1 **Can Double Graft Improve Affinity In Melon? Effects of Simple and**

2 **Double Grafting Melon Plants on Mineral Absorption,**

3 **Photosynthesis, and Biomass**

4

5 Alberto San Bautista,[a] Angeles Calatayud,[b] Sergio Gonzalez-Nebauer,[a] Bernardo

6 Pascual,[a] José Vicente Maroto,[a] Salvador López-Galarza,[a*]

7 [a] Departamento de Producción Vegetal, Universitat Politècnica de València, Camino

8 de Vera 14, 46020 Valencia, Spain.

9 [b] Departamento de Horticultura, Instituto Valenciano de Investigaciones Agrarias,

10 46113 Moncada, Valencia, Spain.

11

12 *Corresponding author: Tel.: +34 963877337; fax: +34 963877339; email address:

13 slopez@prv.upv.es

14

15   **Abstract**

16

17   The spanish type cultivar 'Piel de Sapo' (*Cucumis melo* L. *var. saccharinus*), has

18   less affinity with the *Cucurbita maxima* x *Cucurbita moschata* hybrids actually used

19   as rootstocks. To improve affinity between rootstock and scion double grafting can

20   be used, by means of an intermediate rootstock compatible with both of them. Non-

21   grafted, single, and double grafted melon plants of the cultivar 'Piel de sapo' were

22   evaluated for water and nutrient absorption to accurately evaluate if the double

23   grafting response is related to the improvement in affinity when *Cucurbita maxima x*

24   *Cucurbita moschata* hybrids are used as rootstocks. The melons were also evaluated

25   for photosynthesis activity and biomass production in early phases. The hybrid

26   'Shintoza' (*Cucurbita maxima* x *Cucurbita moschata*) was used as rootstock, and the

27   cantaloupe type melon cultivar 'Sienne' as an intermediate scion. Double grafted

28   plants showed higher increased fresh and dry weights, and also showed a higher

29   capacity for uptaking beneficial minerals (particularly NO_3^-, P, K, Ca, Mn, and Zn)

30   with respect to non-grafted and simple grafted plants. Grafting did not affect net

31   photosynthetic values, but significantly increased water use efficiency. Double

32   grafted plants also revealed higher quantum efficiency PSII photochemistry values.

33   Consequently, double grafting on a vigorous rootstock such as 'Shintoza' (with an

34   intermediate scion that confers better affinity) results in improved mineral and water

35   absorption and achieves an increase in ion influx to the scion – so enabling an

36   increase in light photosynthetic reaction and biomass.

37

38   *Keywords:* biomass; *Cucurbita*; melon; mineral uptake; grafting; photosynthesis;

39   water uptake.

1. Introduction

Cucurbit grafting is becoming a common practice worldwide, particularly in Japan, Korea, Spain, China, and Italy. In all these countries except Korea, grafting in watermelon is significantly more important than grafting in melon (Lee et al., 2010). Currently in Spain, 48.2 million watermelon plants and 2.5 million melon plants (90% and 1% of the total, respectively) are annually grafted on interspecific *Cucurbita maxima* x *Cucurbita moschata* hybrids (Hoyos, 2010). For watermelons, grafting helps control soil-borne pathogens and increases yields without affecting quality (Miguel et al., 2004; López-Galarza et al., 2004). These benefits compensate in part for the extra costs of grafting. Grafted melon plants have also proven effective in controlling soil-borne diseases and the *melon necrotic spot virus* (MNSV). This virus causes vine decline (Davis et al., 2008), although its appearance is less frequent and more erratic than the pathogens affecting watermelon (Cohen et al., 2004; Hassel et al., 2008). Melon plants grafted on *Cucurbita maxima* x *Cucurbita moschata* hybrids show poor compatibility, do not consistently increase yields, and show no improvement in vigour. As a result, this technique is used by few growers and only for cantaloup and Galia types. Melons grafted on melon-resistant cultivars have higher affinity but less resistance to soil-borne diseases, especially to MNSV (King et al., 2010).

The most common melon cultivars in Spain belong to the Spanish melon type 'Piel de sapo' (*Cucumis melo* var. s*accharinus*). These cultivars have little affinity with the *Cucurbita maxima* x *Cucurbita moschata* hybrids used as rootstocks (Miguel et al., 2007). These circumstances explain the different use of grafting in both species.

65   It is known that grafting directly affects plant physiology through the interaction of

66   some or all of the biochemical processes. These processes include increases in

67   water and mineral uptake, improvements in the synthesis of endogenous hormones,

68   stimulated antioxidant systems, and greater resistance to abiotic and/or biotic stress.

69   The result is a more vigorous root and shoot system (Martínez-Ballesta et al., 2010).

70   Poor vascular connections between rootstock and scion produce a deleterious effect

71   on plant growth and create graft incompatibilities (Tiedemann, 1989).

72   Previous information shows that *Cucurbita* spp. rootstocks cause a deleterious

73   graft on melon scion (Lee, 1994; Oda, 1995; Traka-Mavrona et al., 2000). These

74   authors attribute this effect to the differences in stem diameter between *Cucurbita*

75   and *Cucumis melo*, which reduces the sites with vascular and phloem connections

76   due the large pith cavity of *Cucurbita* (Traka-Mavrona et al., 2000; Tiedemann,

77   1989).

78   To improve affinity between rootstock and scion, Hartman et al. (1997) indicated

79   that double grafting by means of a mutually compatible intermediate rootstock is

80   useful. Kwon et al. (2005) sought to improve quality and evaluated the performance

81   of this technique in watermelon, using *Lagenaria* as an intermediate rootstock

82   between 'Shintoza' (*Cucurbita maxima* x *Cucurbita moschata* hybrid) and the scion.

83   Our group evaluated the performance of double grafting melon on 'Shintoza' during

84   four years in field conditions using various intermediate rootstocks. We recorded an

85   increase in yields compared with single grafting, particularly with the Galia-type

86   cultivar 'Sienne'.

87   Double grafting is not currently used in the 'Piel de sapo' type melon. In this study,

88   we used a simple graft with *Cucurbita maxima* x *C. moschata* and double grafted

89   *Cucurbita maxima* x *C. moschata* / melon 'Sienne' as rootstocks on scion melon

90 'Ricura' to identify metabolic and physiological factors that could be associated with

91 the level of grafting compatibility. If double grafting improves compatibility in

92 comparison with simple grafting, then double grafting could be used as a tool to

93 improve the affinity of some melon cultivars such as 'Piel de sapo' and obtain a better

94 yield and resistance to pathogens.

95

96 **2. Materials and methods**

97

98 *2.1. Plant growth*

99

100 Melon plants of the open pollinated 'Piel de sapo' type, cultivar 'Ricura' (Semillas

101 Batlle) were directly grafted on squash (simple graft) (*Cucurbita maxima* x *Cucurbita*

102 *moschata* cv. Shintoza, Intersemillas) or using an intermediate melon rootstock

103 (double graft) (*Cucumis melo* sp. *cantalupensis* cv. Sienne, De Ruiter Seeds).

104 Melon seeds were sown on 14 March 2009 and squash on 29 March 2009 in 200

105 polystyrene trays filled with peat-based substrate that were kept under a Venlo-type

106 glasshouse. Both types of grafting were made on 9 April using the tongue grafting

107 method and the plants were transplanted to 54-cell trays. 'Ricura' variety plants

108 without grafting were also transplanted to 54-cell trays and used as control plants.

109 Double, single, and non-grafted plants were placed in 2 L polyethylene pots

110 covered with aluminium sheets on 29 April (the root system having been previously

111 washed clean of substrate). Pots were filled with a nutrient solution containing (in

112 mmol L^{-1}): 12.3 NO_3^-; 1.02 $H_2PO_4^-$; 2.45 SO_4^{2-}; 3.24 Cl^-; 5.05 K^+; 4.23 Ca^{2+}, 2.55 Mg^{2+}

113 and 2.81 Na^+ that had been artificially aerated. Micronutrients were also provided

114 (15.8 µM Fe^{2+}, 10.3 µM Mn^{2+}, 4.2 µM Zn^{2+}, 43.5 µM B^{5+}, 1.4 µM Cu^{2+}). The electrical

115   conductivity and pH of this nutrient solution were 2.2 dS m^{-1} and 6.5, respectively.

116   Nutrient solution was added daily to compensate for absorption. The layout was in a

117   completely randomised design of 15 plants per treatment.

118   After 13 days of plant acclimation to the pots, all plants were weighed and an

119   exact volume of 2L of nutrient solution was added to each pot. An exact volume of

120   nutrient solution was also added daily and annotated to compensate uptake. The

121   system was watertight, so all volume losses were attributed to water and nutrient

122   uptake.

123   The volume remaining in each pot was measured on 22 May, a 100 mL sample

124   per pot was kept for analysis and each plant was separated in root and aerial parts to

125   determine fresh and dry weights.

126   The environmental greenhouse range during the measurements was: temperature

127   (21-24ºC); relative humidity (52-72%); and solar radiation (610-870 J s^{-1} m^{-2}).

128

129   *2.2. Water and nutrient absorption*

130

131   At the end of the experiment water uptake, expressed as mL dry weight plant^{-1}

132   day^{-1} for each hydroponic unit was calculated from the difference between the

133   remains on the final day and the sum of all the exact volumes added daily.

134   Nitrate concentration in the nutrient solution was determined by the Kjeldahl

135   method at the end of the experiment (Bremner, 1965). Other nutrients were

136   measured using simultaneous ICP emission spectrometry (iCAP 6000, Thermo

137   Scientific. Cambridge, United Kingdom). Results were expressed as mg dry weight

138   plant^{-1} day.$^{-1}$

139

2.3. Photosynthetic activity and chlorophyll fluorescence

Net CO_2 fixation rate (Amax, μmol CO_2 m^{-2} s^{-1}), stomatal conductance to water vapour (gs, mol H_2O m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}), and substomatal CO_2 concentration [Ci, μmol CO_2 mol^{-1} (air)] were measured at steady-state under conditions of saturating light (1200 μmol m^{-2} s^{-1}) and 400 ppm CO_2 with a LI-6400 (LI-COR, Nebraska, USA). The water use efficiency parameters (WUE, μmol CO_2/mol H_2O) were calculated from Amax/E. To evaluate the presence of chronic photoinhibitory processes, the maximum quantum yield of PSII (Fv/Fm: (Fm-Fo)/Fm) was measured on leaves after 30 minutes in darkness using a portable pulse amplitude modulation fluorometer (MINI PAM, Walz, Effeltrich, Germany). The background fluorescence signal for dark adapted leaves (Fo) was determined with a 0.5 μmol photon m^{-2} s^{-1} measuring light at a frequency of 600 Hz. The application of a saturating flash of 10000 μmol photon m^{-2} s^{-1} enabled estimations of the maximum fluorescence (Fm). The steady state fluorescence signal (Fs) and maximum fluorescence yield (F'm) were determined in the same leaves after adapting to light (1200 μmol m^{-2} s^{-1}). The quantum efficiency of PSII photochemistry (ϕ_{PSII}), closely associated with quantum yield of non-cyclic electron transport, was estimated from (F`m-Fs)/Fm` (Genty et al. 1989) and used for the calculation of the relative linear transport rate, ETR (μmol electron m^{-2} s^{-1})=(ϕ_{PSII} *PAR*α*β). PAR is the active photon flux density (1200 μmol m^{-2} s^{-1}), α is the leaf absorbance (0.84), and β is the distribution of absorbed energy between photosystems (0.5).

Gas exchange and chlorophyll fluorescence measurements were performed on 21 May from 9:00 am to 11:00 am (GMT). One measurement per plant was performed

7

164   on a fully expanded mature leaf (third or fourth leaf from the shoot apex). Ten plants

165   were measured for each treatment.

166

167   **3. Results**

168

169   *3.1. Biomass production*

170

171   Total number of leaves by plant, aerial dry weight (DWa), and the aerial and root

172   part dry weight ratio (DWa/DWr) (Table 1) were significantly influenced by grafting.

173   However, at the end of experiment no significant differences were observed for fresh

174   weights (FW), DWr, nor the aerial and root fresh weight (FWa/FWr) ratio between

175   control and graft plants. Nevertheless, double grafted plants showed increased

176   values for these parameters. The number of leaves was higher in double and simple

177   grafted than non-grafted plants (51% and 30% respectively). The aerial fresh weight

178   (FWa) showed no significant differences but the higher value was in double grafting.

179   As a result of grafting, the plants showed a higher aerial dry weight (DWa). These

180   changes meant a higher DWa/DWr ratio for simple and double graft (Table 1).

181

182   *3.2. Water and nutrient absorption*

183

184   Table 2 shows the water and nutrient absorption versus influence of grafting after

185   ten days in the hydroponic system. Water absorption was statistically higher in

186   double grafted plants with an increase of 40% compared to non-grafted plants. The

187   N-NO$_3^-$, P, K, Ca, Mn and Zn uptakes were statistically higher in double grafted

188   plants than in simple grafted and control plants. The opposite occurs with Na, Cu, Fe

8

189   and B uptakes, in these cases a large decrease was observed in double grafted

190   plants (p values between 0.045 and 0.024). The ratio K/Na quantification is a major

191   ionic parameter relation with salt tolerance. The ratio was higher in the double graft

192   plant, lower in control plants, and intermediate in simple graft. There were significant

193   differences between control and grafted plants (3.41, 6.18 and 10.05 respectively,

194   data not shown in the Table 2). Sulphur and Mg absorption showed similar values in

195   the three types of plants (Table 2).

196

197   *3.3. Relation between nutrient absorption and biomass*

198

199   To understand the variations in the plant nutrient content in grafted (simple and

200   double) plants, we compared the nutrient concentration (calculated from absorption)

201   in the DW plants with non-grafted plants as a reference (Table 3). The use of graft

202   induces differences in the mineral content of all plants. With respect to $N-NO_3^-$, P, Fe,

203   Mn, Zn, B, Cu and Na the double graft shows a lower value and significant

204   differences with control plants, due to a higher dry weight in double graft plant. The

205   response of some nutrients such as K, Ca, S, and Mg, was mainly homogenous in all

206   plants.

207

208   *3.4. Graft effect on photosynthetic activity and chlorophyll fluorescence*

209

210   CO_2 assimilation rates (Table 4) at light saturation level (Amax) were similar in

211   the three types of plants without significant differences; however, the grafted plants

212   had lower values of intercellular CO_2 concentration (Ci) with significant differences for

213   double grafted plants. Leaves of shoots grafted on the simple or double rootstocks

214   had lower values of stomatal conductance (gs) and transpiration rate (E). Changes in

215   E but with similar A values in graft plants were associated with statistically significant

216   increases in WUE by about 35% (Table 4).

217   Chlorophyll fluorescence parameters for dark-adaptation for mature leaves

218   subjected to graft and non-graft are shown in Table 4. The maximum quantum yield

219   of PSII photochemistry estimated using the Fv/Fm ratio is similar in all plants. An

220   apparently steady-state was examined after four minutes of actinic illumination for the

221   quantum yield of PSII (ϕ_{PSII}) electron transport rates in all the leaves on the control

222   and graft plants. The highest values for ϕ_{PSII} were obtained by double graft plants

223   with an increase of 12% (with significant differences) compared to control plants.

224

225   **4. Discussion**

226

227   In this study, we describe how simple and double graft affects water and nutrient

228   uptakes and the photosynthetic processes in order to identify metabolic and

229   physiological factors that may be associated with the level of grafting compatibility in

230   plants in early development (when grafting incompatibilities usually occur). The aim is

231   to evaluate the possibility of using double graft in 'Piel de sapo' type melons.

232   Grafting did not affect net photosynthetic values, and similar results were obtained

233   by Salehi et al. (2010) with 'Khatooni' melon (*Cucumis melo* var *inodorus*) grafted on

234   three *Cucurbita* rootstocks. However, grafted plants had more net CO_2 assimilation

235   due to an increase in gs and Ci parameters. Consequently, the WUE values were

236   lower in 'Khatooni' melon grafted plants. In our experimental conditions and with

237   other melon scion and rootstocks, the gs and E values for scion leaves with simple

238   and double grafts are lower. This effect implies that minor gs values are responsible

10

239   for the diminishing intercellular CO_2 concentration when compared with non-grafted

240   plants assayed under the same conditions. This can be explained by the fact that

241   only very critically low levels of gs affect photosynthesis, which is in agreement with

242   Flexas et al. (2004); and/or decreasing stomatal conductance can be compensated

243   by higher photosynthetic capacity (ϕ_{PSII}). The parameter ϕ_{PSII} closely correlates with

244   the quantum yield of non-cyclic electron transport (Genty et al., 1989) being

245   increased by double graft; and therefore indicates a stimulation of electron flow

246   around PSII when compared to simple graft and control plants. Considering the

247   beneficial double grafted effect on the electron transport rate (ETR), the similar

248   values of CO_2 fixation obtained with grafted and non-grafted plants could be a result,

249   at least to some extent, of an increase of ATP and reduced power in graft plants,

250   mainly in double grafted plants with lower gs values. An increase in ϕ_{PSII} can help

251   CO_2 fixation in double grafted plants. These facts occur without signs of

252   photoinhibition in leaves, as indicated by the unchanged Fv/Fm ratio (Calatayud and

253   Barreno, 2001). The Fv/Fm ratio has often appeared as a sensitive parameter in

254   tomatoes grafted under saline conditions (Albacete et al., 2009); in grafted cucumber

255   leaves at low root temperatures (Ahn et al., 1999); and for the water temperature

256   effects on the graft union of tomato and eggplant (Shibuya et al., 2007).

257   Higher water use efficiency is a desired plant characteristic (Ruiz et al., 1997).

258   The WUE parameter in scion leaves is highest in graft plants as a result of lower

259   transpiration and stomatal conductance. The minor E value in grafted plants can be

260   compensated by a higher number of leaves: increasing the transpiration stream

261   (mainly in double graft); and/or by an enhancement of vigour in the rootstock root

262   system (Rouphael et al., 2008a) that encourages water and mineral uptake (Ruiz and

263   Romero, 1999; Davis et al., 2008; Salehi et al., 2010). The higher water absorption

11

264 obtained in double graft shows that a sufficient connection of vascular bundles

265 between scion and the *Cucurbita-Cucumis* rootstocks is obtained. Alterations in

266 growth scion are observed when water absorption by roots is suppressed at the graft

267 interface (Torii et al., 1992; Atkinson and Else, 2001; Oda et al., 2005). Double graft

268 plants produced the highest increase in fresh and dry weight. Dry mass is an

269 important factor when determining how the environment is affecting the growth rate

270 (Justus and Kubota, 2010) and is related to nutrient uptake capacity (Colla et al.,

271 2010a).

272   Double graft plants show a higher capacity for uptaking beneficial minerals

273 compared to non-graft and simple graft plants. Nitrogen is considered a limiting factor

274 to growth, and the development and production of amino acids and proteins in plants

275 (Ruiz and Romero, 1999; Calatayud et al., 2008). In melon, N uptake was more

276 influenced by the rootstock genotype than by the scion (Ruiz et al., 1997). In earlier

277 studies, the influence of rootstocks on N uptake was described (Castle and Krezdorn,

278 1975; Heo, 1991; Jang, 1992). More recently, Salehi et al. (2010) showed that

279 mineral concentration of NO_3^- in the xylem exudates in *Curcurbita* rootstocks on

280 'Iranian' melon was higher than in the non-grafted melons. Plants of *Cucumis melo*

281 grafted on three *Cucurbita maxima* x *C. moschata* cultivars contained higher

282 amounts of organic N than the controls (Ruiz and Romero, 1999); and a higher N

283 uptake efficiency were observed in melon (cv. Proteo) grafted on *Cucumis melo*

284 (Colla et al., 2010a).

285   The characteristics of the rootstocks and a good rootstock-scion interaction can

286 determine increased uptake, as well as the subsequent transport and accumulation

287 of NO_3^- in scion leaves (Ruiz et al., 1996-1997-1999; Martínez-Ballesta et al., 2010).

288 One of the major metabolic check-points coordinating nitrogen assimilation in leaves

289   is nitrate reductase (NR). NR activity is governed by multiple factors, the most

290   important being light, nitrates, and carbohydrates (Sitt et al., 2002; Calatayud et al.,

291   2007). The regulatory effect of light on leaf NR is closely linked to light

292   photosynthesis reaction (Iglesias-Bartolomé et al., 2004): NR located in the cytosol of

293   a leaf catalyses the NADPH-dependent reduction of NO_3^- to NO_2^-. Nitrite is converted

294   into ammonium by nitrite reductase in the chloroplast and accounts for the ability of

295   nitrite to support non-cyclic electron transport (ϕ_{PSII}) in the chloroplast (Heber et al.,

296   1995). A higher ϕ_{PSII} in double graft can also explain the higher NO_3^- uptake and we

297   assume that nitrate in scion leaves is incorporated in the biomass (superior growth of

298   melon on double rootstocks).

299   It has been reported that grafted plants increase the absorption of phosphorus in

300   melon (Ruiz et al., 1996) for compatible grafts with *Solanaceous* plants (Kawaguchi

301   et al., 2008), in watermelon (Colla et al., 2010b), in tomato (Fernandez-Garcia et al.,

302   2004), in eggplants (Leonardi and Guiffrida, 2006), or cucumber (Rouphael et al.,

303   2008b). Due to the low mobility of P, a more vigorous root system characterised by a

304   higher density of root hair and/or increase in exudation of organic acid by the roots

305   can be responsible for increasing the P uptake (Gent et al., 2005; Savvas et al.,

306   2010; Colla et al., 2010b). Higher P depletion in a nutrient solution was obtained with

307   double grafted melon plants. A positive effect has been seen between the foliar level

308   of total P and greater shoot vigour (Lee, 1994; Ruiz et al., 1996) as reflected in the

309   double graft melon plants.

310   Contradictory results have been obtained in graft plants in relation with Ca

311   acquisition (see Martínez-Ballesta, 2010; Savvas et al., 2010). Increased Ca uptake

312   has been observed in watermelon grafted on *Cucurbita maxima* (Ruiz et al., 1997); in

313   tomato grafted on the rootstock of tomato (Fernandez-Garcia et al., 2004); or in

eggplant and tomato plants grafted on interspecific rootstock 'Beaufort' (Leonardi and Guiffrida, 2006). However, melon grafted on *Cucurbita* rootstocks showed no significant differences in comparison with non-grafted plants (Edelstein et al., 2005) or in cucumber grafted on *Cucurbita maxima* x *C. moschata* (Rouphael et al., 2008b). In our results, a significant increase in Ca uptake was obtained in double grafted plants; but not in simple grafted plants. Many reports indicate that Ca absorption can be significantly influenced by rootstocks, but the scion generally has no effect on uptake (Martínez-Balleta et al., 2010).

The uptake of micronutrients does not usually decrease in grafted plants (Huang et al., 2010). The Mn and Zn uptake increases in double grafted melon. The Zn is directly involved in the synthesis of heavy nitrogenous compounds (Cakmak, 1988). Pulgar et al. (1998) indicated that grafted watermelon plants have a higher efficiency in integrating Zn into nitrogenous compounds that form chelates with Zn, thereby also explaining the greater foliar biomass in grafted plants. In our results, a higher Zn uptake is correlated with higher NO_3^- depletion in a nutrient solution. As a catalytically active metal, manganese has an important function and plays an activating role on enzymes and with Fe-involved chlorophyll synthesis. Under saline conditions, cucumber plants grafted on figleaf gourd enhances tolerance by maintaining higher Mn concentrations that promote higher antioxidant enzyme activity (Huang et al., 2010). A stimulated Mn uptake observed in double grafted melon possibly compensates for the lower Fe uptake.

Many results suggest that grafted plants can limit Cu and B transport from root to leaves reducing their toxic effect (Martínez-Ballesta et al., 2010; Savvas et al., 2010). Graft can partly mitigate Cu toxicity in environments with Cu concentrations that are too high. This is due to the ability of the rootstock to restrict Cu accumulation in the

shoots, for example, in cucumber graft on *C. maxima* x *C. moschata* (Rouphael et al., 2008b); or in tomato grafted on *Solanum lycopersicum* x *S. habrochaites* rootstock (Savvas et al., 2009). Excessive boron can be a problem in dry Mediterranean soils. Many results in melon suggest that grafting may alleviate or even prevent growth and yield decrease due to B toxicity (Edelstein et al., 2005, 2007). Double grafted melon shows lower uptake of Cu and B – indicating that these plants could be more effective in excluding both elements.

K concentration in the plant and the K/Na ratio have been the most studied parameters related to salt tolerance, and these parameters are positively correlated with leaf biomass and chlorophyll fluorescence (Cuartero and Fernandez-Muñoz, 1999; Foolad, 2004; Albacete et al., 2009). There are conflicting results when determining the beneficial graft effect of salinity resistance in melon (Romero et al., 1997; Rivero et al., 2003; Edelstein et al., 2005; Colla et al., 2006). The diversity of outcomes may be due to the different effects of the *Cucurbita* rootstocks that were used in these studies (see Colla et al., 2010c). A higher Na exclusion is observed in double graft melon plants compared to simple graft – given that the root system is the same. The bibliography places great importance on the role of rootstocks in conferring resistance to abiotic and biotic stress.

In general, we observed that double graft achieved higher beneficial nutrient uptakes. This superiority in nutrient and water uptake was found in growth plants. In this experiment, we used *Cucurbita maxima* x *Cucurbita moschata* 'Shintoza' as rootstock grafted directly on melon (simple graft), or we used an intermediate rootstock (*Cucumis melo* in double graft). In both cases, the root system developed by *Cucurbita* rootstock was the same. So then why did double graft show the better physiological conditions? Our results confirm that grafted plants on vigorous root

364   systems can improve mineral and water absorption – but good graft compatibility is

365   also important. In our case, an intermediate graft achieved an increase in ion influx to

366   the scion that enabled increased light photosynthetic reaction and biomass.

367   More studies should be undertaken to understand the mechanism underlying the

368   rootstock/scion interaction.

369

370   **Acknowledgements**

371

376

377

References

378

379

380 Ahn, S.J., Im, Y.J., Chung, G.C., Cho, B.H., Suh, S.R., 1999. Physiological

381   responses of grafted-cucumber leaves and rootstocks roots as affected by low

382   temperature. Sci. Hortic. 81, 397: 408.

383 Albacete, A., Martínez-Andujar, C., Edmond, M.G., Acosta, M., Sanchez-Bravo, J.,

384   Asins, M.J., Cuartero, J., Lutos, S., Dodd, I.C., Pérez-Alfocea, F., 2009.

385   Rootstocks mediated changes in xylem ionic and hormonal status are correlated

386   with delayed leaf senescence, and increased leaf area and crop productivity in

387   salinized tomato. Plant Cell Environ 32, 928-938.

388 Atkinson, C.J., Else, M.A., 2001. Understanding how rootstocks dwarf fruit trees. The

389   Company Fruit Tree 34, 46-49.

390 Bremner, J.M. 1965. Total Nitrogen Part 2, in: C.A. Black; D.D. Evans; J.L. While;

391   L.E. Ensminger (Eds). Methods of Soil Analysis, American Society of Agronomy:

392   Madison, Wisconsin: USA, pp. 1149-1178.

393 Cakmak, I., 1988. Morphologische und physiologische vernänderumgen bei

394   zinkmangelpflanzen. Ph. D. Thesis. Universität Hohenheim. Stuttgart. Germany.

395 Calatayud, A., E. Barreno., 2001. Chlorophyll *a* fluorescence, antioxidant enzymes

396   and lipid peroxidation in tomato in response to ozone and benomyl. Environ.

397   Pollut. 115, 283-289.

398 Calatayud, A., Roca, D., Gorbe, E., Martínez, P.F., 2007. Light acclimation in rose

399   (*Rosa hybrida* cv. Grand Gala) leaves after pruning: effects on chlorophyll *a*

400   fluorescence, nitrate reductase, ammonium and carbohydrates. Sci. Hortic. 111,

401   152-159.

402 Calatayud, A., Roca, D., Gorbe, E., Martinez, P.F., 2008. Effect of two nutrient

403   solution temperatures on the uptake of nitrate, nitrate reductase activity, NH_4^+

404   concentration and chlorophyll *a* fluorescence in rose plants. Environ. Exp. Bot.

405   64, 65-74.

406   Castle, W.S., Krezdorn, A.H., 1975. Effects of citrus rootstocks on root distribution

407   and leaf mineral content of Orlando Tangelo trees. J. Amer. Soc. Hort. Sci. 100,

408   1-4.

409   Cohen, R., Burger, Y., Horev, C., Porat, A., Saar, U., Edelstein, M., 2004. Reduction

410   of *Monosporascus* wilt incidence using different Galia-type melons grafted onto

411   *Cucurbita* rootstock. Progress in cucurbit genetics and breeding research, in:

412   Proc. Cucurb. The 8th EUCARPIA Meeting on cucurbit genetics and breeding,

413   pp 313-317.

414   Colla, G., Rouphael, Y., Cardarelli, M., Rea, E., 2006. Effect of salinity on yield, fruit

415   quality, leaf gas exchange, and mineral composition of grafted watermelon

416   plants. HortSci. 41, 622-627.

417   Colla, G., Suarez, C.M.C., Cardelli, M., Rouphael, Y., 2010a. Improving nitrogen use

418   efficiency in melon by grafting. HortSci. 45, 559-565.

419   Colla, G., Rouphael, Y., Cardarelli, M., Salerno, A., Rea, E., 2010b. The

420   effectiveness of grafting to improve alkalinity tolerance in watermelon. Environ.

421   Exp. Bot. 68, 283-291.

422   Colla, G., Rouphael, Y., Leonardini, C., Bie, Z. 2010c. Role of grafting in vegetable

423   crops grown under saline conditions. Sci. Hortic. 127, 147-155.

424   Cuartero, J., Fernández-Muñoz, R., 1999. Tomato and salinity. Sci. Hortic. 78, 83-

425   125.

426   Davis, A.R., Perkins-Veazie, P., Sakata, Y., López-Galarza, S., Maroto, J.V., Lee,

427   S.G., Huh, Y.C., Sun, Z., Miguel, A., King, S.R., Cohen, R., Lee, J.M., 2008.

428   Cucurbit grafting. Crit. Rew. Plant Sci. 27, 50-74.

429   Edelstein, M., Ben.Hur, M., Cohen, R., Burger, Y., Ravina, I., 2005. Boron and

430   salinity effects on grafted and non-grafted melon plants. Plant Soil 269, 273-284.

431   Edelstein, M., Ben-Hur, M. 2007. Preventing contamination of supply chains by using

432   grafted plants under irrigation with marginal water. In: Wilson J. (Ed.),

433   Proceedings of the International Symposium on Water resources Management.

434   Honolulu, Hawai, USA, pp. 150-154.

435   Fernández-García, N., Martínez, V., Carvajal, M., 2004. Effect of salinity on growth,

436   mineral composition, and water relations of grafted tomato plants. J. Plant Nutr.

437   Soil Sci. 167, 616-622.

438   Flexas, J., Bota, J., Cifre, J.M., Escalona, J., Galmés, J., Gulías, E.K., Lefi, S.F.,

439   Martínez-Cañellas, M.T., Moreno, M., Ribas-Carbo, D., Riera, B., Sampol,

440   Medrano, H., 2004. Understanding down-regulation of photosynthesis under

441   water stress: Future prospects and searching for physiological tools for irrigation

442   management. Ann. Appl. Bot. 144, 273-283.

443   Foolad, M.R., 2004. Recent advances of salt tolerance in tomato. Plant Cell, Tissue

444   Organ Cult. 76, 101-119.

445   Gent, M.P.N., Parrish, Z.D., White, J.C., 2005. Nutrient uptake among subspecies of

446   *Cucurbita pepo* L. is related to exudation of citric acid. J. Am. Soc. Hortic. Sci.

447   130, 782-788.

448   Genty B., Briantais, J.M., Baker, N.R., 1989. The relationship between the quantum

449   yield of photosynthetic electron transport and quenching of chlorophyll

450   fluorescence. Biochem. Biophy. Acta 990, 87-92.

451 Hartman, H. T., Kester, D.E., Davies, F. T. Jr, Geneve, R.L. 2002. Hartman and
452   Kester's plant propagation: principles and practices. Prentice Hall, NJ, USA.

453 Hassell, R.L., Memmoott, F., Liere, D.G., 2008. Grafting methods for watermelon
454   production. Hort. Sci. 43,1677-1679.

455 Heber, U., Bukhov, N.G., Neimanis, S., Kobayashi, Y., 1995. Maximum $H^+/h\nu_{PSI}$
456   stoichiometry of proton transport during cyclic electron flow in intact chloroplasts
457   is at least two, but probably higher than two. Plant Cell Physiol. 36, 1639-1647.

458 Heo, Y.C., 1991. Effects of rootstocks on exudation and mineral elements contents in
459   different parts of oriental melon and cucumber. MS thesis, Kyung Hee
460   University, Seoul, South Corea, pp. 53.

461 Hoyos P., 2010. Spanish vegetable production : processing and fresh market.
462   Chronica Hortc. 49,27-30.

463 Huang, Y., Bie, Z., He, S., Hua, B., Zhen, A., Liu, Z., 2010. Improvement cucumber
464   tolerance to major nutrients induced salinity by grafting onto *Cucurbita ficifolia*.
465   Environ. Exp. Bot. 69, 32-38.

466 Iglesias-Bartolomé, R., González, C.A., Kenis, J.D., 2004. Nitrate reductase
467   dephosphorylation is induced by sugars and sugars-phosphates in corn leaf
468   segments. Physiol. Plant. 122, 62-67.

469 Jang, K.U., 1992. Utilization of sap and fruit juice of *Luffa cylindrical* L. Research
470   report of Korean Ginseng and Tobacco Institute, Taejon.

471 Justus, I., Kubota, C., 2010. Effects of low temperature storage on growth and
472   transplant quality of non-grafted and grafted cantaloupe-type muskmelon
473   seedlings. Sci. Hortic. 125, 47-54.

474   Kawaguchi, M., Taji, A., Backhouse, D., Oda, M., 2008. Anatomy and physiology of

475   graft incompatibility in Solanaceous plants, J. Hortic. Sci. Biotechnol. 83, 581-

476   588.

477   King, S.R., Davis, A.R., Zhang, X., Crosby, K., 2010. Genetic, breeding and selection

478   of rootstocks for Solanaceas and Cucurbitaceae. Sci. Hortc. 127, 106-111.

479   Kwon, J.K., Keon,G. B., Kang, K.H., Choi, Y.H., Kang, N.J., Lee, J.H., Jeong, H.J.,

480   Park, J.M., 2005. Effect on different rootstocks and double grafting on the fruit

481   quality and withering occurrence of greenhouse watermelon. Kor. J. Sci.

482   Technol. 23, 382-387.

483   Lee, J.M., Kubota, C., Tsao, S.J., Bie, Z., Hoyos Echevarria, P., Morra L., Oda M.,

484   2010. Current status of vegetable grafting: Diffusion, grafting techniques,

485   automation. Sci. Hortic. 127, 93-105.

486   Lee, J.M., 1994. Cultivation of grafted vegetables. I. Current status, grafting methods,

487   and benefits. HortSci. 29, 235-239.

488   Leonardi, C., Guiffrida, F., 2006. Variation of plant growth and macronutrient uptake

489   in grafted tomatoes and eggplants on three different rootstocks. Eur. J. Hortic.

490   Sci. 71, 97-101.

491   López-Galarza, S., San Bautista, A., Pérez, D.M., Miguel, A., Baixauli, C., Pascual,

492   B., Maroto J.V., Guardiola, J.L., 2004. Effects of grafting and cytokinin-induced

493   fruit setting on colour and sugar-content traits in glasshouse-grown triploid

494   watermelon. J. Hortic. Sci. Biotech. 79, 971-976.

495   Martínez-Ballesta, M.C., Alcaraz-López, C., Muries, B., Mota-Cadenas, C., Carvajal,

496   M., 2010. Physiological aspects of rootstok-cion interactions. Sci. Hortic. 127,

497   112-118.

498   Miguel A., Maroto J.V., San Bautista A., Baixauli C., Cebolla V., Pascual B., López

499   S., Guardiola J.L., 2004. The grafting of triploid watermelon is an advantageous

500   alternative to soil fumigation by methyl bromide for control of *Fusarium* wilt. Sci.

501   Hortic. 103, 9-17.

502   Miguel, A., De la Torre, F., Baixauli, C., Maroto, J.V., Jordá, M.C., López, M.M.,

503   García-Jiménez, J., 2007 Injerto de hortalizas, ed. Ministerio de Agricultura,

504   Pesca y Alimentación and Fundación Ruralcaja, Valencia.

505   Oda M., 1995. New grafting methods for fruit-bearing vegetables. Japan. Agr. Res.

506   Q. 29,187-198.

507   Oda, M., Maruyama, M., Mori, G., 2005. Water transfer at graft union of tomato

508   plants grafted onto *Solamun* rootstocks. J. Japan. Soc. Hort. Sci. 74(6), 458-

509   463.

510   Pulgar, G., Rivero, R.M., Moreno, D.A., López-Lefebre, L.R., Villora, G., Baghour, M.,

511   Romero, L., 1998. Micronutrientes en hojas de sandia injertadas. In: VII

512   Simposio nacional-III Ibérico sobre nutrición mineral de las plantas. Gárate, A.

513   (Ed.), Universidad Autónoma de Madrid, Madrid, p. 255-260.

514   Rivero, R.M., Ruiz, J.M., Romero, L. 2003. Role of grafting in horticultural plants

515   under stress conditions. Food, Agri. Environ, 1: 70-74.

516   Romero, L., Belakbir, A., Ragala, L. Ruiz, J.M. 1997. Response of plant yield and leaf

517   pigments to saline conditions: effectiveness of different rootstocks in melon

518   plants (*Cucumis melo* L.). Soil Sci. Plant Nutr., 43: 855-866.

519   Rouphael, Y., Cardarelli, M, Colla, G., Rea, E., 2008a. Yield, mineral composition,

520   water relations, and water use efficiency of grafted mini-watermelon plants

521   under deficit irrigation. HortSci. 43, 730-736.

522   Rouphael, Y., Cardarelli, M, Rea, E., Colla, G., 2008b. Grafting of cucumber as a

523   jeans to minimize copper toxicity. Environ. Exp. Bot. 63, 49-58.

524   Ruiz, J.M., Belakbir, A., Romero, L., 1996. Foliar level of phosphorus and its

525   bioindicators in Cucumis melo grafed plants: A posible effect of rootstocks. J.

526   Plant Physiol. 149, 400-404.

527   Ruiz, J.M., Belakbir, A., López-Cantarero, I., Romero, L., 1997. Leaf-macronutrient

528   content and yield in grafted melon plants: a model to evaluate the influence of

529   rootstock genotype. Sci. Hortic. 71, 227-234.

530   Ruiz, J.M., Romero, L., 1999. Nitrogen efficiency and metabolism in grafted melon

531   plants. Sci. Hortic. 81, 113-123.

532   Salehi, R., Casi, A., Lee, J.M., Cabalar, M., Delshad, M., Lee, S.G., Huh, Y.C., 2010.

533   Leaf gas exchange and mineral ion composition in xylem sap of Iranian melon

534   affected by rootstocks and training methods. HortSci. 45, 766-770.

535   Savvas, D., Papastavrou, D., Ntatsi, G., Ropokis, A., Olympios, C., Hartmann, H.,

536   Schwarz, D., 2009. Interactive effects of grafting and manganese supply on

537   growth, yield, and nutrient uptake by tomato. HortSci. 44, 1978-1982.

538   Savvas, D., Colla, G., Rouphael, Y., Schwarz, D., 2010. Amelioration of heavy metal

539   and nutrient stress in fruit vegetables by grafting. Sci. Hortc. 127, 156-161.

540   Shibuya, T., Nakashima, H., Shimizu-Maruo, K., Kawara, T., 2007. Improvement of

541   graft development in tomato and eggplant grafted cutting by supplying warmed

542   water to graft union during low-air-temperature storage. J. Japan. Soc. Hort. Sci.

543   76, 217-223.

544   Stitt, M., Müller, C., Matt, P., Gibon, Y., Carillo, P., Morcuende, R., Scheible, W.R.,

545   Krapp, A., 2002. Steps towards an integrated view of nitrogen metabolism. J.

546   Exp. Bot. 53, 959-970.

547 Tiedemann, R., 1989. Graft union development and symplastic phloem contact in the

548   heterograft *Cucumis sativus* on *Cucumis ficifolia*. J. Plant Physiol. 134, 427-440.

549 Torii, T., Kawazaki, M., Okamoto, T., Kitani, O., 1992. Evaluation of graft-take using a

550   thermal camera. Acta Hortic. 319, 631-634.

551 Traka-Mavrona, E., Koutsika-Sotiriou M., Pritsa, T., 2000. Response of squash

552   (*Cucurbita* spp.) as rootstock for melon (*Cucumis melo* L.). Sci. Hortic. 83, 353-

553   362.

554

Tables

Table 1. Vegetative parameters: number of leaves per plant (n⁰ plant⁻¹), aerial and root fresh weight (FWa and FWr respectively in g plant⁻¹), aerial and root dry weight (DWa, DWr in g plant⁻¹), and aerial and root fresh and dry ratios (FWa/Fwr and DWa/Dwr respectively) of single grafted, double grafted, and non-grafted plants at the end of the experiment.

	Leaves	FW_a	FW_r	DW_a	DW_r	FW_a/FW_r	DW_a/DW_r
N	35.80 c	166.54 -	31.82 -	12.09 b	1.69 -	5.60 -	7.19 b
S	46.07 b	165.19 -	22.33 -	15.31 ab	1.59 -	7.56 -	9.61 a
D	54.27 a	226.41 -	34.77 -	20.09 a	2.13 -	6.83 -	9.54 a
Significance F(values)	0.001	0.210	0.402	0.020	0.230	0.445	0.008

Different letters in columns indicate significant differences at $P<0.05$ using the LSD test.

N: Non-grafted plants; S: Simple grafted plants; D: Double grafted plants.

Table 2. Water absorption (WA) (mL plant^{-1} d^{-1}) and nutrient uptake (mg plant^{-1} d^{-1}) of single grafted, doubled grafted, and non-grafted plants during the experiment.

	WA	N-NO$_3$	P	K	Ca	S	Mg	Fe	Mn	Zn	B	Cu	Na
N	324.2 b	210.2 b	11.4 b	491.9 b	47.5 b	12.0 -	15.1 -	0.157 ab	1.36 b	0.13 c	0.12 a	0.034 a	14.4 a
S	374.4 ab	213.0 b	9.6 b	531.4 ab	47.4 b	15.8 -	13.3 -	0.258 a	1.54 b	0.15 b	0.10 ab	0.029 a	8.6 ab
D	453.6 a	266.4 a	15.5 a	630.5 a	66.0 a	12.6 -	17.6 -	0.049 b	1.83 a	0.17 a	0.05 b	0.006 b	6.3 b
Significance F(values)	0.047	0.044	0.013	0.049	0.045	0.844	0.609	0.024	0.007	0.005	0.033	0.033	0.045

Different letters in columns indicate significant differences at *P<0.05* using the LSD test.

N: Non-grafted plants; S: Simple grafted plants; D: Double grafted plants.

Table 3. Nutrient uptake per dry weight (mg g^{-1}) of single grafted, doubled grafted, and non-grafted plants at the end of the experiment.

	N-NO$_3$	P	K	Ca	S	Mg	Fe	Mn	Zn	B	Cu	Na
N	153.88 a	8.37 a	36.31 -	34.35 -	8.76 -	10.80 -	0.113 a	0.997 a	0.094 a	0.089 a	0.025 a	10.50 a
S	127.13 ab	5.76 ab	31.61 -	28.35 -	9.33 -	7.94 -	0.150 a	0.917 ab	0.088 ab	0.056 ab	0.017 a	5.09 b
D	119.77 b	6.96 b	28.41 -	29.64 -	5.70 -	7.93 -	0.022 b	0.822 b	0.075 b	0.023 b	0.003 b	2.89 b
Significance F(values)	0.045	0.058	0.156	0.182	0.762	0.552	0.015	0.041	0.040	0.010	0.007	0.013

Different letters in columns indicate significant differences at $P<0.05$ using the LSD test.

N: Non-grafted plants; S: Simple grafted plants; D: Double grafted plants.

Table 4. Influence of grafting (simple and double) on photosynthetic activity and chlorophyll fluorescence parameters.

	Amax	gs	Ci	E	WUE	φPSII	ETR	Fv/Fm
N	24.4	0.62 a	302 a	13.0 a	2.0 b	0.336 b	171 b	0.77
S	21.8	0.34 b	241 ab	9.0 b	2.7 a	0.343 ab	182 ab	0.79
D	23.3	0.39 b	239 b	9.7 b	2.7 a	0.376 a	192 a	0.78
Significance F(values)	0.710	0.040	0.010	0.040	0.020	0.010	0.010	0.590

Different letters in columns indicate significant differences at $P<0.05$ using the LSD test.

N: Non-grafted plants; S: Simple grafted plants; D: Double grafted plants.

Amax: net CO_2 fixation rate (μmol CO_2 m^{-2} s^{-1}); gs: stomatal conductance to water vapour (mol H_2O m^{-2} s^{-1}); Ci: substomatal CO_2 concentration [μmol CO_2 mol^{-1} (air)]; E: transpiration rate (mol H_2O m^{-2} s^{-1}) WUE: water use efficiency (μmol CO_2 mol^{-1} H_2O); φPSII: quantum efficiency of PSII photochemistry [(F`m-Fs)/Fm`]; ETR: relative linear transport rate (μmol electron m^{-2} s^{-1}); Fv/Fm: maximum quantum yield of PSII.