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Growth Dynamics and Water Potential Components of Three Summer Squash (*Cucurbita pepo* L.) Cultivars

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Abstract

Summer squash fruit is a horticultural crop that possesses a very short postharvest life due to its high rates of metabolism and transpiration along with a low cuticle resistance exhibited mainly when the fruit is harvested at horticultural maturity. This research was realized following the fruit growth of the summer squash cultivars: 'Enterprise', 'Pascola' and 'Hurakan F1', whose seeds were germinated in polystyrene trays and their seedlings were subsequently transferred to pots for optimum growth under greenhouse conditions. Fruits were sampled at 3, 5, 7 and 9 days after anthesis (DAA). Physical (weight, diameter, and length of fruit), chemical (pH, titratable acidity and total soluble solids), hydric status (water, osmotic and pressure potentials), and histological analysis were done. The highest number of fruits having marketing quality were shown in both 'Pascola' and 'Hurakan F1' cultivars at 7 DAA, whereas, in 'Enterprise' was shown at 9 DAA. Marketing quality fruits from the three cultivars showed similarities on pH (about 6.6), titratable acidity (TA) decreases in 'Enterprise' and 'Hurakan F1', whereas total soluble solids (TSS) decreases in 'Pascola' and 'Hurakan F1' ($p \leq 0.5$). From 3 to 9 DAA, in all cultivars, the water potential was close to -1.0 MPa, the osmotic potential showed an increasing pattern ranging between -1.59 and -1.15 MPa, and the pressure potential remained in the positive range. Tissue water stability was histologically related to a well-defined parenchyma tissue showing thin-walled, polygonal, intact and turgid cells during fruit growth.

Keywords: histological analysis, F1 hybrids, pressure potential, osmotic potential, summer squash genotypes

Introduction

Mexico is among the ten major squash (*Cucurbita pepo* L.) producing countries all over the world, with about 400,000 tons produced in 2013. Sinaloa State contributed with the 11.2% of the national production from which the 93.92 % were summer squash type (SIAP-SAGARPA, 2014). Squash fruit is rich in vitamins, minerals with low calorie supply (Danilchenko *et al.*, 2001). It is well known about the growth behaviour exhibited for squash fruit according to Sedano *et al.* (2005) who reported that squash fruit of 'Tala' variety (*Cucurbita pepo* L.) showed a sigmoid-like continuous growth behaviour, a high concentration of solids and good organ integrity in the stage of maximum development. However, this crop shows a high perishability and may be damaged at harvest and/or suffer rotting during postharvest, which can drastically reduce its quality and marketability period (Fornaris, 2012). Crop sensitivity during postharvest handling is closely related to the immature status at harvest (Cantwell and Suslow, 2004) providing high metabolic activity and high transpiration rate (Bafeel and Moftah, 2008). In the case of squash (*Cucurbita maxima* D.), texture is influenced by changes in water content (Sunil *et al.*, 1999). Fruit quality loss is often expressed in terms of firmness loss and appearance (wilt symptoms) along with changes in tissue water status (Brew *et al.,* 2006). Therefore, it is very

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important to take good care of the quality status during fruit development. Water status of fruit during pre-harvest, along with chemical changes has been scarcely studied in summer squashes. Beecher *et al*. (2001) stated that Ψ_p is responsible for providing turgor/freshness to the plant tissues. For such a purpose, Ψ_p must be kept higher than zero as long as possible in both pre- and postharvest. For that, summer-squash fruits must be harvested at a Ψ_p level higher than zero along with a proper fruit edible quality in order to keep turgor for longer after harvest. Hence, this work aimed in monitoring physical, chemical and water status changes (water-, osmotic- and pressure potential) of fruits for three different squash cultivars during its growth till horticultural maturity.

Materials and Methods

Materials and general crop conditions

Experiments were carried out in greenhouses at "Centro de Investigación en Alimentación y Desarrollo, A.C, Unidad Culiacán" (CIAD) in 2012. Three summer squash cultivars were studied: 'Enterprise', 'Pascola' and 'Hurakan F1'. The 'Enterprise' cultivar whose seeds were purchased from Syngenta is a yellow coloured straightneck type; 'Pascola' cultivar (seeds from Syngenta) is a dark green coloured zucchini type, whereas 'Hurakan F1' is a gray zucchini type according with the supplier (Harris Moran), with very similar characteristics to the green-grayish coloured vegetable-marrow type according with the classification of Paris (1986). The three summer squash hybrid seeds varieties were cultivated under controlled environmental conditions (average temperature: 19.8 °C and RH: 64.8 %) and a drip irrigation system. At the beginning of pollination, female flowers were labeled for development tracking. During growth, five fruits were randomly and manually collected at 3, 5, 7 and 9 days after anthesis (DAA) and measured in length (mm), diameter (mm) and weight (g) for each squash cultivar. In the same manner, pH, titratable acidity, total soluble solids, water- (Ψ_w), osmotic- (Ψ_s) and pressure potential (Ψ_p) were determined. Finally, a histological analysis was also performed by observing fruit tissues (epicarp and mesocarp) with optical microscopy.

Dynamics of crop growth

Squash seeds were placed them in 128-hole polystyrene trays and incubated in a climatic chamber Barnstead Iowa 52001 (USA) at 25 ± 1ºC during eight days until reaching 85% of germination. Afterwards, the seedlings were placed in a high technology greenhouse for 17 days. When plants showed three true leaves they were immediately planted in a composed substrate of coconut fiber and perlite (75:25) in two rows of 30 plants for each hybrid. The nutrient solution consisted of 13.0 Meq NO_3, 1.5 H_2PO_4, 7.0 SO_4, 7 K, 9.0 Ca and 4.0 Mg, properly applied by the drip irrigation system every 40 minutes at a rate of 2 Liters per day, with an electrical conductivity of 2 dS m^{-1}, at 64.8% RH (average) and 19.8 °C. Pollination was enhanced by releasing bees (*Apis mellifera*) into the greenhouse. The fruit collection was started at three days after anthesis for growth dynamic measurement.

Chemical analysis

The pH, TA and TSS were determined according to AOAC (2000). Ten grams of the sample were blended in 50 ml of distilled water and filtered through an organza cloth. The pH and titratable acidity were measured employing an automatic titrator Metler Modelo T-50 (Mexico) using 50-mL aqueous extracts from the fruit samples. Firstly, pH was read and thereafter sample were titrated with 0.1 N sodium hydroxide until reaching a pH= 8.2. TA data were reported in % of malic acid. TSS were analyzed in filtered drops using a refractometer ABBE Leyca Mark II (Buffalo, NY) and expressed in Brix degrees.

Water (Ψ_w), Osmotic (Ψ_s) and pressure (Ψ_p) potential

The Ψ_w of the tissue was measured using the constant volume method (Shibairo *et al*., 1997). Three fruits were selected and four cylinders of mesocarp tissue (20x25 mm) were obtained, from each fruit. The cylinders were initially weighed and then immersed in four sucrose solution (0.10, 0.20, 0.30 and 0.40 Molal) for 110 minutes. Subsequently, the excess of water on tissue surface was removed from all samples using paper towels and weighed again on a digital scale AND GFH2000. All data were reported in terms of percentage of weight loss or weight gain because of all samples currently show some variation respect to their initial weight due to the differences in Ψw between samples and sucrose solutions. Weight loss or weight gain versus Ψs was registered and the intersection at zero (neither weight gain nor weight loss) was determined as well. Previously, the Ψ_s for all sucrose solutions were calculated using the following equation:

$$\Psi_{s=}-CiRT$$

Where:
C = molal concentration
i = ionization constant
R= gas constant (0.00831 kg Mpa mol^{-1} $°K^{-1}$)
T= temperature (in °K);

Since $\Psi_w = \Psi_s + \Psi_p$, and because of Ψ_p for a solution at atmospheric pressure is zero, then for the sucrose solutions $\Psi_s = \Psi_w$ and according to this, Ψ_w of the sample is known when neither gain nor loss of weight is observed on samples. In this point the Ψ_s of the solution correspond to Ψ_w of the samples (Salisbury and Ross, 2000).

The direct Ψ_s of the tissue was determined using a vapor pressure osmometer (5520 Wescor® USA) following Turner's methodology (1981). Ten µL of fruit sap was added in filter paper (0.32 cm^2) and placed it in a reception chamber. All sap was obtained from frozen tissue samples (- 20 °C) which were defrosted at room temperature in order to get cell-wall rupture and set the turgor potential to zero. The calibration of the equipment was done with osmotic potential solutions of 290, 1000 and 100 mmol·kg^{-1} of NaCl (results were expressed in mmol·kg^{-1}). Conversion of molality to osmotic potential was carried out using the Van't Hoff equation: $\Psi_{s=} -CiRT$, (Salisbury and Ross, 2000), where the ionization constant, i, is 1.8 for NaCl. The Ψ_p was calculated according to Turner (1981), as the difference among water potential and osmotic potential ($\Psi_p = \Psi_w - \Psi_s$). All these variables were analyzed in freshly cut fruits at 3, 5, 7 and 9 DAA.

Histological analysis

For squash tissue fixation, 1-cm^3 pieces of pericarp (mesocarp with epicarp) were cut, and placed them in a jar with 50 mL of FAA fixative solution (500 mL ethanol 96%, 50 ml acetic acid,

100 ml formaldehyde and 350 ml of water). Afterwards, samples were rinsed with tap water, and dried with alcohol and xylene solutions at different concentrations. Dehydrated tissues were embedded in paraffin and finally, sections of 10 μm thick were made with a rotary microtome LEICA RM 2125 RT China. All sectioned samples were placed in glass microscope slides using adhesive Haupt (1% gelatin, 13% glycerol, 2% phenol and water). Furthermore, they were stained with safranin and solid Green dyes, covered with a coverslip and mounted with Canadian balsam for getting permanent preparations (Osuna *et al*., 2008). At last, cuts were observed in optical microscope 1122-100 Zeiss ® Axiostar Mexico. Cell development was followed in epicarp and mesocarp tissues.

Experimental design

Experiments were conducted under a two-factor randomized design (time and type of squash). Time was set at four levels: 3, 5, 7 and 9 DAA and the type of squash were the cultivars: 'Enterprise', 'Pascola' and 'Hurakan F1'. Means were subjected to comparison according to the Tukey mean separation test ($\alpha = 0.05$) (MINITAB 2004, version 14.0).

Results and Discussion

Dynamics of crop growth

All of the studied cultivars reached horticultural maturity in 42-58 days after sowing (DAS). The whole period to achieve horticultural maturity was composed by a germination stage of 8 days, a vegetative growth stage of 27-35 days and a fruit growth stage of 7-9 days. The fruit growth (measured by weight, length and diameter) showed a sigmoidal behaviour (Fig. 1). Lin and Varner (1991) reported that the growth of a typical zucchini fruit showed a typical sigmoid growth pattern when measured both circumference and length against time. Salisburi and Ross (2000) reported that a sigmoid growth curve is exhibited by numerous annual plants or individual parts of plants and that fruit growth is made up by consecutive stages: cell division, cell elongation and seed formation. Also, Sedano *et al.* (2005) reported a sigmoidal behaviour in dry matter accumulation for the organs and the whole plant of *Cucurbita pepo* L. (hybrid 'Tala'). Furthermore, in a growth dynamic study for 8 hybrids of 'gray zucchini', Lopez *et al.* (2011) reported a sigmoidal pattern for the growth of the whole plants for all hybrids. In this study, 'Pascola' and 'Hurakan F1' showed their greatest size at 7 DAA, whereas the 'Enterprise' cultivar showed it at 9 DAA (Fig. 1). 'Enterprise' cultivar showed a slightly earliness in full flowering and fruit development initiation but commercial size was achieved two days later than 'Pascola' or 'Hurakan F1'. The highest number of fruits per plant having marketing quality was shown at 7 DAA in 'Pascola' and 'Hurakan F1' cultivars, whereas at 9 DAA for 'Enterprise' (Data not shown). All cultivars developed a cylindrical shape with diameters around 46 mm and length around 168 mm in their horticultural maturity (Fig. 1). In the study of growth dynamic for the 8 hybrids of 'gray zucchini', Lopez *et al.* (2011) reported a value of 125 mm reached in 5-7 DAA, as the greatest fruit length for all the studied materials ('Terminator', 'Lolita', 'WA9041', 'Tala', 'Dolarzini', 'Hurakan', among others). This value of fruit length resulted shorter than any of the cultivars of this study which ranged from 142 to 173 mm. In relation to fruit diameter, the gray-zucchini hybrids reached similar values to data of this study at 7 DAA, all of them around 44 mm. All of the three cultivars exhibited their completely flowering period from 38 to 52 DAS. This reproductive period was clearly made up by three phases based on the predominance of flower sex: The first, with the highest number of masculine flowers (about 80%), followed by a phase of uniformity in percentage (50:50), and at the end the female flowers predominated (about 80%). The flowering initiation was at the 38, 38 and 40 DAS for 'Enterprise', 'Hurakan F1' and 'Pascola', respectively, whereas the full flowering was at 40, 42 and 43 for 'Enterprise', 'Hurakan F1' and 'Pascola', respectively. The flowering initiation is the number of days elapsed after sawing when the 50% of total number of plants plus one exhibits at least one flower, whereas full flowering is when the 100% of plants had developed at least one flower).

Chemical characteristics

At horticultural maturity (HM), all of the studied cultivars showed similar levels of pH, ranging between 6.64 and 6.67. TA was lower ($p \leq 0.05$) for 'Enterprise' (0.043%) versus 'Pascola' (0.064%) or 'Hurakan F1' (0.061%). However, TA was not different between 'Pascola' and 'Hurakan F1' (Table 1).

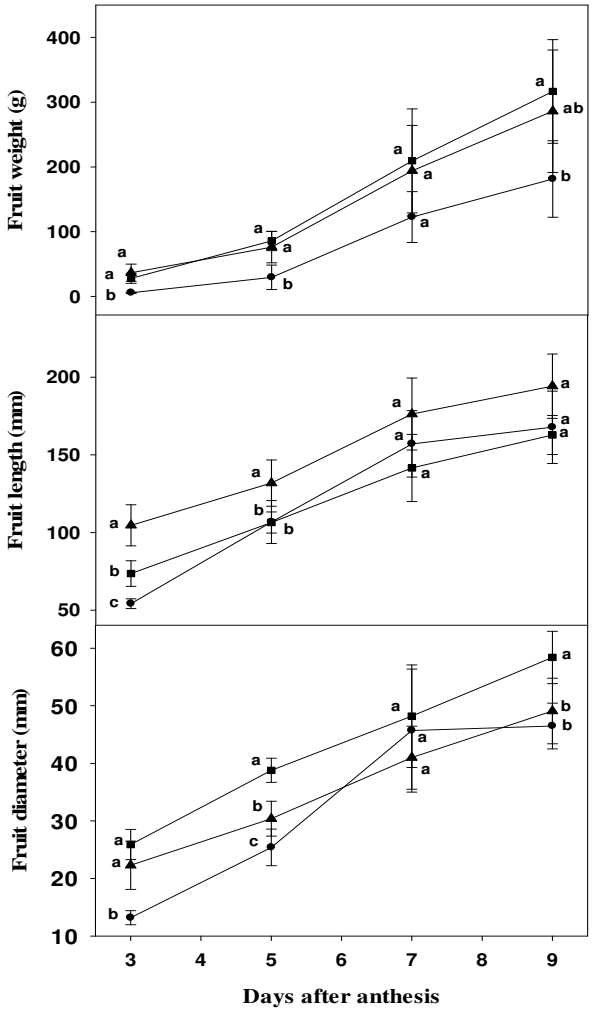

Fig. 1. Fruit weight, length and diameter in three summer squash cultivars during growth

'Enterprise' (●), 'Pascola' (▲) and 'Hurakan F1' (■). Data are means of five replicates ± standard deviation. Different letters among cultivars denote significant differences (Tukey test, $P < 0.05$).

Table 1. Physicochemical characteristics of three summer squash cultivars

Cultivar	Days after anthesis (DAA)	pH	Titratable Acidity	Total soluble solids, in °Brix
'Enterprise'	3	$6.62^{a,x} \pm 0.07$	$0.071^{a,y} \pm 0.003$	$5.40^{a,x} \pm 0.01$
	5	$6.60^{a,x} \pm 0.03$	$0.071^{a,x} \pm 0.003$	$5.55^{a,x} \pm 0.30$
	7	$6.64^{a,x} \pm 0.05$	$0.049^{b,x} \pm 0.006$	$4.95^{a,x} \pm 0.30$
	9 (HM)	$6.67^{a,y} \pm 0.04$	$0.043^{b,x} \pm 0.005$	$4.50^{a,x} \pm 0.60$
'Pascola'	3	$6.79^{a,y} \pm 0.02$	$0.062^{a,x} \pm 0.004$	$5.70^{a,x,y} \pm 0.35$
	5	$6.79^{a,y} \pm 0.02$	$0.062^{a,y} \pm 0.003$	$5.70^{a,x} \pm 0.35$
	7 (HM)	$6.64^{a,x} \pm 0.06$	$0.064^{a,y} \pm 0.004$	$4.65^{b,x,y} \pm 0.30$
	9	$6.63^{a,y} \pm 0.02$	$0.049^{b,x} \pm 0.003$	$3.60^{b,y} \pm 0.01$
'Hurakan F1'	3	$6.75^{a,y} \pm 0.01$	$0.072^{a,y} \pm 0.003$	$6.15^{a,y} \pm 0.30$
	5	$6.77^{a,y} \pm 0.05$	$0.046^{b,z} \pm 0.002$	$5.70^{a,x} \pm 0.35$
	7 (HM)	$6.65^{a,x} \pm 0.03$	$0.060^{a,xy} \pm 0.005$	$4.05,^{b,y} \pm 0.30$
	9	$6.56^{b,x} \pm 0.03$	$0.044^{b,x} \pm 0.005$	$3.45^{b,y} \pm 0.30$

Different lowercase letters (a, b) between DAA for each cultivar denote significant differences (Tukey test, $P < 0.05$).
Different lowercase letters (x,y,z) between cultivars for the same DAA denote significant differences (Tukey test, $P < 0,05$).
HM = Horticultural maturity

Total Soluble Solids (TSS)

At horticultural maturity, TSS showed similar levels ranging from 4.05 to 4.65 °Brix, among cultivars. These chemical properties can be described as it was reported by Kader (2002) for fruits harvested at horticultural maturity. He mentioned that these fruits possess chemical characteristics close to neutrality and are low in sweetness. Derossi *et al.* (2011) reported approximate values for pH (6.42 ± 0.16) and for TSS (4.8 °Brix) from a zucchini-type squash. On the other hand, it seems that although with similar pH values, pumpkins or winter squashes are sweeter than zucchini type judging by the higher °Brix in their fruits. Zinash *et al.* (2013) reported for 20 accessions of fresh pumpkin grown in Ethiopia, a pH of 6.64 ± 0.32, and a TSS of 6.7 ± 1.5 °Brix. In the same manner, Valenzuela *et al.* (2011) reported for a Winter squash (*Cucurbita moschata* D.) a pH of 6.77 ± 0.7, a TA of 0.04 ± 0.04 and TSS of 6.4 ± 2.2. The difference in TSS is believed to be due to differences in the developmental stages at harvest between summer and winter squashes. The harvest stage for winter squashes is carried out at their fully mature fruit stage at which TSS reaches its maximum potential, whereas zucchini type squashes are harvested at horticultural maturity, which is an immature fruit stage of development. During fruit growth, pH kept constant for the three cultivars. TA decreases in 'Enterprise' and 'Hurakan F1', whereas TSS decreases in 'Pascola' and 'Hurakan F1' ($p \leq 0.5$).

Water-, pressure- and osmotic potential

The Ψ_w was measured during fruit growth at 3, 5, 7 and 9 DAA. The horticultural maturity was reached at 7 DAA for both 'Pascola' and 'Hurakan F1', and at 9 DAA for 'Enterprise'. The Ψ_w for all the cultivars was kept with no significant changes during fruit growth measured until harvest at horticultural maturity (Fig. 2). Ψ_w oscillated around -0.99 MPa whereas Ψ_p showed always positive values, however its tendency was decreasing since the beginning, lowering from 0.59 to 0.15, from 0.49 to 0.27 and from 0.41 to 0.19 MPa for, 'Enterprise', 'Pascola' and 'Hurakan F1', respectively. Beecher *et al.* (2001) stated that Ψ_p is responsible for providing cell shape and tissue stiffness due to the pressure that water exerts on cell walls. However, Ψ_p must be kept higher than zero as long as possible in both pre- and postharvest in order to keep tissue-freshness/turgor for longer. Irremediably, plant tissues lose weight in postharvest storage and Ψ_p tends to drop under zero. Urias *et al.* (2012) reported cell plasmolysis (Ψ_p less than zero) when squashes had lost 6% of their weight after the third day of storage at 20 °C and at the sixth day of storage at 10 °C. On the other hand, Ψ_s showed an increasing pattern rising from -1.59 to -1.15, from -1.49 to -1.27, from -1.41 to - 1.19 MPa, for 'Enterprise', 'Pascola' and 'Hurakan F1', respectively. This behavior in Ψ_s suggests that the supply of water and solids from the plant (source) to the fruit (sink) exhibited an increased water/solids ratio during fruit growth, The increased Ψ_s along with the decreasing TSS levels during fruit growth can be seen as a cell solute dilution due to an increased water absorption by the cell and the concomitantly cell growth by enlargement as suggested by Bidwell (1979). The decreasing Ψ_p is also explainable by cell enlargement. When harvested at horticultural maturity, all the cultivars showed a turgid texture with Ψ_p, of 0.15, 0.27 and 0.19 for 'Enterprise', 'Pascola' and 'Hurakan F1', respectively. 'Pascola' and 'Hurakan F1' cultivars were evaluated at 9 DAA (2 days after horticultural maturity) and it was found that Ψ_p decreased slightly in 'Pascola' (reaching 0.21) with respect to 7 DAA, whereas in 'Hurakan F1', Ψ_p decreased approximating to zero (0.06). These latter results suggest that 'Pascola' fruits might be harvested at 9 DAA instead of at 7 DAA provided that quality characteristics (less TSS) do not become a commercial drawback. Harvesting 'Hurakan F1' at or after 9 DAA must be avoided. A Ψ_p higher from zero at harvest is also desirable in summer squash due to its high susceptibility to suffer weight loss and shriveling on postharvest storage according to Sherman *et al.* (1985).

Histology

Fruit ground tissue for all cultivars at all growth stages showed a typical parenchyma tissue. All samples showed thin-walled, polygonal, intact and turgid cells. However, cells from all cultivars at 3 DAA were smaller than at 7 DAA. This clearly suggest that mesocarp cells at 3 DAA were at their proliferation stage (in cell division) and afterwards the cells experienced enlargement, as can be seen at 7 DAA for all cultivars. A similar behaviour was reported by Higashi *et al.* (1999) for two melon genotypes. In this study the cell enlargement was concomitant with a drop in Ψ_p and an increase in Ψ_s, whereas Ψ_w kept unchanged. Although Ψ_p exhibited a drop, its level was always kept in the positive range allowing the tissue to stay turgid at harvest and not so close to zero at which incipient plasmolysis is presented in plant tissues. From Fig. 3 is also clear that cells outward the mesocarp were smaller than cells inward. Epicarp was clearly thinner at 3 DAA than at 7 DAA in 'Enterprise' and 'Pascola' cultivars.

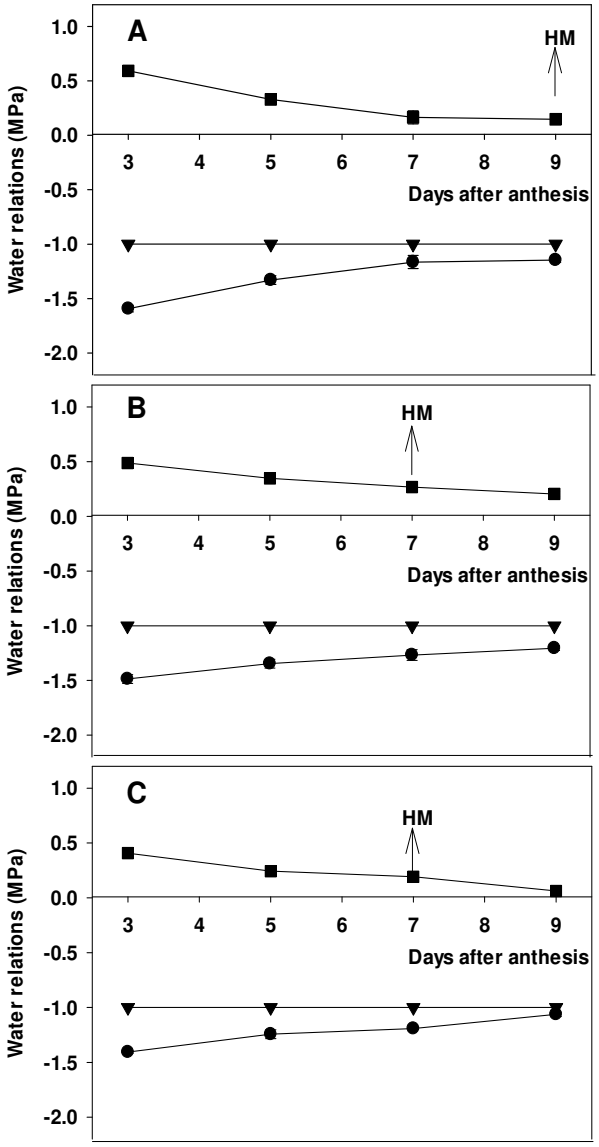

Fig. 2. Water (▼), osmotic (●) and pressure potential (■) for three summer squash cultivars during growth: 'Enterprise' (A), 'Pascola' (B) and 'Hurakan F1' (C). HM = Horticultural maturity.

Conclusions

'Enterprise' cultivar showed a slightly earliness in full flowering and fruit development initiation but its commercial size was achieved later than 'Pascola' and 'Hurakan F1' (after 2 days). However, since 'Enterprise' showed unchanged levels in pH, TA, TSS and pressure potential during the period from 7 to 9 DAA, these results suggest that 'Enterprise' fruits might be harvested at 7 DAA instead of at 9 DAA provided that smaller fruit sizes do not become a commercial drawback. All of the three cultivars showed a sigmoidal pattern of growth mainly when measured by whole fruit weight. Chemical (mainly TSS) and physicochemical (Y_p) variables decreased concomitantly to the increment in osmotic potential, cell enlargement and fruit size suggesting a dilution of solutes into tissue cells during fruit growth.

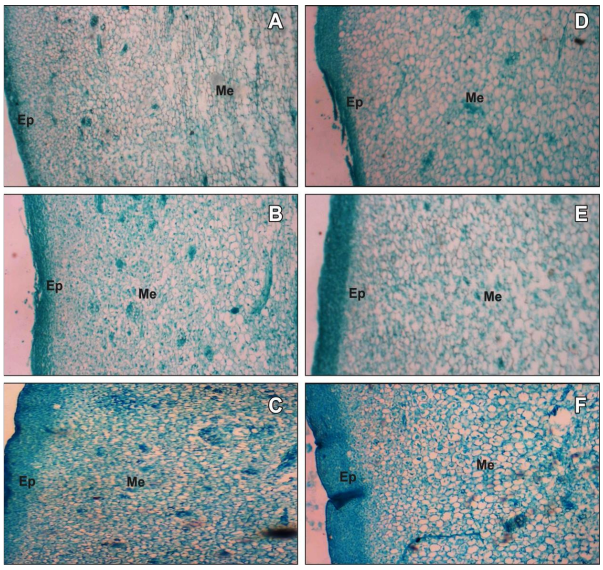

Fig. 3. Fruit transect microphotography for 'Enterprise' (top) 'Pascola' (middle) and 'Hurakan F1' (bottom) summer squash cultivars at 3 (A, B, C) and 7 (D, E, F) days after anthesis. Epicarp (Ep), Mesocarp (Me). Focus: 4X= 40 increases

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