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A Major Gene Conferring Reduced Ethylene Sensitivity and Maleness in *Cucurbita pepo*

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Abstract External treatment with ethylene had indicated earlier that this hormone is the main factor controlling sex determination in *Cucurbita pepo*. Up to now, however, there was no genetic evidence that supported the relationship between ethylene production, or perception, and sexual expression in this species. Here we demonstrate that the extreme male phenotype of the *Vegetable Spaghetti* (*Veg*) inbred line of *C. pepo* subspecies *pepo* is determined by a major gene that confers reduced ethylene sensitivity in plants. The production of female flowers in the *Veg* line is very delayed and reduced with respect to the contrasting *Bolognese* (*Bog*) line, ranging between 5 and 35% of female flowers per plant. This enhanced maleness trait segregates as a single gene in the F_2 and backcross (BC) generations, and co-segregates with a weak ethylene-insensitive phenotype in the F_2 population, suggesting that the gene responsible for the *Veg* phenotype could be the result of a mutation in a receptor or response gene for ethylene. Although the etiolated seedlings of the *Veg* line, and the most androecious plants in the F_2 generation, produce more ethylene than those of the contrasting line, they are less sensitive to this hormone, as indicated by a weaker triple response and a delayed abscission of ethylene-treated male flowers. Given that the sexual phenotype of F_2 plants is correlated with ethylene sensitivity, with the more sensitive plants producing the higher number of female flowers, our results demonstrate that the ethylene response is directly involved in the control of sex determination in *C. pepo*. It regulates the induction of female flower production, and therefore the extension of the initial phase of development in which the plant produces only male flowers, as well as the number of female flowers per plant.

Keywords *Cucurbita pepo* · Sex expression · Ethylene production · Ethylene sensitivity

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Introduction

Cultivated species of the family Cucurbitaceae, including cucumber, melon, watermelon, and squash develop separate male, hermaphrodite, and female flowers that are distributed differently in the leaf axils of both the main and the secondary shoots (Rudich 1990). There are varieties of *Cucumis sativus* that show all the different sex phenotypes: hermaphrodites (only hermaphrodite flowers), monoecious (male and female flowers), andromonoecious (male and hermaphrodite flowers), gynoecious (only female flowers), and androecious (only male flowers). By contrast, varieties of *Cucurbita pepo* are all monoecious and produce male or female flowers in the leaf axils. Sex expression of *C. pepo*, as in other species of the family, varies throughout plant development and it is possible to distinguish three different developmental phases (Peñaranda and others 2007). During the first phase plants produce only male flowers. After the induction of female flower production, the second phase is characterized by the alternation of female and male

flowers, and in the third phase plants produce only female flowers. This last female phase has been observed in only some varieties under certain growing conditions (Peñaranda and others 2007).

The expression of sex in the Cucurbitaceae is known to be controlled by various genetic, environmental, and hormonal factors. In *C. sativus* and *C. melo* it is controlled by three major independent genes and their particular combination explains the main sex phenotypes found in these two species (Galun 1961; Kubicki 1969; Kenigsbuch and Cohen 1990; Pierce and Wehner 1990; Rudich 1990; Perl-Treves 1999). Sex expression in this family can be modified by environmental factors, including light intensity, photoperiod, and temperature. Winter conditions, with short days, low light intensity, and low night temperatures, promote the production of female flowers, whereas summer conditions favor the production of male flowers in different cucurbit species (Wien 1997; Peñaranda and others 2007). In *C. pepo* low temperature inhibits the development of male flowers and increases the number of female flowers per plant (Wien and others 2004), whereas high temperature induces the production of male flowers (Peñaranda and others 2007).

Phytohormones are the main modulators of sex expression in cucurbit species. The effects of the exogenous application of hormones and hormone inhibitors on sex expression and the measurements of the levels of the different hormones in varieties with different sex phenotypes have demonstrated that gibberellins, auxins, brassinosteroids, and especially ethylene play an important role in sex expression in these species. Treatments with gibberellins or gibberellin inhibitors promote the production of male or female flowers, respectively (Rudich and others 1972; Wien 1997). Auxins induce the development of carpels and increase the production of female flowers per plant, although their effects may be mediated by ethylene (Takahashi and Jaffe 1984; Trebitsh and others 1987; Rudich 1990). Nevertheless, the main hormone in the control of sex expression in the Cucurbitaceae is undoubtedly ethylene. The level of ethylene in flower buds seems to be essential for both sex determination and female flower development and differentiation. Thus, ethylene treatments promote the production of female flowers, whereas treatments with inhibitors of ethylene biosynthesis and perception, such as aminoethoxyvinylglycine (AVG) or silver thiosulfate (STS), increase the number of male flowers per plant (Rudich and others 1969; Byers and others 1972; DenNijs and Visser 1980; Owens and others 1980; Rudich 1990). Besides external treatments with phytohormones, there is increasing molecular evidence that ethylene production or perception is essential for sex determination and flower development in species of Cucurbitaceae. In cucumber, gynoecious lines produce more ethylene than monoecious or andromonoecious lines (Owens and others 1980; Yamasaki and others 2001) and have an additional gene for ACS1 (1-aminocyclopropane-1-carboxylate synthase 1) (Trebitsh and others 1997; Mibus and Tatlioglu 2004; Knopf and Trebitsh 2006). In melon, gynoecious lines also produce more ethylene than andromonoecious or monoecious lines (Manzano and others 2008), and the reduction in the production of ethylene in andromonoecious lines results from a mutation in an ACC-synthase gene (Boualem and others 2008). The expression of sex in transgenic melon lines constitutively expressing *ACS*, or the mutant ethylene receptor *At-etr1* of *Arabidopsis,* has also indicated that ethylene production and perception both have an important role in sex expression and flower development (Papadopoulou and others 2005; Little and others 2007).

Because the varieties of *C. pepo* are all monoecious, there have been no studies that have correlated the sexual expression of a genotype with its ethylene production and/or perception. We have identified a *C. pepo* inbred line (*Vegetable Spaghetti*) with an enhanced maleness phenotype. The sex phenotype of this line co-segregates with a weak ethylene-insensitive phenotype determined by a single major gene. The genetic and physiological results of this study demonstrate that ethylene sensitivity of *C. pepo* plants, assessed by the triple response of etiolated seedling or by the abscission of cut male flowers when treated with ethylene, is directly correlated with its sexual expression, with more sensitive plants producing the highest percentage of female flowers.

Materials and Methods

Plants, Growth Conditions, and Evaluation of Sexual Expression

Two inbred lines of *Cucurbita pepo* subspecies *pepo* of the *Veg*etable Marrow Group, *Vegetable Spaghetti* (*Veg*) and *Bolognese* (*Bog*), kindly provided by Dr. Harry S. Paris of the Newe Yaár Research Center, Israel, were studied. The *Veg* and *Bog* lines are the results of 10 and six generations of selfing, respectively.

The evaluation of sex expression in the inbred lines was carried out under two different greenhouse environments: fall-winter 2006-2007 (average temperature and daylength of 14.7°C and 8 h) and spring-summer 2007 (average temperature and daylength of 24.8°C and 14.5 h). Seeds were germinated on wet filter paper in Petri dishes and then transplanted to 1-m rockwool slabs at a density of two plants per slab. Plants were grown in a greenhouse in Almería (Spain) following standard local commercial practices for both plant nutrition and pest and disease control.

Fruits were harvested once or twice a week, depending on the growing season. The field plan was a randomized complete block design with three blocks, and six replicates per block, for each treatment. The F_2 and backcross (BC) generations from an initial cross between *Veg* and *Bog* were obtained, and the sex expression of individual plants of each population determined under fall-winter conditions for 2006-2007. *Bog*, *Veg*, and F_1 populations were also evaluated under spring-summer conditions in 2007. At least 15 plants per generation and growing season were tested.

Sex expression in each plant was estimated as both the number of initial nodes with male flowers before the production of the first female flower (earliness) and the percentage of female or male flowers per plant in the first 20 nodes of the main stem (femaleness or maleness).

Evaluation of Ethylene Production and Sensitivity

For ethylene measurements organs were excised from the plants and incubated at room temperature for 24 h in hermetic containers in the dark, and ethylene production was determined by gas chromatography. To assess the ethylene sensitivity of each inbred line, germinated seeds were incubated in air with 20 ppm of ethylene for 4 days in darkness and the ethylene response was determined by comparing the length and the thickness of hypocotyls and roots in ethylene-treated and nontreated seedlings after six additional days in darkness. To test for ethylene response in plants in the F_2 generation, male flower buds (1-2 days before anthesis) were removed from the plant and placed in glass jars with water in a 30-l hermetic container. The floral buds were incubated in air with 20 ppm of ethylene during 2 days, and the number of hours to abscission was scored in each flower during a total of 3 days post-treatment. The sensitivity to ethylene was evaluated in at least 15 flowers of each sex phenotype in the F_2 population.

Statistical Analysis

Differences between lines in each season trial (fall-winter or spring-summer) were determined by an analysis of variance (ANOVA), followed by Tukey's multiple-comparison test by using the STATISTIX 8.0 software package. The χ^2 test was used to determine if the observed phenotypic proportions in the F_2 and BC generations fit the expected Mendelian segregation ratios for a single gene.

Results

The Predominantly Male Phenotype of the *Veg* Inbred Line Is Controlled by a Major Gene

The sex expression of *C. pepo* is characterized by two different phases of development, one initial male phase in which the plant produces only male flowers, and a second intermediate phase characterized by the alternate production of both male and female flowers. The sex expression was evaluated as the number of male flowers in the initial male phase of development and the number of male and female flowers per plant. Table 1 summarizes the results in the inbred lines *Veg* and *Bog* under both fall-winter and spring-summer conditions. The sex expression of *Bog*, although strongly female, was comparable to that in other varieties, developing the first female flowers between nodes 2 and 4 and producing a mean of 69 and 75% female flowers in spring-summer and fall-winter, respectively (Table 1; Fig. 1). In contrast, the sex phenotype of *Veg* was strongly male in both growing seasons. The development of the first female flower in the *Veg* inbred line was very delayed (over node 10), and the production of female flowers was low, with a mean of 20 and 22% female flowers per plant in summer-spring and fall-winter conditions, respectively (Table 1; Fig. 1). Fall-winter conditions had a small effect on the production of female flowers in both inbred lines but differences between the two growing seasons were not statistically significant (Table 1).

A formal genetic analysis was performed to assess the inheritance of the predominantly male phenotype in the *Veg* inbred line. A cross between *Bog* and *Veg* was made using the *Veg* inbred line as pollen donor, and the F_1, F_2,

Table 1 Sex expression of two inbred lines of *C. pepo* and the F_1 hybrid

Genotype	Number of initial male flowers per plant		Number of female flowers per plant (%)	
	Fall-Winter 2006–2007	Spring-Summer 2007	Fall-Winter 2006–2007	Spring-Summer 2007
Bolognese	2.60 ± 0.51 a	2.57 ± 0.66 a	15.33 ± 0.44 a (75.1%)	14.16 ± 0.56 a (69.1%)
Vegetable spaghetti	8.70 ± 0.51 c	10.16 ± 0.36 b	4.60 ± 0.42 c (22.2%)	4.16 ± 0.29 b (20.8%)
F_1 (*Veg* × *Bog*)	4.29 ± 0.39 b	Nd	12.29 ± 0.52 b (57.3%)	nd

nd not determined

The number of male and female flowers was scored in the first 20 nodes of the main stem of each plant. Values are means ± SD. Values followed by a different letter in each column are statistically different by Tukey's test at $P \leq 0.05$. $n \geq 15$ for each population and growing season

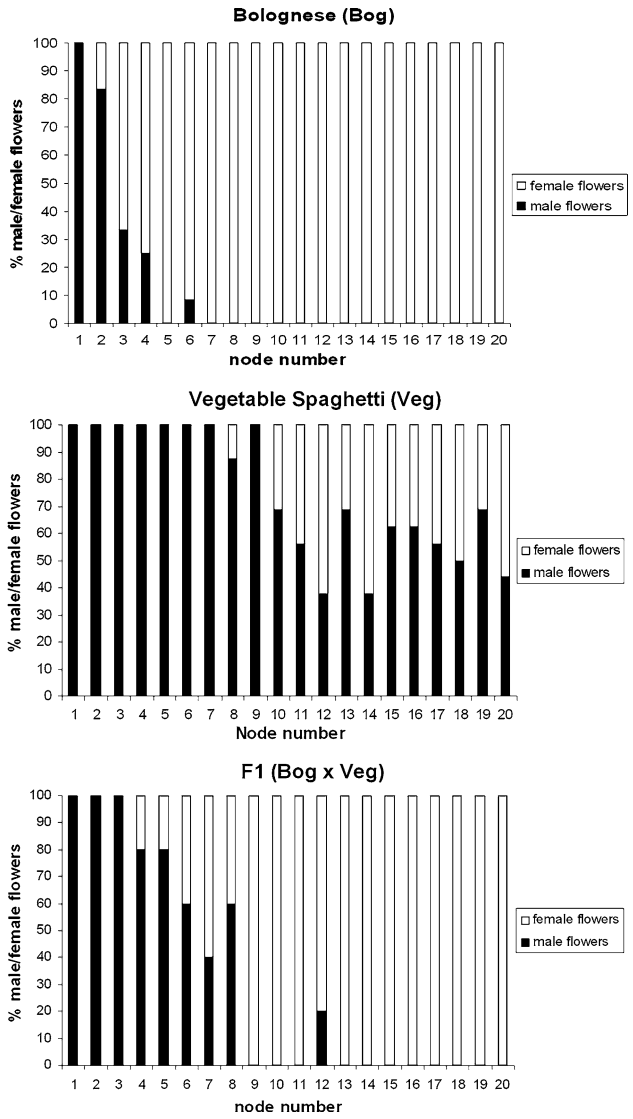

Fig. 1 Sexual expression of the inbred lines Bolognese (*Bog*) and Vegetable Spaghetti (*Veg*) as well as the F_1 (*Bog* × *Veg*) in the first 20 nodes of the plants. In each node, black and white bars represent the percentage of male and female flowers in the total of plants analyzed ($n \geq 15$ for each population). Evaluation was carried out in a greenhouse during fall-winter 2006–2007

and backcrossing (BC, F_1 × *Veg*) generations were obtained and characterized. The sex phenotype of F_1, although intermediate, was closer to the *Bog* parental line (Table 1; Fig. 2), suggesting that the male phenotype could be considered recessive. Nevertheless, the fact that the mean number of initial male flowers and the mean number of female flowers per plant in the F_1 generation differed significantly from *Bog* values (Table 1) indicates that the gene is not completely recessive.

Figure 2 shows a comparison of the distribution of the number of female flowers per plant in the F_2 and BC populations and also in the F_1 and in the parental lines *Veg* and *Bog*. The F_2 and BC generations showed the highest

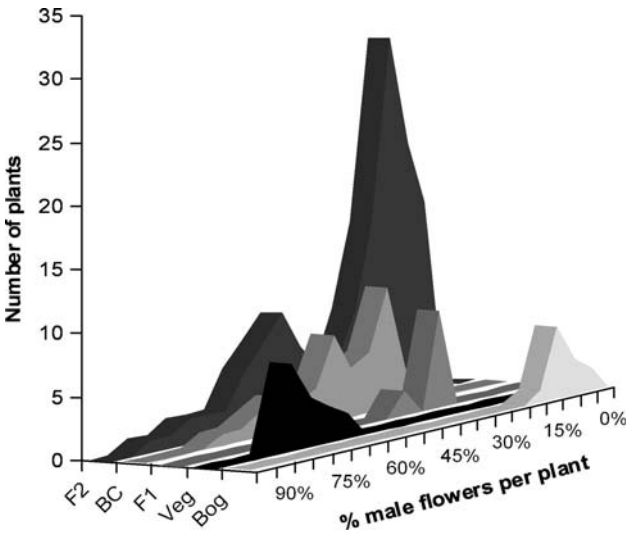

Fig. 2 Frequency distribution of the number of male flowers per plant in parental lines *Veg* and *Bog* as well as in the F_1, F_2, and BC generations. Note the bimodal distribution of F_2 and BC populations, with a clear separation between plants with less and more than 50% male flowers. All the generations were grown under the same conditions during fall-winter 2006–2007

variance for this trait compared with the respective parents and F_1, indicating segregation for the number of female flowers per plant. Moreover, the F_2 and BC generations did not fit a normal distribution but showed bimodality (Fig. 2) with two separated groups. Accordingly, plants were classified into two distinct classes: male-monoecious (MM, plants producing more than 50% male flowers) and female-monoecious (FM, plants producing less than 50% male flowers). The F_2 segregation (99:35) revealed a good fit to a 3:1 FM:MM segregation ratio ($\chi^2 = 0.022$; $P > 0.85$), whereas the BC segregation (22:20) fit a 1:1 ratio ($\chi^2 = 0{,}095$; $P > 0.75$). Because no group with an intermediate phenotype was distinguishable in the F_2 population, data indicate that the predominantly male phenotype of the *Veg* inbred line seems to be controlled by a single recessive gene which we named *Cucurbita pepo WEAK ETHYLENE INSENSITIVE* (*CpWEI*). However, the variation of the sexual phenotype of MM and FM groups in the F_2 and BC populations with respect to those of *Veg* and *Bog* also suggests that the major gene *VEG* would be interacting with other minor segregating genes.

Ethylene Sensitivity Is Reduced in the *Veg* Line

Given that ethylene is the main factor controlling sex determination in species of the Cucurbitaceae, we compared the level of internal ethylene and the sensitivity to this hormone in plants of both the *Bog* and *Veg* lines. Ethylene production was determined in complete seedlings with one true leaf and in 4-5 cm leaves from plants having

Table 2 Ethylene production and ethylene sensitivity of *Cucurbita pepo* inbred lines *Veg* and *Bog*

Inbred line	Ethylene production (nl C_2H_4/g FW * *h*)		Ethylene sensitivity Hypocotyl length (cm)			Ethylene sensitivity Root length (cm)		
	Seedlings	Leaves	Control	Ethylene 20 ppm	Reduction hypocotyl length	Control	Ethylene 20 ppm	Reduction root length
Bolognese	73.6 ± 6.7 a	19.9 ± 2.2 a	4.82 ± 1.27	0.44 ± 0.13	90.9%	6.48 ± 1.88	0.90 ± 0.26	86.1%
Vegetable spaghetti	123.6 ± 18.5 b	15.6 ± 3.9 a	3.24 ± 0.80	1.40 ± 0.38	56.8%	3.34 ± 0.59	1.58 ± 0.36	52.7%

Ethylene production was measured in individual seedlings with one true leaf as well as in 4-5 leaves from plants having 6-7 leaves. Measurements were carried out in at least ten seedlings of each inbred line and in leaves from 10 plants. Values followed by a different letter in each column are statistically different by Tukey's test at $P \leq 0.05$. Ethylene sensitivity was assessed as the reduction of hypocotyl and root length in 12 ethylene-treated etiolated seedlings in comparison with 12 control nontreated plants. Experiments were repeated three times

6-7 leaves. In contrast with what we expected according to their sex phenotypes, seedlings of the *Veg* line, during their earliest stages of development, produced significantly more ethylene than those of the *Bog* line (Table 2), although in later stages its production in young leaves was not statistically different from that of *Bog* (Table 2). On the other hand, the sensitivity to ethylene, assessed on the basis of the triple response of etiolated seedlings, was reduced in the *Veg* line (Table 2). The triple response, characterized by an inhibition of hypocotyl and root elongation, a radial swelling of the hypocotyl, and an exaggeration of the curvature of the apical region of the hypocotyl (apical hook), was evaluated in the *Veg* and *Bog* lines in three independent experiments with 12 replicates per variety (Table 2; Fig. 3). In the presence of ethylene the reductions in hypocotyl and root lengths were much less pronounced in the *Veg* line (Fig. 3), indicating that this line is less sensitive to ethylene. Whereas in the *Bog* line ethylene treatment reduced the length of hypocotyls and roots to 9.1 and 13.9%, respectively, in the *Veg* line the lengths of hypocotyls and roots were reduced to only 43.2 and 47.3%, respectively (Table 2).

We have also assessed the sensitivity to ethylene in *Bog* and *Veg* lines by comparing the abscission of ethylene-treated male flowers of the two lines. Twenty male flowers at the same stage of development of each line (1-2 days before anthesis) were treated with ethylene for 48 h. The number of flowers that reached abscission was recorded every 12 h for 3 days following the treatment (Fig. 4). The number of flowers that reached abscission was similar in the two lines immediately after treatment, but 3 days later the percentage of abscised flowers was 100% in *Bog* but only 60% in the *Veg* line (Fig. 4). As expected, in the non-ethylene-treated controls, the rate of abscission of male flowers was very low in both *Bog* and *Veg* lines (Fig. 4). Considering that the abscission time of the nondropping flowers of the *Veg* line was 5 days, we estimated that the abscission time of *Veg* male flowers (82.71 ± 6.20 h) was significantly higher than that of *Bog* (64.94 ± 5.06 h) (Fig. 5a). Given that the flower abscission process in *C. pepo* is sensitive to ethylene, these results confirmed the triple response experiment, indicating that the *Veg* line has reduced ethylene sensitivity.

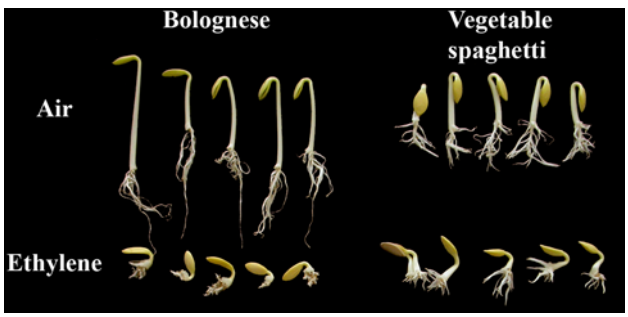

Fig. 3 Triple response of *Veg* and *Bog* inbred lines to ethylene. Germinated seeds were placed in hermetic containers with no ethylene or with 20 ppm of ethylene for 4 days

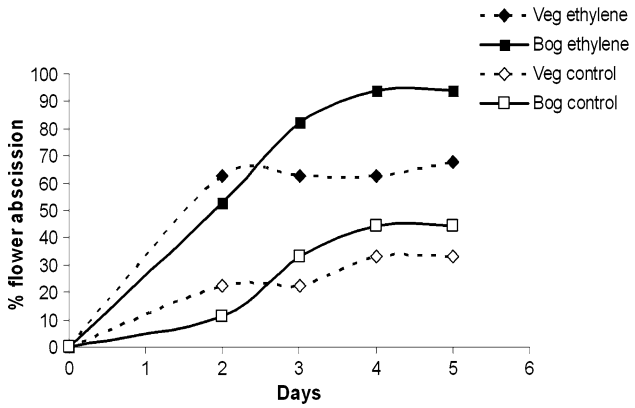

Fig. 4 Evolution of male flower abscission of *Bog* and *Veg* inbred lines during 5 days after cutting. Twenty male flowers at the same stage of development of each line were treated with ethylene or air (control) for 48 h in hermetic containers, and the abscission of each flower was recorded during 3 days after the treatment at intervals of 12 h. Because flowers were not all available in the same day, the experiment was repeated three times to analyze at least 20 male flowers for each genotype and treatment

The Predominantly Male Phenotype of *Veg* Co-segregates with a Weak Ethylene Sensitivity in the Segregating Population F_2

To determine whether the sexual phenotype of the *Veg* line is a response to its reduced ethylene sensitivity, we studied the relationship between ethylene sensitivity and sexual phenotype in the segregating F_2 population of the cross *Bog* × *Veg*. The sexual expression of 135 F_2 plants was individually recorded as the percentage of female flowers in the first 20 nodes of the plant. The sensitivity to ethylene was assessed by treating cut male flowers from each individual F_2 plant with ethylene for 48 h and recording the number of flowers that reached abscission at intervals of 12 h. From these data we estimated the abscission time of ethylene-treated male flowers from plants of the F_1 and F_2 generations. The abscission time of F_1 male flowers was intermediate but not significantly different from the abscission time of *Bog* flowers (Fig. 5a). When plants of the F_2 population were classified separately into FM and MM, as we did previously for the segregating analysis, the abscission of those male flowers derived from MM plants was significantly delayed compared with that from FM plants (Fig. 5a). Moreover, when F_2 plants were classified on the basis of their percentage of male flower production, separating them into groups that differ in 5% of male flowers (ranging from 5 to 95% male flowers per plant), a significant correlation was found between the percentage of male flowers per plant and the sensitivity to ethylene, which was assessed as the mean abscission time of cut male flowers after ethylene treatment ($R^2 = 0.723$; $P = 0.0005$) (Fig. 5b). Those F_2 plants that produced the highest number of male flowers were those that showed the lowest ethylene sensitivity and therefore the highest abscission time of ethylene-treated male flowers (Fig. 5b).

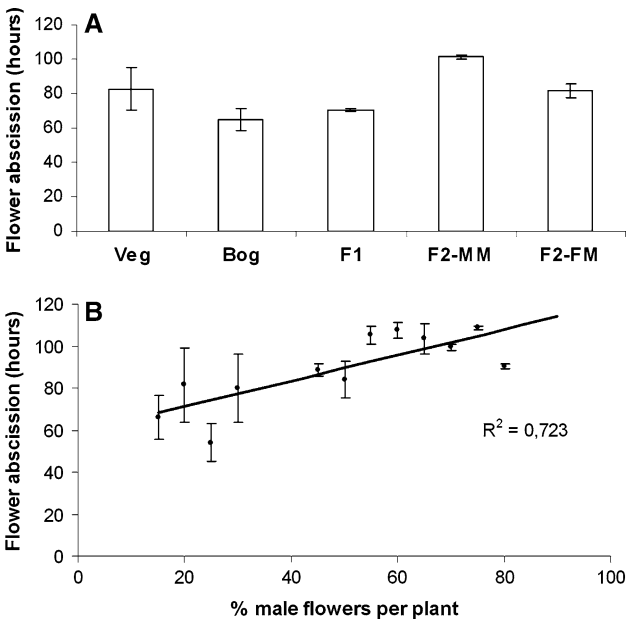

Fig. 5 **a** Comparison of abscission time of ethylene-treated male flowers derived from *Veg* and *Bog* inbred lines and F_1 as well as from female-monoecious (FM) and male-monoecious (MM) plants of the F_2 population. Male flower buds in the same stage of development were treated with ethylene during 48 h and their abscission evaluated every 12 h after treatment. Note that the abscission time of F_1 is very similar to that of *Bog* and that the flowers derived from most male plants of the F_2 population (MM) were less sensitive to ethylene and therefore delayed in abscission compared with most female plants of the F_2 population (FM). **b** Regression of the percentage of male flowers per plant onto abscission time of male flower buds treated with ethylene in the F_2 generation of *Bog* × *Veg*. The relationship between the two parameters is linear, with a correlation of $R^2 = 0.723$ ($P = 0.0005$). *Bars* represent standard error

Discussion

We have provided genetic evidence that sensitivity to ethylene is correlated with the induction and development of female flowers in *C. pepo*. Various studies have previously shown that ethylene is the principal component in the control of sex determination in *C. pepo*, and that its application has a feminizing effect on various *C. pepo* varieties, reducing the male phase of development and increasing the number of female flowers per plant (Matlob and Basher 1983; Payán and others 2006; Peñaranda and others 2007). Accordingly, the application of ethylene inhibitors lengthens the initial male phase of development and reduces the number of female flowers per plant (Payán and others 2006; Peñaranda and others 2007). To date, however, no ethylene mutant has been isolated and characterized in this species.

The phenotypic and genetic analysis of the *Veg* inbred line of *C. pepo* indicates that its extremely male sex phenotype could be the result of a single mutation in a major gene that regulates ethylene perception, or response, that we have named *Cucurbita pepo WEAK ETHYLENE INSENSITIVE* (*CpWEI*). In fact, the predominantly male phenotype and the reduced ethylene sensitivity of the *Veg* line co-segregates as a major gene in both the BC and F_2 generations. The phenotypic alterations in both the *Veg* line itself and most male plants of the F_2 are comparable to those found in other species such as *Arabidopsis*, petunia, tomato, and melon with reduced response to ethylene, including reduced triple response to exogenous ethylene treatments of etiolated seedlings, delayed flower senescence, and abscission and elevated ethylene production (Wilkinson and others 1997; Clark and others 1999; Alonso and others 2003; Little and others 2007). In addition to supporting the idea that the *Veg* inbred line has a reduced ethylene sensitivity, the fact that the *Veg* line is altered in diverse developmental processes, including seedling

growth (triple response), sex determination (number of male and female flower per plant), and abscission of male flowers, indicates that the function of *CpWEI* is not tissue-specific but can interfere with general ethylene signaling in *C. pepo*. The *Veg* inbred line can therefore be used as a tool to study the effects of the inhibited ethylene signal transduction pathway in different developmental processes, including sex determination and flower development. It is likely that the mutation in the *CpWEI* gene affects an ethylene receptor or a response gene of *C. pepo*, but we cannot exclude the possibility that the mutant gene is connected with the biosynthesis or response to other hormones such as auxins. The relationship between ethylene and auxins has been documented extensively (Stepanova and Alonso 2005), and it has recently been reported that certain weak ethylene-insensitive mutants of *Arabidopsis* affect genes that mediate the biosynthesis of auxins (Stepanova and others 2005, 2008).

The major alteration of the *Veg* line is its severe male sex phenotype, suggesting that the ethylene sensitivity conferred by the *CpWEI* gene is essential for inducing female flower development in undifferentiated asexual buds. The implication of the role of the ethylene signal pathway in the sex determination in species of Cucurbitaceae is documented mainly in cucumber and melon. In cucumber, the andromonoecious plants (mmff) exhibit a weaker triple response than monoecious (MMff) or gynoecious plants (MMFF) (Yamasaki and others 2001), and it has recently been found that the *M* locus co-segregates with an *ETHYLENE INSENSITIVE3* (*EIN3*)-like gene in a segregating population of cucumber (Liu and others 2008). This supports the suggestion of Yamasaki and others (2001) that the *M* locus governs the sensitivity of male and female flowers to ethylene. On the other hand, the *F* locus appears to regulate ethylene production, which has been confirmed by molecular as well as differential expression among gynoecious, monoecious, and andromonoecious varieties (Kamachi and others 1997; Trebitsh and others 1997; Yamasaki and others 2001). In melon, however, the andromonoecious sex phenotype is the result of a reduction in ethylene production conferred by a mutation in an ACC-synthase gene (Boualem and others 2008), although the inhibition of ethylene perception in transgenic plants for the mutant ethylene receptor *At-etr1-1* of *Arabidopsis* under the control of different promoters has shown that ethylene perception in the melon is also essential for the proper development of carpels and maturation of female flowers (Little and others 2007). The involvement of ethylene production and sensitivity in pistil and stamen development has also been documented in flowers of the hermaphrodite species *Arabidopsis* (Guo and Ecker 2003; Duan and others 2008) and tobacco (Takada and others 2005, 2006; Hibi and others 2007). Our results in *C. pepo* indicate that ethylene sensitivity is not only important for maintaining female flower identity, driving the development of stamens and carpels in female and male flowers, but it is also essential for the control of the transition from a male to a female phase of development in the main axis of the plant, as well as for the sex determination of individual floral buds. In fact, we found that reduced ethylene sensitivity conferred by the *Veg* mutation is correlated with both an extended male phase of development and an increase in the number of male flowers per plant.

The altered ethylene response of *Veg* does not affect the development of male or female flowers. We have previously shown that the inhibition of either ethylene production or perception by aminoethoxyvinylglycine (AVG) or silver thiosulfate (STS), respectively, produces a partial or complete conversion of female into bisexual flowers. These bisexual flowers develop partial or complete stamens and have delayed or arrested flower maturation and abscission (Payán and others 2006; Peñaranda and others 2007). As in the melon (Little and others 2007), ethylene production and perception are required for proper development of *C. pepo* female flowers, maintaining the arrest of stamen development and promoting female flower maturation (Peñaranda and others 2007). The proper development of female flowers in both the *Veg* line and most male-type plants of the F_2 and BC populations indicates that the *CpWEI* gene does not appear to be responsible for this function during female flower development. The function of *CpWEI* seems to be more restricted to apical or floral meristems at an earlier key stage of sex determination, promoting the transition from a male to a female phase of development in the apical meristem and inducing the acquisition of female flower identity in the earliest stages of flower meristem development.

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