Chlorophyll fluorescence imaging as a tool to evaluate calyx senescence during the ripening of persimmon fruit treated with gibberellic acid

Ayoub Fathi-Najafabadi[1*], Cristina Besada[1], Rebeca Gil[1], Mª Angeles Calatayud[2], Alejandra Salvador[1]

[1] Postharvest Department, Valencian Institute for Agricultural Research. Ctra. Moncada Náquera Km. 4.5, 46113 Moncada, Valencia, Spain.

[2] Horticulture Department, Valencian Institute for Agricultural Research. Ctra. Moncada Náquera Km. 4.5, 46113 Moncada, Valencia, Spain.

***** Correspondence: Ayoub Fathi-Najafabadi

E-mail: ayfatna@upv.es; Tel.: +34-675062438

Abstract:

The effect of gibberellic acid (GA) on retarding loss of persimmon firmness and fruit coloration has been previously reported. Nevertheless, information about the effect of this treatment on calyx senescence is lacking. In this study, chlorophyll fluorescence imaging (CFI) was used to evaluate calyx senescence in persimmon fruit treated with GA. At three harvest times, physico-chemical parameters measured on persimmon fruit and CFI parameters (Fo, Fm and Fv/Fm) on calyx sepals were evaluated on the fruit treated once or twice with GA, and also on untreated fruit (CTL). A decline in the chlorophyll fluorescence parameters correlated with calyx senescence and progressed during fruit ripening. Spatial images heterogeneity in the Fm/Fv measurements illustrates senescence and necrosis dynamics, which began in the apical area of sepals and progressed to the basal area. Besides retarding fruit ripening, GA treatments delayed the calyx senescence process, and hence improved external fruit quality maintenance. The CFI parameters measured on the calyx correlated with both external color evolution and firmness loss during fruit ripening. Consequently, these chlorophyll fluorescence parameters could act as a potential non-intrusive tool to determine persimmon harvesting time.

Keywords: Calyx senescence, ripening, Fo, Fm, Fv/Fm, fruit quality

1. Introduction

Persimmon (*Diospyros kaki* L.f.) 'Rojo Brillante' is an astringent cultivar that is cultivated mainly in Mediterranean countries like Spain and Italy. Under Mediterranean agroclimatic conditions, commercial fruit maturation lasts between mid-October and December. As this short harvest period implies a major commercialization problem for growers and persimmon producers, pre- and postharvest treatments are usually applied to prolong harvest period and to extend the shelf-life of fruit once harvested.

One of the most studied preharvest treatments to delay the harvest time of some persimmon cultivars is gibberellic acid (GA) (Agustí et al., 2004; Besada et al., 2008). GA, applied at the fruit color-break, delays fruit maturity and postpones the green-to-orange color change and, thus, retards flesh softening (Ben-Arie et al., 1996; Agustí et al., 2004).

Fruit external color and firmness are the most important attributes used as a maturity index to decide about the 'Rojo Brillante' harvest. Nevertheless, calyx status is also an important fruit quality attribute. In persimmon, the calyx is a very characteristic fruit part from the visual point of view. Persimmon fruit possess a large green four-lobed calyx around the fruit stem-end that darkens during fruit ripening and is associated with commercial quality loss. Previous studies have indicated that the photosynthesis of sepals contributes to persimmon fruit development (Yonemori et al., 1996; Nakano et al., 1998). During calyx senescence, desiccation and browning start from the apical part of sepals toward the base. Leaf senescence in plants is related to the breakdown of chloroplast and the degradation of chlorophylls (Lim et al., 2007). To date, information about the relation between calyx senescence and physico-chemical changes in persimmon during fruit ripening, and whether pretreatments delay ripening, e.g.,

GA, affect calyx senescence, is lacking. This information could be useful for determining the harvest time with maximum fruit quality.

Chlorophyll fluorescence imaging (CFI) is a widespread rapid non-intrusive technique to evaluate photosynthetic activity. The heterogeneity of photosynthetic activity over the whole leaf area can be analyzed by CFI (Gorbe and Calatayud, 2012). Some spatial-temporal variations in CFI may be caused by different stress factors, which can be visualized easily by CFI, but cannot be detected by conventional fluorimeter point measurements by non-imaging CF (Ellenson and Amundson, 1982; Omasa and Takayama, 2003, Chaerle et al., 2007; Chen et al., 2009; Weber et al., 2017; Dong et al., 2019).

The intensity of the fluorescence signals emitted by photosynthetically active chlorophyll molecules of photosystem II (PSII) is related to the integrity and activity of the photosynthetic apparatus in vegetable samples. One of the most CF parameters used to study the effects of environmental factors on PSII is the Fv/Fm ratio, which is related to the maximum photosynthetic efficiency of PSII. Hence the application of the CFI technique to measure the Fv/Fm ratio could be used to provide information about the heterogeneous changes in the photosynthetic efficiency of the calyx during persimmon fruit ripening.

The present research aimed to characterize the calyx senescence process that occurs during fruit ripening by CFI, and to establish the relation between CF parameters and fruit color/flesh firmness, and the effect of GA treatment on calyx senescence.

2. Materials and Methods

2.1. Experimental design

The study was carried out in a commercial ‘Rojo Brillante’ persimmon orchard located in Albal, Valencia, Spain (39°23'06.6"N 0°25'13.7"W) at an altitude of 10 m from sea level. One row of six trees, and two trees as one replicate, were taken for each evaluated treatment. Each row was separated by another row that was not taken into account to avoid crossed-contamination between GA treatments.

The trees of one row were sprayed with GA (30 µL L^{-1}) when the fruit skin color index (CI =1000a/Lb, ‘L’,’a’, ‘b’ Hunter parameters) was close to -1.8 on October 10 (one GA treatment) (GA1). The trees of another row were sprayed at the same time as GA1, and another GA treatment was applied fifteen days later on October 25 (two GA treatments) (GA2). Finally, the trees of a third row were selected as the control (CTL) to which no GA treatment was applied.

Three harvests of 120 fruit per treatment (40 fruit per replicate) were carried out on November 13 (first harvest or H1), on November 27 (second harvest or H2) and on December 4 (third harvest or H3). After each harvest, fruit were transported to the Instituto Valenciano de Investigaciones Agrarias (IVIA, Moncada), Spain, where flesh firmness, pulp and skin color, soluble tannin content and ethylene and CO_2 production were measured on each fruit. Ethylene and CO_2 production were also determined in the calyx once carefully separated from fruit. Moreover, the CF parameters were measured in the fruit calyx.

2.2. Physico-chemical parameters

Skin color, firmness and pulp color were individually determined with 20 fruit per replicate for each GA treatment and harvest period. Skin color was evaluated by a CR-300 Chroma Meter (Konica Minolta Sensing Americas, Inc., Ramsey, NY, USA) on two opposite fruit sides. Hunter parameters ‘L’ ‘a’ ‘b’ were measured. The results were expressed as color index (CI = 1000a/Lb), which reflects color

changes in persimmon fruit (Salvador et al., 2007). Fruit firmness, indicated by the maximum force (N) required to break pulp, was measured on opposite equatorial sides by a texturometer (model 4301, Instron Corp., Canton, Mass., USA) with an 8-mm plunger. The crosshead speed during firmness determinations was set at 10 mm/min. In order to measure pulp color, fruit were cut in half and the two opposite equatorial fleshy parts were measured by the CR-300. The results were also expressed as color index.

Soluble tannins (ST) in pulp were evaluated on three samples of five fruit per treatment and harvest period by the Folin-Denis method (Taira, 1996), as described by Arnal and Del Río (2004). The results were expressed on a fresh mass basis (g kg^{-1}).

CO_2 and ethylene production were individually measured on four fruit, and also on their separated calyxes. Both fruit and calyx samples were sealed in 1 L and 100 mL jars, respectively. Jars were kept for 2 h at 20 ºC. Then 1 mL of air from the headspace was extracted with a BD Plastic pack syringe and injected into a model 2000 gas chromatograph (Perkin-Elmer, Norwalk, Conn, USA) fitted with a Poropak QS 80/100 column, a thermal conductivity detector (for CO_2) and a flame ionization detector (for ethylene). Helium was used as the carrier gas at 63.4 kPa. The injector, oven and detector temperatures were 115, 35 and 150 ºC, respectively, to measure CO_2. To determine ethylene concentration, helium was employed as the carrier gas at 55.2 kPa. The injector, oven, and detector temperatures were 175, 75 and 175 ºC, respectively. The CO_2 released from fruit and calyxes was expressed as mmol kg^{-1} h^{-1}, while ethylene was expressed as nmol kg^{-1} h^{-1}.

2.3. Chlorophyll fluorescence parameters

During each harvest period, ten calyxes per treatment were carefully removed from fruit. The CF images were taken on two opposite sepals by an IMAGING-PAM CF system (Heinz Walz GmbH, Effeltrich, Germany). Sepals were incubated in very dim light in the laboratory for at least 1 h after this; individual sepals were placed under the fluorometer cover with black cloth for 10 min to measure Fo and Fm. With this protocol, we are certain that samples were dark-adapted. Fo was determined using light pulses at 1 Hz and 0.5 µmol quanta m^{-2} s. Fm was established by applying a blue saturation pulse at 10 Hz and 2400 µmol quanta m^{-2} s according to the IMAGING-PAM software. The Fv/Fm ratio was determined as (Fm-Fo)/Fm and CF images were taken. To evaluate spatial heterogeneity, the PAM software was used to select three areas per sepal (basal, middle, apical) to determine the Fo, Fm and Fv/Fm parameters inside each area . The pixel values for each image of the fluorescence parameters were displayed with the help of a false color code ranging from black (0.000), red, yellow, green, blue to pink (ending at 1000) (Berger et al., 2004). The means of Fo, Fm and Fv/Fm were calculated for every single sepal.

2.4. Statistical analysis

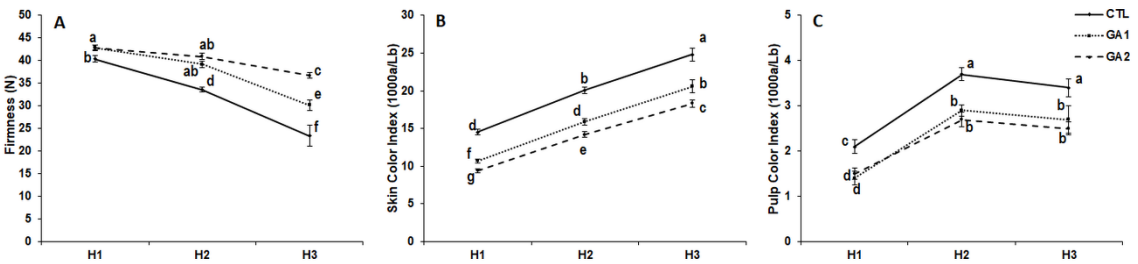
Data were subjected to an analysis of variance, and multiple comparisons between means were determined by the LSD test ($P \leq 0.05$) using the Statgraphics Centurion XVII.I software (Manugistics Inc., Rockville, MD, USA). The results are presented as means ±standard errors (SE). Pearson correlation

and simple linear regression were used to relate both fruit firmness and color to the CF parameters.

3. Results and Discussion

3.1. Physico-chemical parameters

The fruit of ‘Rojo Brillante’ are commercialized with a crisp texture, which means that good firmness at harvest is crucial for preserving this quality parameter during the postharvest period (Salvador et al., 2007). Upon the H1, the firmness of CTLs (40 N) was significantly lower than that of the GA1- and GA2-treated fruit, whose values came close to 43 N (Figure 1A). During the following harvests, the firmness of the CTL fruit gradually decreased to 23 N. In the fruit undergoing both GA treatments, firmness did not change during H2, but a significant change was seen during H3 (GA1: 30 N; GA2: 37 N). The effect of the preharvest GA treatments on delaying firmness fruit was in persimmon cultivars like ‘Rojo Brillante’ and ‘Triumph’ (Besada et al., 2008; Agustí et al., 2004; Ben-Arie et al., 1996). This effect has been related to the maintenance of an organized cell wall and a delay in the cell wall changes that accompany fruit softening (Ben-Arie et al., 1996).



183 **Figure 1.** Flesh Firmness (A), skin color index (B) and pulp color index (C) of the
184 'Rojo Brillante' persimmons without any treatment (CTL), treated once with
185 gibberellic acid (GA1) and treated twice with gibberellic acid (GA2) at three
186 harvest times (H1, H2 and H3). Vertical bars represent SE intervals. Different
187 letters indicate statistical differences ($P \leq 0.05$).

188

189 An increase in skin fruit coloration occurred in parallel to a reduction in firmness
190 as harvest progressed (Figure 1B). At H1, the CTL fruit were picked with an
191 external color index of +14.5, while the fruit of both GA treatments had
192 significantly lower values, close to +10. The color index of all the fruit increased
193 with harvest time, and the CTL fruit had the highest color indices, followed by the
194 GA1 and GA2 fruit for each harvest time. In persimmon, the color index increased
195 during fruit development, and was related to chlorophyll degradation, and also to
196 carotenoid and lycopene accumulation (Zhou et al., 2011; Novillo et al., 2016). In
197 'Rojo Brillante' persimmons, a strong negative correlation between skin color and
198 firmness values during ripening has been reported (Salvador et al., 2006;
199 Tessmer et al., 2016; Besada and Salvador, 2018). Indeed, color measurements
200 have been established as the usual non- destructive method to predict flesh
201 firmness in 'Rojo Brillante' persimmons (Salvador et al., 2006, 2007). In the
202 present work, the correlations between skin fruit color and flesh firmness were
203 significant and negative (-0.93) (data not shown).

204 At H1, a significantly higher color index of +2.1 was found for pulp coloration in
205 the CTL fruit compared to the GA1 and GA2 fruit, which had similar values that
206 came close to +1.4 (Figure 1C). All the fruit exhibited a marked rise in pulp color
207 at H2, which remained almost constant during H3. The maximum internal color
208 index was +3.7 in the CTL fruit, and it came close to +2.8 in the fruit from GA

treatments, with no differences noted between GA1 and GA2. Carotenoid compounds have been reported to be responsible mainly for persimmon fruit flesh color, and their content increases during ripening (Bing et al., 2006; Novillo et al., 2016).

A significant characteristic of some persimmon cultivars is fruit astringency at harvest due to high soluble tannin contents. During ripening, gradual tannin insolubilization brings about the progressive decline in soluble tannins with consequent astringency reduction (Tessmer et al., 2016). In astringent persimmon cultivars like 'Rojo Brillante', fruit flesh concentrations of soluble tannins remain high in the commercial maturity stage. Only in very soft overripe fruit is tannin insolubilization complete, and no sensorial astringency is detected (Tessmer et al., 2016; Besada and Salvador, 2018). In the present study, the fruit of all the treatments had similar tannins contents, which lay between 4.3 and 4.9 g kg^{-1} (data not shown). This is the range reported for this cultivar by previous studies (Novillo et al., 2015, 2016).

Fruit CO_2 production significantly lowered from H1 to H2, but no change was observed during H3 (Table 1). These values fell within the range previously seen for 'Rojo Brillante' (Novillo et al., 2014, 2016). Nakano et al. (1997) demonstrated that the fruit of cultivar 'Hiratanenashi' treated with GA had lower respiration values than the untreated fruit. Nevertheless, the present study found no statistical differences among treatments. No ethylene production was detected in any fruit from any treatment (data not shown). This coincides with other studies that have reported undetectable ethylene concentrations in the same maturity stage (Salvador et al., 2007; Novillo et al., 2014, 2016).

Table 1. CO_2 production in fruit and ethylene production in the fruit calyxes of

235 ‘Rojo Brillante’ persimmons without treatment (CTL), treated once with gibberellic
236 acid (GA1) and treated twice with gibberellic acid (GA2) at three harvest times
237 (H1, H2 and H3). Different letters in each parameter indicate statistical
238 differences (P ≤ 0.05).

	Fruit CO_2 (mmol kg^{-1} h^{-1})			Calyx Ethylene (nmol kg^{-1} h^{-1})		
	H1	H2	H3	H1	H2	H3
CTL	0.38a	0.28b	0.22b	0	0	20.98a
GA1	0.33a	0.25b	0.27b	0	0	14.73b
GA2	0.33a	0.24b	0.26b	0	0	7.58c

239

240

241 **3.2. Calyx senescence and CF parameters changes**

242 Figure 2 illustrates the visual aspect of the CTL, GA1 and GA2 fruit sepals during
243 the three harvests. Although no marked differences appeared among treatments
244 at H1, the CTL fruit sepals displayed slight dehydration symptoms in apical parts.
245 During H2, the CTL fruit presented severe senescence symptoms, which were
246 more evident during H3 when sepals were completely desiccated and necrotic.
247 The GA-treated fruit sepals presented no senescence symptoms until H3, when
248 slight browning was observed mainly in apical parts. These symptoms were more
249 marked in the GA1 fruit than the GA2 fruit.

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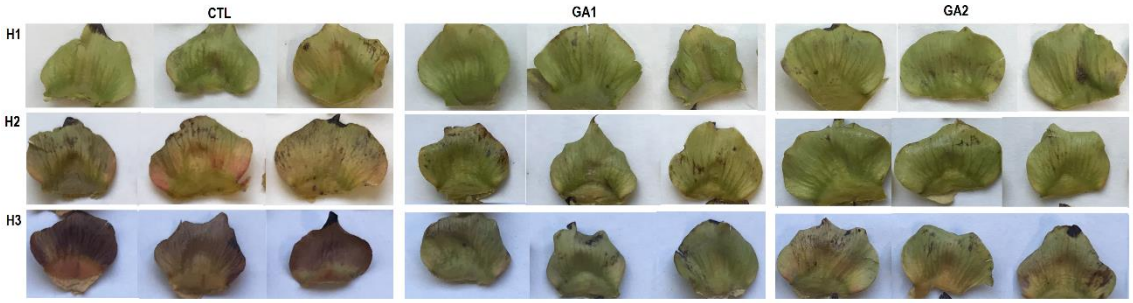

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252 **Figure 2.** Visual aspect of the sepals of ‘Rojo Brillante’ persimmons without any

253 treatment (CTL), treated once with gibberellic acid (GA1) and treated twice with
254 gibberellic acid (GA2) at three harvest times (H1, H2 and H3).

255

256 Nakano et al. (2003) reported that ethylene biosynthesis of the persimmon calyx
257 in fruit detached from trees was almost zero at harvest but increased during fruit
258 ripening. In the present study, the ethylene concentration was detected only in
259 the calyx at H3 (Table 1), and the highest ethylene values were obtained in the
260 fruit in the most advanced maturity stage (CTL fruit).

261 The CF parameters were measured in the sepals of the calyxes from the CTL-
262 and GA-treated fruit (Figure 3). At H1, the mean Fv/Fm in the whole sepal was
263 similar and came close to 0.7 for the fruit sepals in all the treatments (Figure 3A).
264 This value is lower than the average Fv/Fm values (0.83) found in a wide range
265 of leaves under control conditions (Björkman and Demmig, 1987), which was
266 similar to the Fv/Fm values reported for sepals of *Dendrobium* (0.71) (Khoo et al.,
267 1998), tomato (0.78) (Hetherington et al., 1998) or *Helleborus niger* (0.75) (Brcko
268 et al., 2012). In the fruit of both GA treatments, the Fv/Fm values did not change
269 at H2 but lowered at H3 (mean values for GA1: 0.50; GA2: 0.58). Nevertheless,
270 in the CTL fruit, a drastic drop occurred during H2 with values of 0.07, but with no
271 significant changes during H3.

272 At H1, the CTL and GA1 fruit sepals had similar Fo (0.06) and Fm (0.21) values,
273 while significantly higher values were obtained for the GA2 fruit (Figure 3B and
274 3C). Both parameters drastically dropped in the CTL fruit after H2 with values of
275 Fo: 0.03 and Fm: 0.04, which remained during H3. Fo and Fm remained constant
276 until H2 for the GA1 and GA2 fruit and then lowered, with similar Fo (0.04) and
277 Fm (0.12) values during H3.

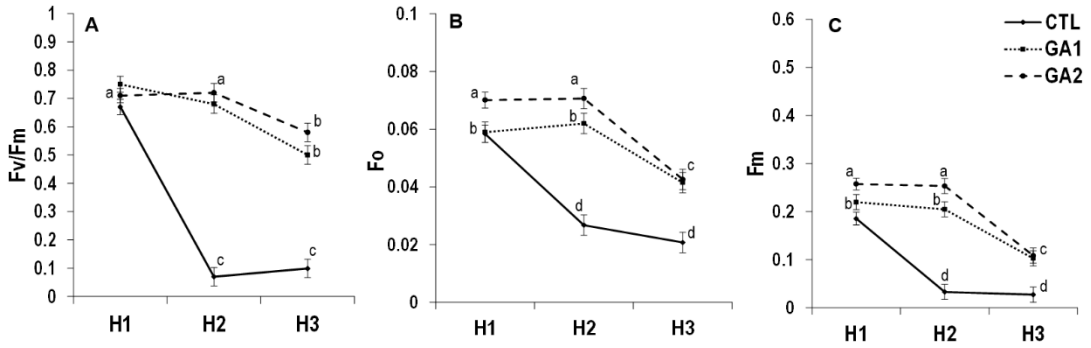

Figure 3. Chlorophyll fluorescence image parameters (Fv/Fm, Fo, Fm) of the whole sepals of the 'Rojo Brillante' persimmons without any treatment (CTL), or treated once with gibberellic acid (GA1) and treated twice with gibberellic acid (GA2) at three harvest times (H1, H2 and H3). Vertical bars represent SE intervals. Different letters in each parameter indicate statistical differences ($P \leq 0.05$).

Figure 4 reveals a different spatio-temporal heterogeneity in the images of Fv/Fm, Fm and Fo for the representative sepals of CTL fruit at H1, where higher values are observed in the basal area. When CFI was analyzed in the different areas inside sepals, a distinct behavior was noted for both treatment and harvest period (Figure 5). At H1, the Fv/Fm in the basal area was close to 0.8 for all the treatments and were 0.63 for CTL and close to 0.7 in the middle areas for both GA treatments. Lower values were found in the apical area, where the CTL value was 0.47, which is significantly lower than that of GA1 and GA2 (0.65). In the CTL fruit, Fv/Fm drastically dropped in all the areas at H2 and remained unchanged at H3. The basal area obtained Fv/Fm values that came close to 0.2, while values came close to zero in the middle and apical areas. In the GA-treated fruit, the decline in Fv/Fm after H1 was much less marked in all the sepal areas than in

299 the CTL fruit. In the basal area, a slight decrease in Fv/Fm was noted, but with
300 no differences between GA1 and GA2. Nevertheless, in the middle and apical
301 areas, this drop was more marked with GA1 showing lower values than GA2 in
302 both areas.

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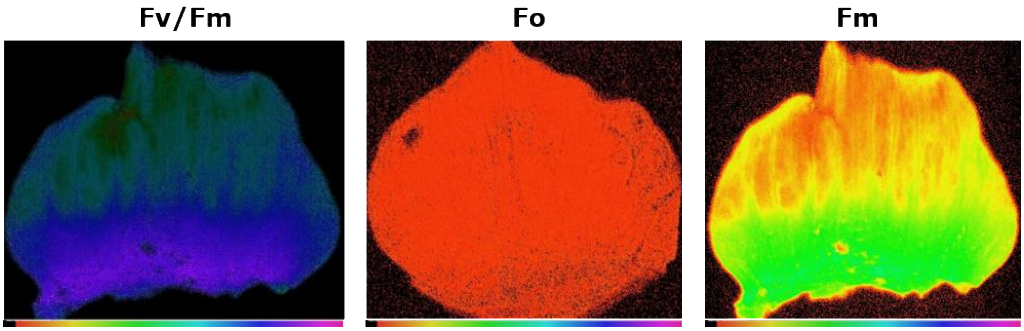

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305 **Figure 4.** Chlorophyll fluorescence image for Fv/Fm, Fo and Fm in the control
306 fruit (CTL) of the representative sepals after first harvest (H1). The false color
307 code depicted at the bottom of each image ranges from 0.000 (black) to 1.000
308 (pink).

309

310 With regard to the Fo parameter, similar behavior was detected in the three
311 measured areas. During H1, CTL and GA1 had values that came close to 0.06,
312 while GA2 obtained higher values. In the CTL fruit, a gradual decrease was
313 detected to values of 0.02 during H3. Nevertheless, no changes in Fo occurred
314 for the GA1 and GA2 fruit until H2, when its values dropped during H3 with values
315 close to 0.04. Similarly to that observed for Fv/Fm, the basal area obtained higher
316 Fm values than the middle area, and the lowest values were for the apical area.
317 In the three CTL areas, Fm drastically dropped to values close to zero during H2.
318 In the GA1 and GA2 fruit, Fm remained almost constant between H1 and H2, but
319 drastically dropped during H3.

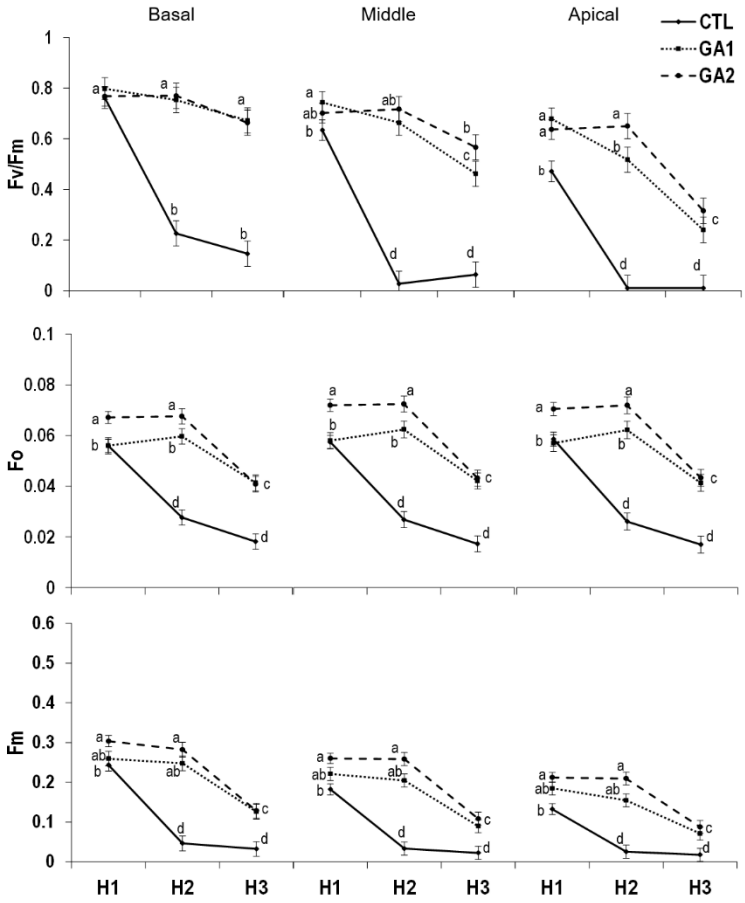

Figure 5. Chlorophyll fluorescence parameters (Fv/Fm, Fo, Fm) measured in three sepal lobe areas (basal, middle, apical) of 'Rojo Brillante' persimmons without any treatment (CTL), treated once with gibberellic acid (GA1) and treated twice with gibberellic acid (GA2) at three harvest times (H1, H2 and H3). Vertical bars represent SE intervals. Different letters in each parameter indicate statistical differences ($P \leq 0.05$).

According to the results, CFI is well suited to visualize spatio-temporal heterogeneity in sepals' responses to applied GA. Throughout harvest times, the Fv/Fm values lowered to a greater extent for the CTL sepals, which was attributed to a decline in Fm and Fo levels. Fm is related to the maximal fluorescence level when the primary acceptor electron, Q_A, maximally reduces. The decrease in Fm

observed throughout the experiment indicates that the photochemistry of PSII, and its ability to reduce Q_A, could be affected by the senescence process of sepals (Oxborough, 2004; Baker, 2008; Gorbe and Calatayud, 2012). Increased Fo in previous studies has been related to stress conditions for several plant species (Havaux, 1993; Lazár, 1999). Nevertheless, in the present study, Fo lowered as senescence advanced. This descent could reflect damage to regulatory processes of PSII, such as impairment in the photoprotective mechanism associated with energy dissipation in sepals, as previously observed in leaves by Angelopoulos et al. (1996) and Hong and Xu (1999). In addition, the Fo measurement in the IMAGING-PAM fluorimeter could imply a limitation associated with not applying far-red for its correct determination to obtain maximal Q_A oxidation. This fact could result in overestimated Fo values, even if the decreasing tendency was clear in all areas and treatments. More studies are necessary to explain this Fo behavior in persimmon sepals.

A common characteristic associated with leaf senescence is disintegration of intracellular organelles (Lim et al., 2007), in which cell membranes lose their physical integrity and photosynthetic capacity diminishes, which consequently lead to a decline in fluorescence parameters. Therefore, the higher values of the fluorescence parameters found in the sepals of the GA-treated fruit, especially for GA2, compared to the CTL fruit, can be associated with GA functions in delaying cell wall degradation and chlorophyll breakdown in leaves and fruit, as previously reported (Ben-Arie et al., 1996; Rosenvasser et al., 2006; Li et al., 2010).

Across sepal areas, the highest CF values were for the basal area, which lowered in the areas furthest away from fruit. This fact could be related to the transfer of water and nutrients from fruit to sepals, which allows photosynthetic reactions to

be maintained in the closest area to fruit. Plant exposure to the GA regulator brought about a marked increase in the photochemical efficiency of PSII in the calyx in all areas, which indicates a suppression of, or delay in, leaf senescence. Several authors have demonstrated that exogenous GA application increases Fv/Fm in pepper leaves (Ouzounidou et al., 2010), in okra (Ilias et al., 2007) and geranium (Currey et al., 2013). The application of GA has been associated with its capacity to preserve photosynthesis and to delay chlorophyll loss (Jordi et al., 1994).

Pearson correlation was used to correlate fruit firmness and color with the CF parameters (Table 2). All the studied CF parameters correlated negatively with external fruit color, while a positive correlation with flesh firmness was found. In both cases, the highest correlation coefficient was detected with Fm in all the measured sepal areas. The Fo of the basal area, and the Fv/Fm of the apical area, also correlated highly with color and flesh firmness.

Table 2. Equations and correlations between external color index (CI) or firmness and the chlorophyll fluorescence parameters (Fv/Fm, Fm, Fo) of sepals.

	Color Index (CI)		Firmness (N)	
	Equation	**r**	**Equation**	**r**
Fv/Fm mean	CI=24.22 - 14.59 × Fv/Fm	-0.767	Firmness= 26.28 + 19.61 × Fv/Fm	0.778
Fv/Fm Basal	CI=25.93 - 15.25 × Fv/Fm	-0.754	Firmness=23.80 + 20.80 × Fv/Fm	0.776
Fv/Fm Middle	CI=23.54 - 13.89 × Fv/Fm	-0.775	Firmness=27.20 + 18.70 × Fv/Fm	0.787
Fv/Fm Apical	CI=22.53 - 15.43 × Fv/Fm	-0.834	Firmness=28.73 + 20.30 × Fv/Fm	0.827
Fm mean	CI=23.87- 47.56 × Fm	-0.853	Firmness= 27.31 + 60.40 × Fm	0.817
Fm Basal	CI= 24.20 - 41.53 × Fm	-0.861	Firmness=26.90 + 52.67 × Fm	0.824
Fm Middle	CI=23.57 - 46.01 × Fm	-0.854	Firmness= 27.64 + 58.72 × Fm	0.822
Fm Apical	CI=23.21 - 54.85 × Fm	-0.840	Firmness=28.16 + 69.47 × Fm	0.802
Fo mean	CI=24.63 - 156.46 × Fo	-0.677	Firmness=25.31 + 218.54 × Fo	0.713
Fo Basal	CI=27.64 - 231.37 × Fo	-0.805	Firmness= 21.95 + 305.76 × Fo	0.803
Fo Middle	CI=26.45 - 200.04 × Fo	-0.775	Firmness=23.36 + 267.51 × Fo	0.781

Fo Apical	CI=26.27 - 198.64 × Fo	-0.766	Firmness=23.44 + 268.99 × Fo	0.782

4. Conclusion

This is the first time that a study aim was the fruit calyx senescence process during the ripening of persimmon fruit by measuring the CF parameters of sepals. Spatial-temporal heterogeneity in the CFI parameters clearly illustrated the onset of senescence and necrosis symptoms in apical sepal areas that progressed toward the basal area. The results also indicated that GA treatments, applied to delay fruit ripening, not only delayed fruit ripening, but also the calyx senescence process and improved external fruit quality maintenance.

Bearing in mind that external color and fruit firmness are the most important parameters during persimmon ‘Rojo Brillante’ harvesting, the high correlation between these physico-chemical parameters and the CF parameters indicated that the CFI measurements in sepals of calyxes could act as a potential non-intrusive tool to determine persimmon quality at harvest.

Acknowledgments

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