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# A screening test for the determination of cut flower longevity and ethylene sensitivity of carnation

**Juan Manuel Sánchez Díaz[1], Silvia Jiménez-Becker[1*], Manuel Jamilena[2]**

[1]*Department of Agronomy, University of Almería, La Cañada de San Urbano, Almería, Spain*

[2]*Department of Applied Biology, University of Almería, La Cañada de San Urbano, Almería, Spain*

**Corresponding author: sbecker@ual.es*

**Abstract**

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Strategies to prevent postharvest losses include the use of genotypes that have a longer life. The objective of this study was to develop a screening test for the estimation of cut flower postharvest life and the response to exogenous ethylene of different carnation cultivars at an early stage of plant growth. Ethylene sensitivity and production in different cut flower cultivars was evaluated, and a similar response in the vegetative stage was studied. Also, the possible relationship between the morphological parameters of cuttings and flower postharvest life was studied. Ethylene production of cuttings may be a useful tool for estimating ethylene production of cut flowers. There is a strong relationship between cut flower vase life and the root length of cuttings, as well as cut flower ethylene sensitivity and the number of internodes the cuttings have. Applications of exogenous ethylene to cutting cultivars have an effect on the growth parameters of the cuttings, but the response to ethylene in cut flowers does not behave in the same way in the vegetative stage.

**Keywords**: cutting; hormones; root growth; postharvest; climacteric flower

Ethylene gas has profound effects on a diverse array of plant growth and development processes, including germination, flowering, senescence, abscission, fruit ripening and yield (Archambault et al. 2006). A well-known effect of ethylene on plant growth is the so-called 'triple response' of etiolated dicotyledonous seedlings. This response is characterized by the inhibition of hypocotyl and root cell elongation, radial swelling of the hypocotyl, and exaggerated curvature of the apical hook (Guo, Ecker 2004). The ethylene-induced triple response was exploited to design an elegant genetic screen that readily identified insensitive mutants such as tall seedlings in a lawn of short seedlings (Muday et al. 2012). Moreover, ethylene modulates responses to plant hormones, such as jasmonic acid, salicylic acid, auxin, abscisic acid (ABA), and cytokinin, but the mechanisms that control each of these critical hormone interactions are largely unknown (Guo, Ecker 2004). The earliest genetic evidence that ethylene and auxin may act through convergent pathways to regulate root growth comes from the identification of ethylene-insensitive mutants with defects in auxin transporters and ethylene insensitive roots (Muday et al. 2012). These observations were supported by the demonstration that mutants with enhanced ethylene signaling, or synthesis, have reduced root elongation (Muday et al. 2012). In this regard, treatment with ethylene or 1-aminocyclopropane-1-carboxylic acid (ACC) reduces lateral root initiation in both Arabidopsis and tomato. There are numerous and often contradictory reports on the effect of ethylene on root growth which appear to depend on the concentrations applied. Low concentrations

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(1 ppm) may enhance root elongation, whereas high concentrations severely inhibit root elongation, but simultaneously increase root diameter and root hair formation (Marschner 1995).

Most horticulturists are involved to some extent in some aspects of postharvest horticulture, at least as consumers desiring ornamentals with an attractive appearance and long postproduction life (Kader 2003). Strategies to prevent losses include the use of genotypes that have a longer postharvest life (Ebrahimzadeh et al. 2008). Flower senescence in most carnation cultivars is characterized by autocatalytic ethylene production and subsequent wilting of the petals (climacteric flower) (Satoh et al. 2005), but some long-lasting cultivar flowers are associated with the reduction, delay or even the absence of ethylene (Wu et al. 1991) and thus, this has an influence on their vase life (Nukui et al. 2004). This means that the production of ethylene and long postproduction life varies among carnation cultivars. The carnation flower is also highly sensitive to exogenous ethylene (Onozaki et al. 2001), but its response to ethylene exposure also depends on the cultivar. Some long-life cultivars, such as 'Chinera' and 'Epomeo', have lower ethylene sensitivities than the normal Sim-type cultivar 'White Sim' (Wu et al. 1991). However, Ebrahimzadeh et al. (2011) observed that some long vase life cultivars show high ethylene responsiveness and, in contrast, cultivars with a short vase life show low responsiveness. In this regard, Onozaki et al. (2004) reported that Mediterranean carnation cultivars show more variation in ethylene production and sensitivity than Standard Sim-type cultivars.

The development of a technology that allows rapid and early detection of the ethylene responsiveness and postharvest life of the different cultivars may be very useful. The objective of this study was to develop a screening test for the determination of cut flower postharvest life and the response to exogenous ethylene of different carnation (*Dianthus caryophyllus* L.) cultivars, at an early stage of the plant growth. Ethylene sensitivity and production in six different cultivars: 'Dover', 'Famosa', 'Fuente', 'Hugo', 'Master' and 'Mundo' of cut flowers was evaluated, and a similar response in the vegetative stage was studied. Also, the possible relationship between the growth parameters of cuttings (stem diameter, root length, internode number, dry weight of radical and aerial part) and flower postharvest life was studied.

## MATERIAL AND METHODS

**Plant material and treatment**. Ten carnation (*Dianthus caryophyllus* L.) flowers of six different cultivars: 'Dover', 'Famosa', 'Fuente', 'Hugo', 'Master' and 'Mundo' were harvested from the Barberet & Blanc Company (Murcia, Spain) and transported to the laboratory on the day of harvest. The flowers were harvested at the early stage of flower opening and immediately transferred to the laboratory at the University of Almeria, Spain. In the laboratory, the flowers were trimmed to a stem length of 30 cm, randomized and divided into two groups. The first group of flowers was exposed to 10 ppm of ethylene for 8 h with the flowers held in distilled water. The other groups of flowers (control) were held in distilled water in an air atmosphere for 8 hours. At the end of the treatments (start of the measurements), the flowers were held in distilled water. The distilled water was replaced every two days. The environmental conditions maintained throughout the experiment were: 12 h light, 12 h darkness at 21 ± 1°C and 60–70% relative humidity.

In addition, 75 cuttings of the same previously mentioned cultivars were harvested. Twenty-five were used to measure ethylene production and the rest were divided into two groups. Half of the cuttings of each cultivar were treated with 10 ppm of ethylene for 24 h, and the rest were held in an air atmosphere for 24 hours. At the end of the treatments (start of the measurements), the cuttings were transplanted into a hydroponics system for 30 days. Experiments were carried out in a propagation tunnel. The growing system consisted of polystyrene panels floating in polystyrene trays filled with 10 l of nutrient solution. The polystyrene panels had holes in them and a cutting was placed in each hole. The nutrient solution was pumped to provide enough oxygen to the systems. The nutrient solutions were replaced with fresh solution every week. The concentrations in the standard nutrient solution for $H_2PO_4^-$, $NO_3^-$, $SO_4^{2-}$, $K^+$, $Ca^{2+}$ and $Mg^{2+}$ were 1.5, 12.0, 1.1, 5.8, 3.8 and 1.0 mmol/l, respectively. Heating was set to maintain a minimum of 22°C and a relative humidity of at least 65% was maintained by a fog-system.

**Carnation vase life**. The vase life of each flower was defined as the number of days after cutting until the petals showed in-rolling or browning, and had no decorative value. Flowers were evaluated daily. Values of vase life were the mean of 5 flowers.

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The ethylene sensitivity of the cultivars was estimated as the ratio between the vase life of the control flowers and the vase life of treated ones. Cut flowers were classified as follows: cultivars with a value around 1 show a low response to ethylene, values around 2 a mid-level response and those with values near to 3 are cultivars with a high rate of ethylene responsiveness.

**Ethylene production**. Ethylene production of flowers was measured in five replicates every day during the experiments. Firstly, petals were separated by hand from other parts of flowers, and then the outer petals of each flower were enclosed in 20-ml glass jars and incubated at 21°C. After 60 min incubation, a 1-ml gas sample was injected into a gas chromatograph (Varian 3900; Varian Analytical Instruments, Walnut Creek, USA) fitted with a flame ionisation detector (FID) and a GS-Q 30 m × 0.25 mm × 25 um ID column (Agilent J&W, Agilent Technologies, Santa Clara, USA) based on the chromatographic conditions reported by Ebarhimzadeh et al. (2011). To measure the ethylene production of the untreated cuttings, 5 cuttings per replicate were enclosed in 1 l jars for 8 hours. 1 ml of gas was then withdrawn and the ethylene concentration was determined by gas chromatography. There were 5 replications.

**Morphological parameters of the cuttings**. Morphological parameters were registered every five days during the trial. The values of these parameters were the mean of 25 cuttings. Stem diameter and root length were measured with a digital LIMIT calliper (Whitworth, New York, USA) with a sensitivity of 0.01 mm. The number of internodes and root initiation development were counted. Fresh and dry weight was measured at the end of the trial. Roots and stems, petioles and leaves (aerial part) were weighed separately on a COBOS series CSC scale (precision 0.01 g) to determine fresh weight. Afterwards, they were dried in a NüveE FN500 oven (Nuve, Ankara, Turkey) (range 30°C to 300°C) at 60°C for 48 h to determine dry weight.

**Experimental design and statistical analysis**. The experimental design was completely randomized with 6 cultivars, 2 treatments (control and exogenous ethylene) and 5 replications per treatment in the case of the flowers, 25 replications per treatment in the case of cuttings morphological parameters and 5 replications in the case of cutting ethylene production.

An analysis of variance (ANOVA) and the Least Significant Difference (LSD) by $P < 0.05$ were conducted. Simple regressions between cut flower vase life, ethylene sensitivity, ethylene production and the morphological parameters of cuttings (stem diameter, root length, internode number, dry weight of radical and aerial part ) were carried out. From all the parameters considered, the one with the highest coefficient of determination was selected. Analysis of data was made using the software packages Excel 7.0 and Statgraphics Centurion XVI.II.

## RESULTS AND DISCUSSION

### Vase life

The studied cut flower cultivars exhibited a wide range of variation in vase life (Fig. 1). 'Famosa' cut flowers, 54 days, have the longest vase life among the six selected cultivars, followed by the 'Dover' and 'Fuente' flowers. The shortest vase life was for 'Hugo' flowers, only 27 days. Treating the carnation cultivars with ethylene reduced the postharvest life of all of the cultivars (Fig. 1). The mean vase life of the studied cultivars when exposed to exogenous ethylene was 15 to 38 days, whereas untreated flowers had a vase life in the longevity range of 27–54 days. The results showed that the 'Fuente' cv. is highly responsive to exogenous ethylene, and 'Master' and 'Mundo' demonstrate a low responsiveness to exogenous ethylene. Cut flower cultivars respond differently to exogenous ethylene; this variation in response to ethylene treatment has a genetic cause (Onozaki et al. 2008). Moreover, the hypothesis that natural longevity was associated with low sensitivity to ethylene was not sustained

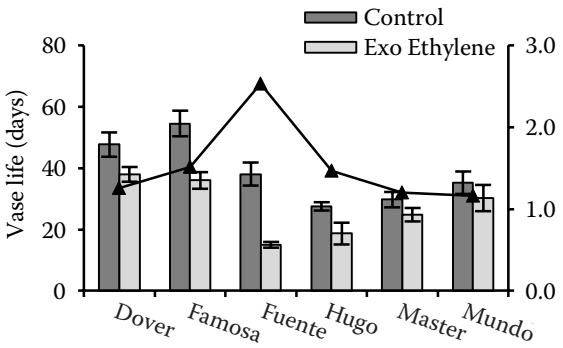


Fig. 1. Vase life and ethylene sensitivity among six carnation cultivars treated and untreated with ethylene
values of vase life are the means of five flowers ($P < 0.05$)

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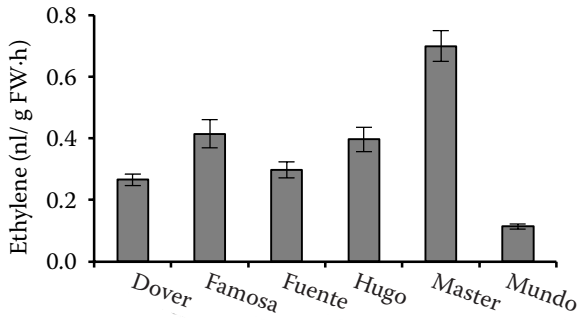


Fig. 2. Cutting ethylene production (nl/g FW·h) among the six cultivars investigated

(Müller et al. 1998). Both the 'Famosa' cv. with the longest vase life, and 'Hugo' with the shortest vase life have a mid-level response to ethylene (1.5).

## Ethylene production

Notable differences in the ethylene production of cut flowers and the ethylene production of cuttings were observed among the six investigated cultivars (Fig. 2). The ethylene production of the cut flowers had a variant in the range of 30.8 to 2.3 nl/g FW·h (Fig. 3). The highest ethylene production for the cuttings was found in the cv. 'Master' (0.70 nl /g FW·h) and the lowest in the cv. 'Mundo' (0.11 nl/g FW·h). In addition, there was a correlation ($R^2$ = 0.78) between the ethylene production of the cut flowers and the ethylene production of the cuttings (Fig. 3) that would permit, knowing the ethylene production in an early stage of the growing crop, an estimation of the ethylene production of cut flowers.

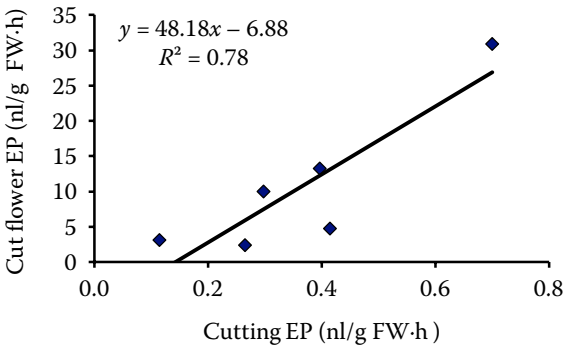


Fig. 3. Correlation between cut flower ethylene production (nl/g FW·h) and cutting ethylene production (nl/g FW·h)

## Morphological characteristics of the vegetative cuttings

There were noticeable differences between cultivar stem diameters (Fig. 4a). For example, 'Master' has the smallest stem diameter and maintained a small stem diameter over a long period, while the 'Hugo' cultivar has a large stem diameter which started to increase after only a few days. A simple regression between stem diameter and vase life, ethylene sensitivity and production was carried out (Table 1). Ethylene production, vase life and ethylene sensitivity had no clear relationship with stem diameters.

Notable differences in root length and rooting initiation were observed among the 6 investigated cultivars (Fig. 4b). The 'Mundo' showed early and long root growth and 'Fuente' shorter root length. Cut flower vase life correlated with root length 10, 15 and 20 days after transplanting ($R^2$ = 0.82, 0.85 and 0.79, respectively) (Table 2). Fig. 5 shows the relationship between root length and vase life 10 days after transplanting. It can be seen that cultivars with quick rooting and long root length showed low cut flower vase life. Auxins play an important role in the regulation of root growth and development; they promote the formation of lateral roots, or at least their initiation (Marschner 1995). However, just the opposite can be found in cut flowers; a high concentration of auxin promotes petal senescence in carnations by enhancing ethylene production (Bhattacharjee, Dee 2005) and that causes a reduction in the vase life of cut flowers. On the other hand, in this assay the relationship between ethylene synthesis and root elongation (Muday et al. 2012) could not be found.

Notable differences in the number of internodes were observed among the six investigated cultivars (Fig. 6). 'Mundo' had the higher number of internodes (9.45) and 'Fuente' the lowest (7.53). This data show a negative correlation between the number of internodes and ethylene sensitivity ($R^2$ = 0.79) (Table 3) (Fig. 7). Cultivars such as 'Fuente' that have a high responsiveness to ethylene, showed a low number of internodes. On the other hand, 'Mundo' showed a high number of internodes and a low responsiveness to ethylene (Fig. 7). This study indicates the possibility of predicting ethylene sensitivity based on the number of internodes.

Differences in root and aerial dry weight were observed among the 6 investigated cultivars (Fig. 8). Arial and root dry weight did not show a clear re-

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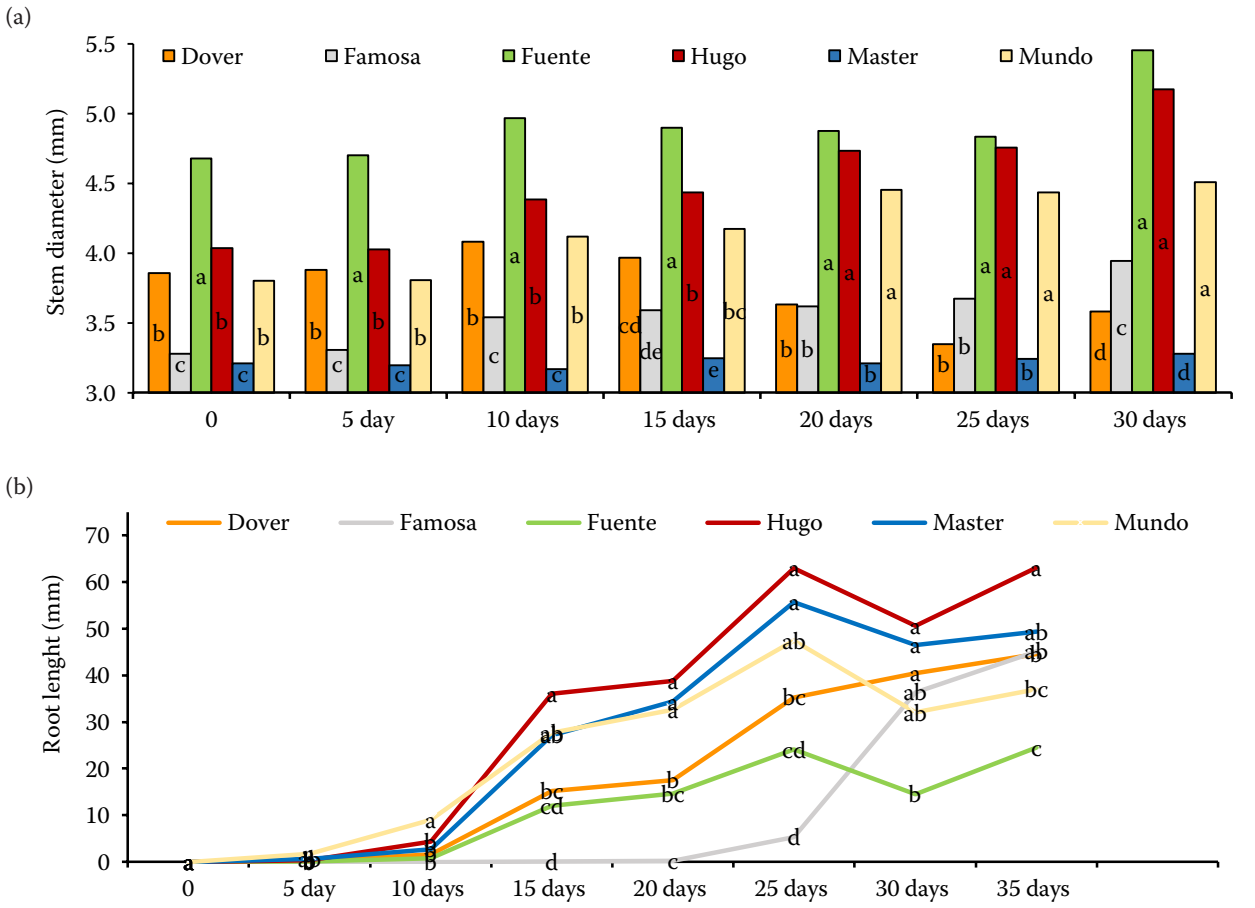


Fig. 4. Temporal change of (a) the stem diameter and (b) root length during the assay among the six cultivars investigated
Bars with different letters are significantly different between cultivars

lationship with vase life, ethylene sensitivity and production (Table 3).

### Response of cuttings and cut flowers to exogenous ethylene among carnation cultivars

Applications of 10 ppm of exogenous ethylene to the cutting of cultivars increased stem diameter, root length, internode number and dry weight of radical and aerial part (data not shown). These observations agree with those of Archambault et al. (2012) who found early reductions in stem lengths of cereal and canola cultivars in response to the application of ethephon. However, Marschner (1995) reports that the effect of ethylene on root growth appears to depend on the concentrations applied. Low concentration may enhance root elongation, whereas high concentrations se-

Table 1. Simple regression between vase life, ethylene sensitivity and production and stem diameter

| Stem diameter | 0 day | 5 days | 10 days | 15 days | 20 days | 25 days | 30 days |
|---|---|---|---|---|---|---|---|
| Vase life | **0.79** | 0.00 | 0.16 | 0.30 | 0.13 | 0.00 | 0.11 |
| Ethylene sensitivity | 0.13 | 0.01 | 0.19 | 0.00 | 0.00 | **0.88** | 0.18 |
| Ethylene production | 0.68 | 0.47 | 0.31 | 0.04 | 0.24 | 0.15 | 0.01 |

values in bold indicate a strong relationship; it is the coefficient of determination, without unit stem diameter (mm)

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Table 2. Simple regression between vase life, ethylene sensitivity and production and root length

| Root length (mm) | 0 day | 5 days | 10 days | 15 days | 20 days | 25 days | 30 days |
|---|---|---|---|---|---|---|---|
| Vase life | 0.16 | 0.28 | **0.82** | **0.85** | **0.79** | 0.08 | 0.10 |
| Ethylene sensitivity | 0.28 | 0.20 | 0.07 | 0.08 | 0.09 | 0.46 | 0.24 |
| Ethylene production | 0.06 | 0.02 | 0.28 | 0.37 | 0.35 | 0.11 | 0.06 |

values in bold indicate a strong relationship; it is the coefficient of determination, without unit

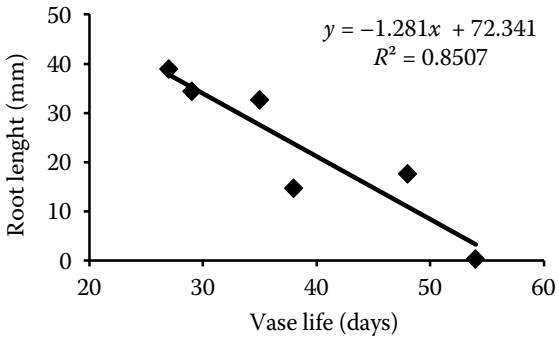


Fig. 5. Temporal change of the root length during the assay among the six cultivars investigated

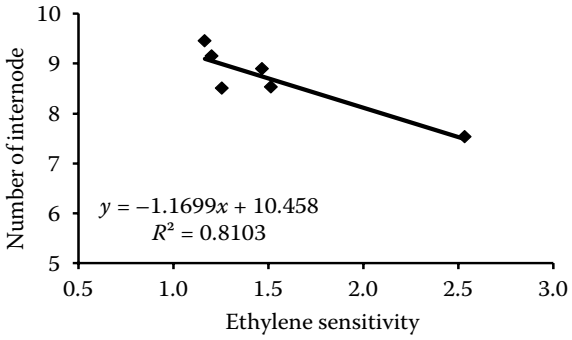


Fig. 7. Number of internodes of the six cultivars investigated

verely inhibit root elongation, but simultaneously increase root diameter and root hair formation (Marschner 1995). Other authors observed few significant differences between treated and control plants in total vegetative above-ground biomass and root mass in cereals (Archambault et al. 2006). The cultivars showed different responses to ethylene treatment in the vegetative stage (cutting) and cut flower stage. Therefore no relationship between the cut flowers' response to ethylene and cuttings' response to ethylene were found. That is, cut flower cultivars that are sensitive to ethylene do not demonstrate the same sensitivity to ethylene in the vegetative stage. These results indicate that the developmental stage influences ethylene sensitivity (Edelman, Jones 2014) and this can vary depending on the developmental age or organ type (i.e. flowers, leaves or roots) (Edelman et al. 2014). Edelman and Jones (2014) found that there was no consistent correlation between seedling and mature plant response within the Solanaceae accessions that they evaluated.

Moreover, all selected cultivars are commercial cultivars with a relatively long vase life. Breeding programs were selected for reduced ethylene sensitivity to create cut carnation varieties with extended vase life (Onazaki et al. 2001). It may be very interesting to extend this study to non-commercial cultivars with extremely short vase lives and ethylene sensitivity, since this screening test is a prelimi-

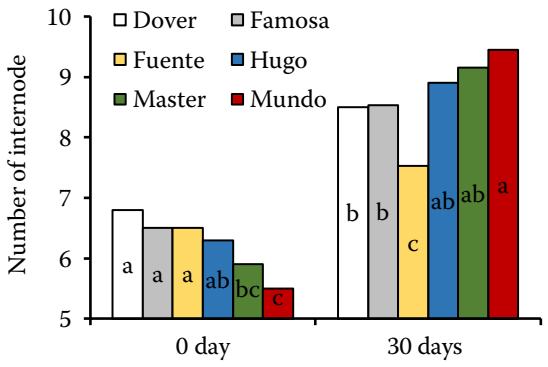


Fig. 6. Simple regression between root length and vase life

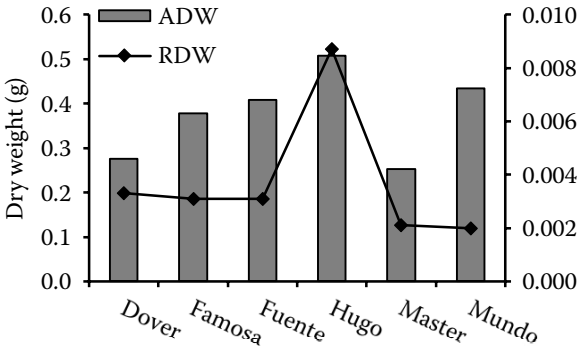


Fig. 8. Simple regression between number of internodes and ethylene sensitivity

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Table 3. Simple regression between vase life, ethylene sensitivity and production and number of internodes, root dry and fresh weight

| | No. of internode | | Root dry weight 30 days (g) | Fresh dry weight (g) | |
|---|---|---|---|---|---|
| | 0 day | 30 days | | 0 day | 30 days |
| Vase life | 0.00 | 0.14 | 0.14 | 0.00 | 0.08 |
| Ethylene sensitivity | 0.19 | **0.79** | 0.05 | 0.11 | 0.12 |
| Ethylene production | 0.30 | 0.15 | 0.01 | 0.51 | 0.08 |

values in bold indicate a strong relationship; it is the coefficient of determination, without unit

nary test to select cultivars with a relatively long vase life and low ethylene sensitivity.

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