Yield and fruit quality response of sweet pepper genotypes grown under soilless cultivation

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Running title: <mark>Response of pepper genotypes to soilless cultivation</mark>

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1
2 **ABSTRACT**

3 Total yield, physical and phytochemical characterization of three yellow and five red-

4 colored sweet pepper genotypes were analyzed under fully controlled environmental

5 and irrigation conditions under soilless culture. Results showed both greater fruit

6 firmness and pericarp thickness in the red-colored genotypes than in the yellow ones.

7 However, not significant differences between these two colors were found for total

8 yield, shape index and dry matter percentage. Additionally, peroxidase activity, total

9 protein and total phenolic compounds were not modified according with the color of the

10 genotype. With respect to the genotypes studied, Cierva, A67, Traviatta, Cabezo and

11 Limona showed the highest yields in the "extra" fruit category whilst Disco and Zar

12 showed the lowest. Additionally, Cierva and Cabezo showed higher protein

13 concentration and peroxidase activity than any other genotype.

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INTRODUCTION

Sweet peppers traditionally have been field-grown and harvested at a mature-green stage. However, there is an increasing consumer demand for high-quality colored pepper (Shaw and Cantliffe, 2002) and concern regarding environmental issues in crop production. Sweet pepper farmers often use soilless cultivation to achieve higher yields and fruit quality, minimizing environmental pollution. The reasons imposing a switch over to hydroponics are increasingly associated with environmental policies. In particular, the recycling of greenhouse effluents in closed hydroponic or soilless systems enables a considerable reduction of fertilizer application, and a drastic restriction or even a complete elimination of nutrient leaching from greenhouses to the environment (Savvas and Passam, 2002). This issue is very important, especially for nitrate and phosphate leaching to surface and groundwater resources (eutrophication) (Martinez et al., 2005). In addition, the upcoming elimination of methyl bromide (soil disinfectant) may lead the sweet pepper industry into hydroponic greenhouse production. Additionally, this technique restricts costs and increase profitability, as it is based increasingly on automation of nutrient and water supply (Savvas, 2003). Nowadays, new sweet pepper genotypes (red or yellow-colored) are in the market to accomplish consumer demand, but not all these new genotypes have been studied under soilless systems and fully controlled environmental and nutritional conditions.

Red and yellow sweet peppers are commonly available in markets where the color of the fruit is the major factor associated with the consumers´ purchasing decisions (Sun et al., 2007). Fresh peppers are an excellent source of vitamins as well as neutral acidic phenolic compounds, important antioxidants involved in a variety of plant defense responses (Howard et al., 2000). These compounds can vary to a large extent

1   according to the genotype (Mejía et al., 1988; Ruiz et al., 2006) or cultivation method

2   (Gómez et al., 1998; Chatterjee and Chatterjee, 2005; del Amor, 2007). Phytochemicals

3   are bioactive compounds ubiquitous in plants; they are mainly secondary metabolites

4   and their occurrence in plants is considered to be the result of natural adaptation and

5   selection via complex, co-evolutionary processes (Wink, 2003). Phytochemicals have

6   also a protective role in human health (Heber, 2004; Meskin et al., 2004). Therefore, it

7   is important to know both the potential fruit yield and the phytochemical response of

8   different genotypes in soilless media. Therefore, the aim of this study was to determine,

9   with full control of plant nutrition and environment, yield and fruit quality parameters

10   for sweet pepper genotypes under soilless cultivation management.

11

12   **MATERIALS AND METHODS**

13   Commercial pepper cultivars (*Capsicum annuum* L.) were grown in the same

14   polyethylene greenhouse at San Javier, Murcia (Spain). Plants were transplanted on 18

15   January 2006, from a commercial nursery. All varieties received the same amount of

16   water and fertilizers. Fertilizer composition in the irrigation water had the following

17   composition in mmol L^{-1}: 12 NO_3^-, 1.8 $H_2PO_4^-$, 5.5 K^+, 5.4 Ca^{2+}, and the appropriate

18   micronutrient concentrations. The greenhouse had 24 lines with 22 bags (containers)

19   filled with coconut coir fiber; each bag had three plants with 3 self-compensating 4 L h^{-1}

20   drip emitters. Irrigation scheduling was based on plant need, to achieve *ca.* 30% daily

21   leaching from the container to avoid both nutrient imbalance and high salinity in the

22   root zone (del Amor and Gómez-López, 2009).

23   Climate parameters (temperature, humidity, and atmospheric CO_2 concentration)

24   inside the greenhouse during the crop season were monitored with a climate station

25   placed inside the greenhouse. Peppers were harvested at full maturity; depending on the

1   genotype, this meant either red or yellow fruit. Yellow pepper genotypes analyzed were

2   cvs. Cierva (Seminis), Disco (Western Seed), and Limona (Syngenta Seeds) and the red

3   pepper genotypes were Cabezo (Syngenta Seeds), Coyote (Syngenta Seeds), Traviatta

4   (Rijz-waan), Zar (Z-Seeds), and A67 (Gautier). Each genotype was grown in 16 bags

5   with a total of 48 plants, with a randomized distribution in 4 blocks inside the

6   greenhouse.

7

8   *Yield and agronomical fruit quality*

9   Marketable fruit quality was determined individually from each plant front each block.

10   Each substrate bag (with three plants) was considered a replicate. Fruit harvesting was

11   performed weekly at the fully-mature, yellow stage of ripening. All fruits from plants

12   were counted, weighed, and graded according to marketable standards (del Amor,

13   2007). Marketable characteristics for California peppers were defined as: *Extra*:

14   uniform color, good health state, square shape, and weight >190 g. *I class*: uniform

15   color, good health state, non-square shape, and weight >225 g; *II class*: uniform color,

16   good health state, non-square shape, and weight of 224-170 g; *III Class*: uniform color,

17   good health state, non-square shape, and weight of 100-170 g; *Non-marketable*: the

18   remaining fruits – rotten fruits with more than 20% of their surface affected by blossom

19   end-rot (BER) or lighter than 100 g.

20

21   *Physical fruit parameters*

22   Fruit firmness was determined on fruit with intact skin in the equatorial region, using a

23   Bertuzzi FT011 penetrometer fitted with an 8-mm-diameter probe. Pericarp thickness

24   was determined from 10 fruits per genotype from the average of three determinations in

25   the apical, equatorial, and basal parts of the fruit. Shape index was calculated as the

1   ratio of maximum height to width. Fruit color was determined with a Konica-Minolta

2   CR-300 colorimeter, with three measurements along the equatorial perimeter. Color

3   data are provided as CIELab coordinates, which define the color in a three-dimensional

4   space: $L*$ indicates lightness and $a*$ and $b*$ are the chromaticity coordinates, green-red

5   and blue-yellow coordinates, respectively. $L*$ is an approximate measurement of

6   luminosity, which is the property according to which each color can be considered as

7   equivalent to a member of the grey scale, between black and white, taking values within

8   the range 0-100: $a*$ takes positive values for reddish color and negative values for

9   greenish ones, whereas $b*$ takes positive values for yellowish color and negative values

10   for bluish ones.

11   $C*$ is chroma [$C* = \sqrt{\left(a*^2\right) + \left(b*^2\right)}$], being 0 at the centre of a color sphere and

12   increasing according to the distance from the center. Finally, h_{ab} is the hue angle

13   [$h_{ab} = arc\ tg\left(\dfrac{b*}{a*}\right)$], which is defined as starting at the $+a*$ axis and is expressed in

14   degrees; 0º would be $+a*$ (red), 90º would be $+b*$ (yellow), 180º would be $-a*$ (green),

15   and 270º would be $-b*$ (blue). The color analyses were run for at least 3 replicates.

16

17   *Peroxidase activity*

18   Pepper peroxidase was extracted and partially purified using the method described by

19   our group in 2003, but with some modifications (Nuñez-Delicado et al., 2003). Fresh

20   peppers were washed and the seeds and peduncle were removed. A 50-g sample was

21   homogenized with 100 ml of sodium phosphate buffer (pH 7.3) for 5 min in an

22   Ultraturrax. Ten milliliters of the homogenate were used for the extraction of phenolic

23   compounds. The rest of the homogenate was filtered through four layers of cheesecloth.

24   This filtrate was subjected to temperature-induced phase partitioning by adding TX-114

1 at 4 ºC so that the final detergent concentration was 4% (w/v). The mixture was kept at

2 4 ºC for 15 min. and then warmed to 35 ºC for 15 min. At this time, the solution became

3 spontaneously turbid due to the formation, aggregation, and precipitation of large,

4 mixed micelles of detergent, which contained hydrophobic proteins and phenolic

5 compounds. This turbid solution was centrifuged at 10,000 g for 15 min, at 25ºC. After

6 discarding the pellet and detergent-rich phase, the clear detergent-poor supernatant

7 which contained the soluble persimmon PPO, was brought to 30% saturation with

8 $(NH_4)_2SO_4$ under continuous stirring at 4ºC. After one hour, the solution was

9 centrifuged at 60,000 g for 30 min, at 4ºC, and the pellet was discarded. $(NH_4)_2SO_4$ was

10 added to the clear supernatant to give 80% saturation and the mixture was stirred for 1

11 hour at 4ºC. The precipitate obtained between 30% and 80% was collected by

12 centrifugation at the same rotor speed and dissolved in a minimum volume of 100 mM

13 sodium phosphate buffer, pH 7.3. The salt content was removed by dialysis and the

14 enzyme stored at –20ºC.

15   The peroxidase activity was followed spectrophotometrically in a Shimadzu

16 model UV-1603 spectrophotometer at the absorption maximum of the ABTS radical

17 cation, 414 nm ($\varepsilon_{414}= 31.1$ M^{-1}cm^{-1}) (Rodriguez-López et al., 2000). One unit of enzyme

18 was defined as the amount of enzyme that produced 1 µmol of ABTS radical per

19 minute. The standard reaction medium at 25 ºC contained 1 ng/mL of partially purified

20 peroxidase, 50 mM sodium citrate buffer (pH 4.5), 1 mM ABTS, and 6 mM H_2O_2, in a

21 final volume of 1 mL.

22 *Protein content*

23 The total protein content was determined according to Bradford's dye binding method,

24 using bovine serum albumin (BSA) as a standard (Bradford, 1976). Analyses were done

25 in triplicate for each sample.

1   *Total phenolics*

2   Total phenolics were determined in 80% ethanol using the Folin-Denis method (Kidron

3   et al., 1978). Analyses were done in triplicate for each sample, using chlorogenic acid

4   for the quantification.

5   *Statistical analyses*

6   The design was a fully randomized block with sixteen replicates and four blocks per

7   treatment. Guard rows were placed between treatments and at both ends. All data were

8   analyzed for significant differences by one-way analysis of variance (ANOVA) and

9   Duncan's multiple range test at $P<0.05$ using the SPSS v. 12.0 statistical package.

10

11   **RESULTS AND DISCUSSION**

12   *Total yield and agronomical fruit quality*

13   The yellow-colored Limona and Cierva and the red-colored Cabezo genotypes showed

14   the highest total yield with 8.36, 7.70, and 7.74 kg m^{-2}, respectively (Table 1),

15   significantly higher than the Coyote genotype (5.72 kg m^{-2}). With respect to the most

16   economically valuable category (Extra class), Cierva, Limona, A67, Traviatta, and

17   Cabezo showed significantly higher extra class yields compared with genotypes Disco

18   and Zar. Especially remarkable was the difference for the yellow genotypes: Cierva

19   produced three times more than genotype Disco in this fruit category. In general, no

20   significant difference was found when comparing yields of plants grouped according to

21   fruit color (Table 1).

22

23   *Fruit physical parameters*

24   Red-colored genotypes showed both greater fruit firmness and pericarp thickness than

25   the yellow-colored genotypes (Table 2). Thus, fruit firmness was almost double (3.36

1   kg^{-1} compared to 6.23 kg^{-1}) for the red genotypes with respect to the yellow ones. The

2   red-colored Coyote and Traviatta genotypes showed the highest fruit firmness, whilst

3   there were no significant differences among the three yellow-colored genotypes (Table

4   2). Red-colored fruits showed an increase of 1.36 mm in the pericarp thickness

5   compared with the yellow fruits. But, no significant differences were found for fruit size

6   (length, width, or shape index) or the dry matter percentage between fruits of these two

7   genotypes. Obviously, each color group showed significant differences for L*

8   (luminosity), a* (reddish to greenish ones), and b* (yellows to bluish ones) color

9   parameters, that consequently affected the C$_{ab}$ (chroma) or h$_{ab}$ (hue angle), But

10   considering each genotype individually, Limona showed the highest luminosity whilst

11   A67, Coyote, and Zar showed the lowest for yellow and red genotypes respectively

12   (Table 2).

13

14   *Peroxidase activity, total protein, and total phenolic compounds*

15   Peroxidase is an oxidoreductase that catalyzes a reaction in which hydrogen

16   peroxide acts as the acceptor and another compound acts as the donor of hydrogen

17   atoms (Adams, 1978; Whitaker, 1994; Rodrigo et al., 1996). It is involved in plant

18   hormone regulation (Greppin et al., 1986), defense mechanisms (Hammerchmidt et al.,

19   1982), indoleacetic acid degradation during maturation and senescence of fruits and

20   vegetables (Brooks, 1986), and lignin biosynthesis (Gross, 1980). Because of its

21   multiple functions, the enzyme is commonly found as several isoenzymes in plants. In

22   the presence of H$_2$O$_2$, peroxidases from plant tissues are able to oxidize a wide range of

23   phenolic compounds (Onsa et al., 2004). Oxidation of a wide range of organic

24   compounds has led to the speculation that the enzyme may be associated with the losses

25   in color, flavor, and nutritional value of raw and processed foods (Nebesky et al., 1950;

1 Bruemmer et al., 1976; Kampis et al., 1984; Robinson, 1987). This enzyme is also of

2 concern to food processors because of its high thermostability. So, peroxidase is used

3 commonly as an index of the adequacy of fruit and vegetable blanching, due to its high

4 concentration in most plant tissues, its high thermal stability, and its ease of assay (Lu

5 and Whitaker, 1974; Anthon and Barrett, 2002).

6 Peroxidase of sweet pepper fruit was extracted using Triton X-114 and

7 quantified using ABTS as a donor of hydrogen atoms. For the studied pepper genotypes,

8 the peroxidase activity was significantly higher in Cierva and Cabezo, whist Coyote,

9 Traviatta, and Zar showed the lowest values (Figure 1). When the protein content of

10 peppers was analyzed, it was shown that the highest values corresponded to those fruits

11 that also had significantly higher levels of peroxidase activity. Additionally, Cierva and

12 Cabezo genotypes, that showed high levels of both protein and peroxidase, had a

13 tendency to reduce their total content of phenolic compounds, especially in Cierva

14 (Figure 1). This decrease in phenolic compounds may be due to the high oxidative

15 activity of peroxidase. Other genotypes did not show a clear and defined relationship

16 between these parameters. Chu et al. (2002) and Sun et al. (2007) found that total

17 phenolic content and total antioxidant activity in red peppers are at the top end of the

18 range, among several vegetable species. Additionally, Sun et al. (2007) also found no

19 differences for yellow- or red-colored peppers, which agrees with the data from this

20 study.

21
22 **ACKNOWLEDGEMENTS**

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2   phylogenetic perspective. *Phytochemistry* 64: 3–19.

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4

1 **Figure captions**

2

3 *Figure 1*. Enzymatic activity of peroxidase, total protein, and total phenolics

4 concentration (mg^{-1}FW) in sweet pepper fruits: yellow-colored (CI: Cierva; DI: Disco;

5 LI: Limona) or red-colored genotypes (CA: Cabezo; CO: Coyote; TR:Traviatta; ZA:

6 Zar; AG: AG67). The main genotype effects were analyzed statistically by Duncan´s

7 multiple range test at the 0.05 level. Means of each color or genotype followed by the

8 same letter are not significantly different. ns: not significant

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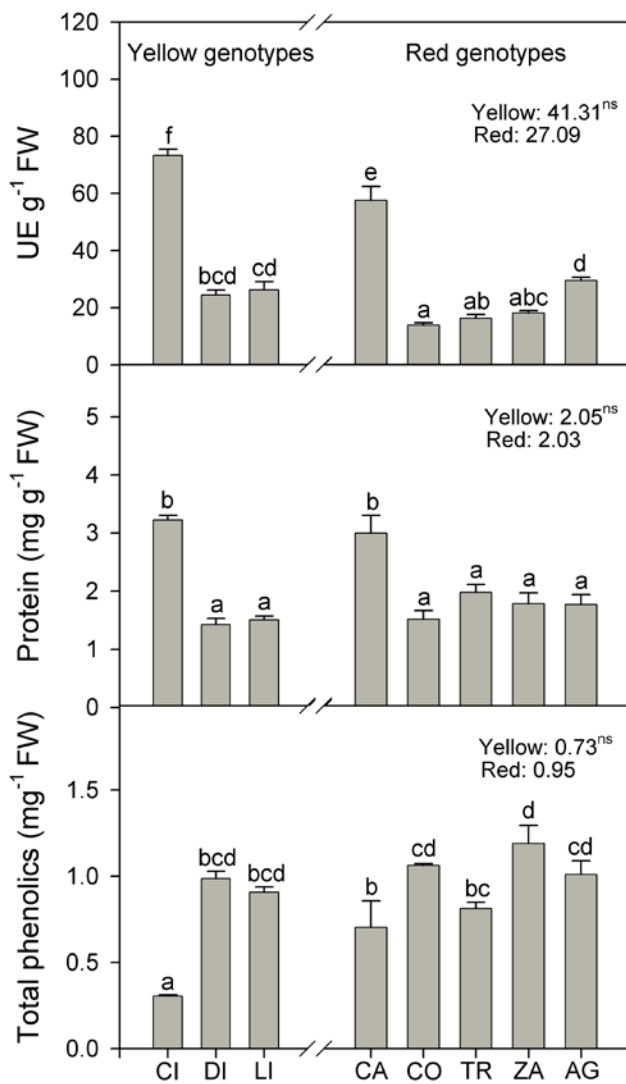
2   Table 1

3   Total fruit yield and agronomic fruit quality categories in different sweet pepper

4   genotypes. Main effects of genotype or color were analyzed statistically by Duncan´s

5   multiple range test at the 0.05 level. Values followed by the same letter are not

6   significantly different. ns: not significant. Y: yellow genotype. R: red genotype.

7

| | kg m^{-2} | | | | | |
	Extra	I	II	III	IV	Total
Genotype						
Cierva[Y]	2.88d	1.53ab	1.72abc	0.71abc	0.71ab	7.70b
Disco[Y]	0.94a	1.31a	2.08c	1.07c	1.08cd	6.80ab
Limona[Y]	2.43cd	1.98b	1.91abc	0.93bc	0.93bcd	8.36b
Cabezo[R]	2.10bcd	1.67ab	1.80abc	0.87abc	1.31cd	7.74b
Coyote[R]	1.64abc	1.43ab	1.34a	0.69bc	0.61a	5.72a
Traviatta[R]	2.67d	1.61ab	1.36ab	0.52a	1.04bc	7.18ab
Zar[R]	1.49ab	1.30a	1.98bc	0.66bc	1.42d	6.85ab
A67[R]	2.82d	1.38a	1.60abc	0.68bc	0.93ab	7.43ab
Color						
Yellow	2.09a	1.61a	1.91a	0.90b	1.06a	7.62a
Red	2.14a	1.47a	1.61a	0.68a	1.10a	6.98a

8

1	Table 2

2	Fruit physical parameters in different sweet pepper genotypes. Main effects of genotype or color were analyzed statistically by Duncan´s multiple

3	range test at the 0.05 level. Values followed by the same letter are not significantly different. Y: yellow genotype. R: red genotype.

4

	Length (mm)	Width (mm)	Shape Index	Firmness (kg^{-1})	Pericarp thickness (mm)	Dry matter (%)	Color				
							L*	a*	b*	C_{ab}	h_{ab}
Genotype											
Cierva[Y]	108.5d	85.23ab	1.28c	3.28a	7.10a	7.29a	49.7d	3.5a	28.1e	28.4b	82.7bc
Disco[Y]	94.27bc	86.27ab	1.09a	3.21a	6.81a	8.19b	45.0c	3.5a	24.0d	24.3b	81.7b
Limona[Y]	85.97a	83.09a	1.04a	3.57a	7.26ab	6.91a	51.6e	2.5a	29.3e	29.4c	85.1c
Cabezo[R]	96.78bc	88.21b	1.09a	4.72b	8.48bc	6.84a	30.1 b	23.5c	10.2c	25.7 b	23.5a
Coyote[R]	102.17cd	81.37a	1.25bc	7.04d	8.60c	8.09b	28.7ab	21.9c	8.7ab	23.6 b	21.7a
Traviatta[R]	90.07ab	87.27b	1.03a	7.05d	8.41bc	6.9a	30.0b	23.7c	9.8bc	25.6b	22.4a
Zar[R]	97.83bc	85.44ab	1.14ab	6.11c	8.02abc	8.31b	28.1a	19.5b	8.1a	21.1a	22.8a
A67[R]	98.53dbc	87.65b	1.13 a	6.05c	8.57c	7.02a	27.5a	19.8b	8.1a	21.4a	22.3a
Color											
Yellow	95.1a	84.83a	1.12a	3.36a	7.05a	7.47a	48.7b	3.2a	27.0b	27.3b	83.2b
Red	95.9a	86.02a	1.13a	6.23b	8.41b	7.48a	28.9a	21.7b	9.0a	23.5a	22.5a

5