmed by the appearance of the positive K band (YUSUF et al., 2010). There was also a reduction in yield ( $\Psi E_0 / (1-\Psi E_0)$ ) and in quantum efficiency ( $\Psi E_0$ ) of the electron transport from the reduced quinone A (Q<sub>A</sub>-) to the electron acceptors of PSI.

The maximum quantum yield of PSII  $(\varphi P_0 = F_V/F_M)$  seems to be the parameter less sensitive to climatic variations that affected the three cultivars analyzed in this study, showing no significant differences either regarding the time of data collection or the development stage. However, the fall in temperature caused damage to PI<sub>total</sub> components (total photochemical performance index): RC / ABS represents the density of active reaction centers,  $\varphi P_0 / (1 - \varphi P_0)$  represents the quantum yield of the first reactions in PSII, ΨE<sub>0</sub>/(1  $-\Psi E_0$ ) represents the ability to maintain electrons in the electron chain between PSII and PSI and  $\delta R_o/(1 \delta R_0$ ) represents the performance of the PSI reactions (GAMA et al., 2013, CHEN et al., 2016a, B). PI has been widely used and discussed in biotic and abiotic stress investigations (STRASSER et al., 2010) because it is a parameter that, by definition, considers the effect of several individual parameters, that is, the density of active reaction centers, the quantum efficiency performance of PSII, the conversion of energy to the transport of electrons and the reduction of the final PSI receptors. The results obtained here showed that the total performance index (PI<sub>TOTAL</sub>) was significantly higher in the Vitoria cultivar both in the reproductive stage (Mother Plant) and in the vegetative stage (Daughter Plant) in the month of June. Japira cultivar also presented higher PI<sub>TOTAL</sub> in the reproductive stage in the month of March. It was concluded that measurements of OJIP fluorescence transients and their analysis using the JIP test can therefore be used as indicators for stress tolerance and physiological disturbances before the appearance of visible signs of water stress.

Table 2 shows a higher chlorophyll indexes in the month of March than in the month of June for

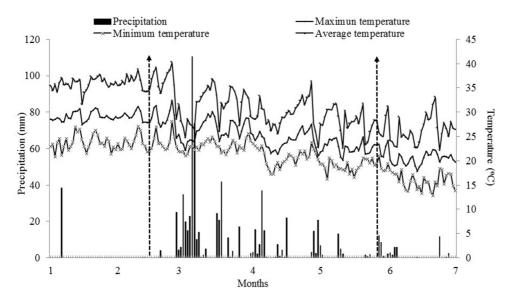
both periods, reproductive and vegetative. Vitória and Japira cultivars showed higher rates compared to Prata cultivar in the reproductive period (47.68 and 31.5 SPAD units, respectively). Similar results were also observed for the malonic aldehyde content (MDA), where values were higher in the reproductive period compared to the vegetative period. However, the increase in MDA was only for Mother Plants in March, since in June, Daughter Plants presented MDA content approximately 40% higher than in March. The high MDA content observed among cultivars at the vegetative stage would be equivalent to the low chlorophyll content observed in Daughter Plants in June. Li et al. (2010) attributes the reduction in chlorophyll content in Chorispora bungeana plants submitted to low temperature, the degradation / destabilization of membranes of the peripheral part of the light-harvesting complex. Thus, it could be inferred that plants at the vegetative stage were more sensitive to low temperatures during the month of June, with a decrease in chlorophyll content and an increase in MDA content. Indeed, the increase in lipid peroxidation, observed by MDA content analysis, has been considered an indicator of the occurrence of oxidative damage in cells (CHEN et al., 2016b).

The results of the post-harvest analysis show that, regardless of cultivar, fruit yield was lower in June (Table 3). Also in June, the lowest quantum efficiency (Figure 4) and highest MDA content (Table 2) were observed, suggesting that the low temperatures and low rainfall observed during this period may have been limiting to the photosynthetic activity, impacting the physiological activities of the plant and cause a decrease in the production of photoassimilates.

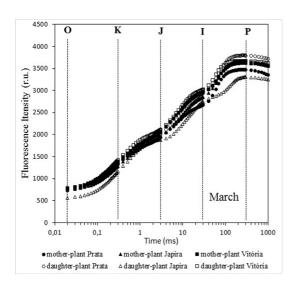
Table 4 shows the results obtained for post-harvest analyses. Japira and Vitória cultivars harvested in the month of June, maintained the pulp firmness until the 8th day after harvest, while Prata cultivar presented decrease in firmness on the 6<sup>th</sup> day. Prata cultivar presented a greater decrease in pulp firmness also in the month of March, which confirms it as a cultivar with shorter shelf life compared to the other cultivars. In terms of soluble solids content, lower values were observed in June, when higher pulp firmness and pH were observed. There was also little variation in titratable acidity during fruit ripening. The results indicate a difference in fruit quality according to the cultivar analyzed and a significant interference of the thermal amplitude and prolonged drought in the banana development. Thus, the decrease in the production of photoassimilates during fruit formation compromised its quality. Japira and Vitória cultivars seem to have more

plasticity to tolerate or even to adapt to such abiotic variations, which was relevant to fruit yield. The results obtained also show the potential of the use of fluorescence techniques in the monitoring of plant

vitality, proving the efficiency of the method in the *in vivo* identification of banana plants with greater agronomic potential.



**FIGURE 1** - Climate diagram of the Incaper Experimental Farm in Alfredo Chaves (20°37'S and 40°43'W - 35m a.s.l.), ES, during the study period (January to July 2010). Precipitation;  $T_{\text{Max}}$  - maximum monthly temperature;  $T_{\text{Min}}$  - minimum monthly temperature;  $T_{\text{Med}}$  - average temperature. Dotted arrows indicate the data collection periods. Data obtained from INMET (National Meteorological Institute) automatic meteorological station.



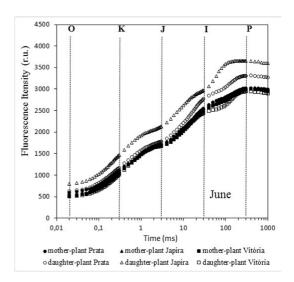
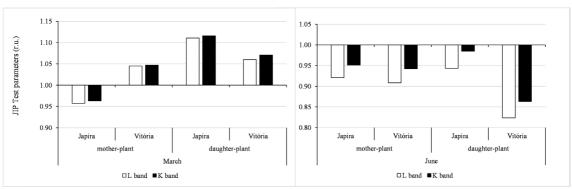
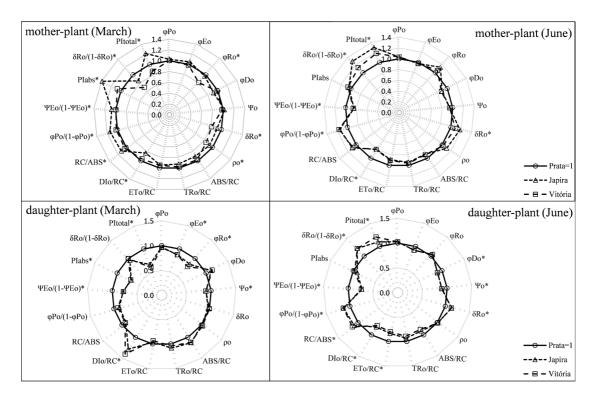


FIGURE 2 - Chlorophyll *a* fluorescence induction curves of three banana cultivars: Prata, Japira and Vitória in two development stages (reproductive = Mother Plant and vegetative = Daughter Plant) using a Handy-PEA fluorometer (Hansatech, UK). The time to adaptation to the dark was 30 min. Data obtained in March (high temperature and excess water in soil) and in the month of June (lower temperatures and water deficit). Data are plotted on a logarithmic time scale and curves are presented without normalization. (n = 7)



**FIGURE 3** - Relative variation of the L Band and K Band values obtained in two development stages: reproductive (Mother Plant) and vegetative (Daughter Plant) of banana trees, in situ, Prata, Japira and Vitória cultivars in the months of March (high water availability and high temperatures) and June (water restriction and low temperatures). The values plotted in the graphs were normalized using the Prata cultivar as reference (= 1.0). (n = 7).



**FIGURE 4** - Parameters of chlorophyll *a* fluorescence from the analysis of the JIP test obtained in leaves of Prata, Japira and Vitória cultivars in two development stages: reproductive (Mother Plant) and vegetative (Daughter Plant) in the months of March (high water availability and high temperatures) and June (water restriction and low temperatures). Significant differences at 5% probability, Tukey's test, are indicated by the asterisk. Each parameter is expressed as a fraction relative to values of Prata cultivar used as reference (= 1.0). (n = 7).

**TABLE 1 -** Values of the basic parameters of the transient fluorescence OJIP of chlorophyll *a* in banana leaves, Prata, Japira and Vitória cultivars adapted to the dark in two development stages reproductive (Mother Plant) and vegetative (Daughter Plant) in the months of March (high water availability and high temperatures) and June (water restriction and low temperatures). Data represent the mean values ± standard deviation (n = 7).

			$F_0$	$F_{_{\mathrm{J}}}$	$F_{I}$	$F_{v}$
		Prata	$0.77 \pm 0.01$	$1.94 \pm 0.04$	$2.67 \pm 0.12$	$2.69 \pm 0.11$
	Mother Plant	Japira	$0.74 \pm 0.02$	$1.95\pm0.06$	$2.85 \pm 0.15$	$2.92 \pm 0.14$
March		Vitória	$0.79 \pm 0.03$	$2.07 \pm 0.10$	$2.96 \pm 0.12$	$2.83 \pm 0.12$
Waren		Prata	$0.75 \pm 0.03$	$2.01 \pm 0.09$	$2.94 \pm 0.11$	$3.04 \pm 0.11$
	Daughter Plant	Japira	$0.79 \pm 0.02$	$2.13 \pm 0.07$	$2.97 \pm 0.10$	$2.86 \pm 0.09$
		Vitória	$0.76 \pm 0.03$	$2.11 \pm 0.10$	$3.02 \pm 0.17$	$2.90 \pm 0.09$
		Prata	$0.59 \pm 0.01$	$1.70 \pm 0.01$	$2.56 \pm 0.11$	$2.42 \pm 0.11$
	Mother Plant	Japira	$0.53 \pm 0.03$	$1.71 \pm 0.03$	$2.49 \pm 0.11$	$2.50\pm0.14$
June -		Vitória	$0.52 \pm 0.04$	$1.68 \pm 0.03$	$2.51 \pm 0.15$	$2.48 \pm 0.13$
	Daughter Plant	Prata	$0.63 \pm 0.03$	$1.79 \pm 0.05$	$2.77 \pm 0.12$	$2.66 \pm 0.13$
		Japira	$0.56 \pm 0.02$	$1.88 \pm 0.04$	$2.75 \pm 0.10$	$2.74 \pm 0.08$
		Vitória	$0.50 \pm 0.04$	$1.70 \pm 0.04$	$2.45 \pm 0.10$	$2.44 \pm 0.09$

**TABLE 2 -** Chlorophyll index (SPAD units) and malonic aldehyde content (MDA) in banana leaves, Prata, Japira and Vitória cultivars adapted to the dark in two development stages reproductive (Mother Plant) and vegetative (Daughter Plant) in the months of March (high water availability and high temperatures) and June (water restriction and low temperatures).

Variable	Cultivar		March	June
		Prata	30.11Ab*	17.50Bbc
	Mother Plant	Japira	35.25Ab	31.50Aa
SPAD		Vitória	47.68Aa	25.40Bb
SPAD		Prata	18.93Ac	16.50Abc
	Daughter Plant	Japira	27.50Ab	12.40Bc
		Vitória	26.80Ab	14.30Bbc
		Prata	10.80Ab	10.60Aab
	Mother Plant	Japira	13.55Aa	11.50Ba
MDA		Vitória	10.75Ab	7.20Bb
(nmol g-1 DM)		Prata	6.80Bbc	13.50Aa
	Daughter Plant	Japira	4.25Bc	13.40Aa
		Vitória	5.26Bc	12.80Aa

<sup>\*</sup> Averages followed by uppercase letters in the row and lowercase letters in the column differ statistically by the Tukey test at 5% probability. (N = 5)

**TABLE 3** - Characterization of the fruits of banana trees cv. Prata, Japira and Vitória collected in the months of March (high water availability and high temperatures) and June (water restriction and low temperatures).

	Cultivars						
Characteristic	Prata		Japira		Vitória		
	March	June	March	June	March	June	
Fresh weigth (g)	90,12a*	25,38b	168,91a	102,72b	175,55a	115,70b	
Diameter (cm)	37,56a	23,78b	43,86a	35,85b	15,59a	13,71b	
Lenght (cm)	11,30a	7,90b	14,92a	12,83b	44,36a	38,57b	
Skin:Pulp relation	2,04a	0,92b	1,62a	1,17b	1,75a	1,27b	

<sup>\*</sup> Means followed by different letters in the line differ statistically by the Tukey test at 5% probability of error (n = 5)

**TABLE 4** - Physicochemical characteristics of banana fruits Prata, Japira and Vitória cultivars during the maturation period, collected in the months of March and June.

		Days after harvest						
Chai	racteristic	4	6	8	10	12		
and	d variety	More yellow than green	Yellow with greed end	Yellow	Yellow with few brown spots	Yellow with many brown spots		
	March							
Firmness (N)	Prata	46.06Ab*	29.59Bb	23.52ABcd	20.97ABb	14.70Ba		
	Japira	52.92Aab	44.49ABab	27.83ABCbcd	20.77BCb	16.46Ca		
ess	Vitória	67.23Aab	44.88Aab	16.07Bd	14.21Bb	14.11Ba		
mné	June							
.E.	Prata	63.70Aab	49.00ABab	48.31ABabc	34.30Bb	33.51Ba		
	Japira	68.90Aab	65.46Aa	65.66Aa	18.81Bb	19.60Ba		
	Vitória	80.26Aa	63.89Ab	68.99Aab	54.29Bab	16.26Ca		
×	March							
Bri	Prata	13.00Ba	23.80Ab	18.60Aba	21.40ABa	24.80Aa		
s (°	Japira	8.00Bab	9.20ABc	17.00ABab	17.20ABab	18.80Aa		
Soluble Solids (°Brix)	Vitória	4.60Cab	11.40BCc	18.00ABa	22.50Aa	20.20Aba		
Sc	June							
ıble	Prata	3.20Ab	6.20Ac	9.40Abc	4.00Ac	7.20Ab		
olt	Japira	4.20Cb	5.40BCc	8.20BCc	10.90Bbc	23.20Aa		
	Vitória	1.30Cb	3.20Aa	3.80Cc	3.20Cc	21.70Ba		
	March							
	Prata	4.58Aab	4.00Ad	4.33Abc	4.36Ac	4.43Aa		
	Japira	4.79Aab	4.57Abc	4.45Abc	4.46Ac	4.43Aa		
Hd	Vitória	4.90Aab	4.30ABcd	4.25Bc	4.31ABc	4.37Aba		
Ω,	June							
	Prata	5.09Aa	4.95Aab	4.82Ab	4.88Aab	4.68Aa		
	Japira	4.41Ab	5.23Ba	4.81ABbc	4.54Ac	4.36Aa		
	Vitória	4.91Bab	0.38De	5.44Aa	5.35ABa	4.27Ca		
	March	0.504	0.604	0.664	0.604	0.561		
Titratable acidity (g.100g <sup>-1</sup> )	Prata	0.59Aa	0.69Aa	0.66Aa	0.60Aa	0.56Aa		
	Japira	0.36Aab	0.51Aab	0.56Aab	0.59Aa	0.58Aa		
table acid (g.100g <sup>-1</sup> )	Vitória	0.30Bb	0.48ABabc	0.52ABab	0.57ABa	0.63Aa		
ntab (g. ]	June	0.01.61	0.215.61	0.4051	0.55.1	0.50 :		
Ĭtre	Prata	0.21Cb	0.31BCbc	0.42Bbc	0.57Aa	0.58Aa		
Ι	Japira	0.36BCab	0.27Cbc	0.46ABabc	0.53Aa	0.47Aba		
	Vitória	0.22Bb	0.25Bc	0.26Bc	0.25Bb	0.44Aa		

<sup>\*</sup> Averages followed by uppercase letters in the row and lowercase in the column differ statistically by the Tukey test at 5% error probability (n = 5)

## **CONCLUSIONS**

Japira and Vitória cultivars presented similar physiological responses, possibly due to their greater phylogenetic proximity. Both cultivars are good alternatives to the planting of the Prata cultivar due to their higher tolerance to abiotic stresses (total photochemical performance index and the MDA content were significantly higher) and presented fruits with shelf life (verified by pulp firmness) equivalent or longer than Prata cultivar.

#### REFERENCES

CASTRICINI, A.; SANTOS, L. O.; DELIZA, E.; COELHO, E. F.; RODRIGUES, M. G.V. Caracterização pós-colheita e sensorial de genótipos de bananeiras tipo Prata. **Revista Brasileira de Fruticultura**, Jaboticabal, v.37, n.1, p.27-37, 2015.

CHEN, S.; YANG, J.; ZHANG, M.; STRASSER, R. J.; QIANG, S. Classification and characteristics of heat tolerance in Ageratina adenophora populations using fast chlorophyll a fluorescence rise OJIP. **Environmental and Experimental Botany**, Oxford, v.122, p.126-40, 2016a.

CHEN, Y. E.; CUI, J. M., LI; G. X.; YUAN, M.; ZHANG, Z. W.; YUAN, S.; ZHANG, H. Y. Effect of salicylic acid on the antioxidant system and photosystem II in wheat seedlings **Biologia Plantarum**, Prague, v.60, n.1, p.139-47, 2016b.

CHOUDHARY, K. K.; AGRAWAL, S. B. Effect of elevated ultraviolet-B on four tropical soybean cultivars: quantitative and qualitative aspects with special emphasis on gas exchange, chlorophyll fluorescence, biomass and yield. **Acta Physiologiae Plantarum**, Copenhagen, v.37, p.31, 2015.

CONGMING, L.; VONSHAK, A. Effects of salinity stress on photosystem II function in cyanobacterial Spirulina platensis cells. **Physiologia Plantarum**, Copenhagen, v.114, p.405-13, 2002.

COSTA, A.N.; COSTA, A. de F.S.; ARAÚJO, J.B.S.; MANGARAVITE, J.C.S.; VENTURA, J. A.; MIRANDA, V. Recomendações técnicas para o cultivo da banana orgânica. Vitória: Incaper, 2006. 48 p. (Documentos, 144)

DONATO, S.L.R.; SILVA, S.O.; FILHO, O.A.L., LIMA, M.B.; DOMINGUES, H.; ALVES, J.S. Comportamento de variedades e híbridos de bananeira (*Musa spp.*), em dois ciclos de reprodução no sudoeste da Bahia. **Revista Brasileira de Fruticultura**, Jaboticabal, v.28, n.1, p.139-44, 2006.

GAMA, V.N.; CUNHA, J.T.; LIMA, I.M.; BACARIN, M.A.; SILVA, D.M. Photosynthetic characteristics and quality of five passion fruit varieties under field conditions. **Acta Physiologia Plantarum**, Copenhagen, v.35, p.941-8, 2013.

GASHI, B.; BABANI, F.; KONGJIKA, E. Chlorophyll fluorescence imaging of photosynthetic activity and pigment contents of the resurrection plants *Ramonda serbica* and *Ramonda nathaliae* during dehydration and rehydration. **Physiology Molecular Biology of Plants**, Lucknow, v.19, n.3, p.333-41, 2013.

GONDIM, A.R.O.; MEDEIROS, J.F.; LEVIEN, S.L.A.; CARMO, G.A.; SENHOR, R.F. Balanço hídrico e da salinidade do solo na bananeira irrigada com de diferentes salinidades. **Acta Scientia Agronomica**, Maringá, v.31, p.1-6, 2009.

HALLIWELL, B.; GUTTERIDGE, J.M.C. Free radicals in biology and medicine. Oxford: Oxford University Press, 1989. 543 p.

HEATH, R.L; PACKER, L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. **Archives in Biochemistry and Biophysics**, New York, v.125, p.189-98, 1968.

HODGES, D.M.; DELONG, J.M.; FORNEY, C.F.; PRANGE, R.K. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. **Planta**, Berlin, v. 207, p.604-11, 1999.

JANSKA, A.; MARSIK, P.; ZELENKOVA, S.; OVESNA, J. Cold stress and acclimation - what is important for metabolic adjustment? **Plant Biology**, Berlin, v.12, p.395–405, 2010.

KALAJI, H.M., JAJOO, A., OUKARROUM, A.,

BRESTIC, M., ZIVCAK, M., SAMBORSKA, I.A., CETNER, M.D., ŁUKASIK, I., GOLTSEV, V., LADLE, R.J. Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. **Acta Physiologiae Plantarum**, Copenhagen, v.38, p.102, 2016.

LI, Y. H.; SUN, Z. L.; XU, X. L.; JIN, M.; LIU, Y. J.; ZHANG, H.; AN, L. Z. Influence of low temperatures on photosystem II photochemistry and expression of the NADPH: protochlorophyllide oxidoreductase in the alpine, subnival perennial, *Chorispora bungeana*. **Photosynthetica**, Praha, v. 48, n.3, p.457-68, 2010.

LUKATKIN, A. S.; BRAZAITYTE, A.; BOBINAS, C.; DUCHOVSKIS, P. Chilling injury in chilling-sensitive plants: a review. **Agriculture**, Switzerland, v.99, p.111-24, 2012.

MELO, A.S.; SILVA J.R.C.D.; FERNANDES, P.D.; SOBRAL, L.F.; BRITO, M.E.B.; DANTAS, J.D.M. Alterações das características fisiológicas da bananeira sob condições de fertirrigação. **Ciência Rural**, Santa Maria, v.39, n.3, p.733-41, 2009.

MENGARDA, L.H.G.; MILANEZ, C.R.D.; SILVA, D.M.; AGUILAR, M.A.G.; CUZZUOL, G.R.F. Morphological and physiological adjustments of Brazilwood (*Caesalpinia echinata* Lam.) to direct solar radiation. **Brazilian Journal of Plant Physiology**, Londrina, v.24, n.3, p.161-72, 2012.

OUKARROUM, A.; MADIDI, S.E.; SCHANSKER, G.; STRASSER, R.J. Probing the responses of barley cultivars (*Hordeum vulgare* L.) by chlorophyll a fluorescence OLKJIP under drought stress and rewatering. **Environmental Experimental Botany**, Oxford, v.60, p.438-46, 2007.

PBMH/PIF. Programa Brasileiro para a modernização da horticultura e produção integrada de frutas. **Normas de classificação de banana**. São Paulo: CEAGESP, 2006 (documentos, 29).

PEREIRA, A.; MARASCHIN, M. Banana (*Musa* spp) from peel to pulp: Ethnopharmacology, source of bioactive compounds and its relevance for human health. **Journal of Ethnopharmacology**, Zurich, v.160, p.149-163, 2015.

SALVATORI, E.; FUSARO, L.; GOTTARDINI, E.; POLLASTRINI, M.; GOLTSEV, V.; STRASSER, R.J.; BUSSOTTI, F. Plant stress analysis: Application of prompt, delayed chlorophyll fluorescence and 820 nm modulated reflectance. Insights from independent experiments. **Plant Physiology and Biochemistry**, Netherlands, v.85, p.105-13, 2014.

STRASSER, B. J., STRASSER, R. J. Measuring fast fluorescence transients to address environmental questions: The JIP-test. In: MATHIS, P. (Ed.). **Photosynthesis**: from light to biosphere. Dordrecht: Kluwer Academic Publishers, 1995. p.977-80.

STRASSER, R.J.; TSIMILLI-MICHAEL, M.; QIANG, S.; GOLTSEV, V. Simultaneous in vivo recording of prompt and delayed fluorescence and 820-nm reflection changes during drying and after rehydration of the resurrection plant *Haberlea rhodopensis*. **Biochemistry Biophisica Acta**, Amsterdam, v.1797, p.1313-26, 2010.

STRAUSS, A.J.; VAN HEERDEN, P.D.R. Effects on both the roots and shoots of soybean during dark chilling determine the nature and extent of photosynthesis inhibition. **Environmental and Experimental Botany**, Oxford, v.74, p.261-71, 2011.

VENTURA, J.A.; GOMES, J.A. Recomendações técnicas para o cultivo de bananeira no Estado do Espírito Santo. Vitória: Incaper, 2005. 42 p. (Documento, 141)

VENTURA, J.A.; SILVA, S.O.; GOMES, J.A.; COSTA, H.; GASPAROTTO, L.; PEREIRA, J.C.R.; PEREIRA, M.C.N. Vitória e Japira-Novas cultivares de bananeira. 2.ed. Vitória: Incaper, 2007. (Documentos, 142)

YUSUF, M.A.; KUMAR, D.; RAJWANSHI, R.; STRASSER, R.J.; TSIMILLI-MICHAEL, M.; GOVINDJEE; SARIN, N.B. Overexpression of γ-tocopherol methyl transferase gene in transgenic *Brassica juncea* plants alleviates abiotic stress: physiological and chlorophyll *a.* **Biochemistry Biophysica Acta**, Amsterdam, v.1797, p.1428-38, 2010.