Theor Appl Genet (2001) 103:282–287

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Ethylene-insensitive related phenotypes exist naturally in a genetically variable population of *Dianthus barbatus*

Received: 25 June 2000 / Accepted: 15 November 2000

Abstract Within the genetically variable population of *Dianthus barbatus* ''Dagan'', we identified genetic lines with flowers possessing ethylene insensitivite-related phenotypes. The phenotypes are: *Caf*$^+$, with a fresh-looking corolla attached to a mature ovary; *Inr*$^-$, with flowers whose petals do not inroll during flower senescence; and *Rfi*$^+$, whose flower petals inroll, but recover from inrolling. The frequencies of *Caf*$^+$, *Inr*$^-$ and *Rfi*$^+$ were 33%, 25% and 75% in the population inspected, respectively. These relatively high frequencies were probably due to continuous selection over the years for flowers with open and long-lasting corollas. By examining the distribution of the genetic lines which express two of these phenotypes, we determined that *Caf*$^+$, *Inr*$^-$ and *Rfi*$^+$ are independent traits. However, these traits might be associated with male sterility. Exposure of a random sample of the population to exogenous ethylene, followed by examination of the resulting inrolling and wilting, revealed five different ethylene response groups. In one group ethylene enhanced both inrolling and wilting, and most genetic lines within this group exhibited a *Caf*$^-$ phenotype. In two other groups ethylene enhanced either the inrolling or the wilting, and both of the *Caf*$^+$ and *Caf*$^-$ phenotypes were included. Two other groups were completely non-responsive to ethylene, but in one of them the flower life span was twice as long as in the other, and all the genetic lines exhibited the *Caf*$^+$ phenotype. We concluded that the *Caf*$^+$ phenotype is most-likely related to ethylene insensitivity and that the inrolling and wilting are controlled by ethylene, probably via different pathways.

Keywords Ethylene sensitivity · *Dianthus* · Genetic lines · Inrolling · Wilting

Communicated by H.C. Becker

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Introduction

Ethylene is involved in the senescence of many flowers, among them species within the family *Caryophylaceae*. The natural-senescence cascade in the carnation flower is initiated by pollination-induced ethylene, leading to an increase of ethylene in the petals, which eventually causes their inrolling and wilting (Halevy 1986; Woltering et al. 1994; Van Altvorst and Bovy 1995). In ethylene-sensitive flowers, senescence is enhanced by exposure to exogenous ethylene.

The ethylene response pathway has been thoroughly studied, and many components of this pathway and the interactions among them have been discovered both in *Arabidopsis* and tomatoes. (Chang et al. 1993; Chang 1996; Lanahan et al. 1994; Ecker 1995; Wilkinson et al. 1995; Bleecker and Schaller 1996; Keiber 1997; Fluhr 1998; Johnson and Ecker 1998; McGrath and Ecker 1998; Solano and Ecker 1998). Mutations in several genes in the pathway give rise to ethylene-insensitive phenotypes not only in seedlings, where these mutations were originally discovered, but also in all stages of plant development (Bleecker et al. 1988; Guzman and Ecker 1990; Chang 1996; Johnson and Ecker 1998; Solano and Ecker 1998). This is perplexing, especially in the light of the diverse processes that are induced by ethylene in the various developmental stages: in seeds, ethylene promotes germination; in roots it induces root hair growth; in seedlings grown in darkness it prevents stem elongation, while in those grown in light it causes stem elongation; and in petals and fruits it initiates senescence (Abeles et al. 1992; Dolan 1997; Smalle and van der Straeten 1997). In order for ethylene to control a plethora of unrelated processes, there may be additional genes in the ethylene-response pathway that contribute to the response specificity. In support of this, many proteins, which bind ethylene-response elements, have been identified (Okamuro et al. 1997), and different *cis* ethylene-response elements are used to activate ethylene-induced genes in various tissues (Deikman et al 1997).

The present study describes ethylene insensitivite-related flower phenotypes in a genetically variable population of *Dianthus barbatus*. Some of the ethylene-insensitive phenotypes are not associated with each other. We also describe five groups differing in their responses to exogenous ethylene.

Materials and methods

Plant material

A genetically variable population of *D. barbatus* ''Dagan'' was created by Umiel et al. over 20 years ago and has since been maintained by seed propagation. This population was constructed by crossing cold-requiring biennial cultivars with cold-indifferent annual garden cultivars, and selecting for cold-indifferent annual phenotypes suitable for cut-flower production (Umiel et al., unpublished data). Therefore, the proportion of phenotypes with open and long-lasting flowers of commercial value was increased in the population by selection over the years. In addition, the population was enriched in male-sterile plants, to ensure a high level of cross-pollination within the population. This population yielded several commercial *D. barbatus* cultivars for cut flowers, which do not require cold treatment for flowering (Shor et al. 1987; Umiel et al., unpublished results). The ''Dagan'' population was grown from seeds in an open field in Bet-Dagan (Israel) and pollinated by natural pollinators (bees, butterflies etc.). The phenotypes observed in this population are described in Fig. 1.

Analysis of the dependency between the various phenotypes

A random sample of 174 plants (genetic lines) was chosen and marked. Each plant was characterized for the phenotypes Caf^+ / Caf^-, Inr^+ / Inr^-, Rfi^+ / Rfi^- and for MS/MF. The frequency of each single phenotype in the population (e.g. Caf^+) was determined (see Table 1). The observed numbers of genetic lines each expressing a double-phenotype combination (e.g. Caf^+ / Inr^-, Caf^+ /MS etc.) was also determined by counting the double phenotype separately in the sample (see Table 2).

In order to determine dependency (and /or association) between different phenotypes, we assumed the null hypothesis that each phenotype within the paired phenotypes is independent of (and/or not associated with) the other phenotype in the pair. The expected frequency of the double-phenotype combination was calculated as the product of the individual frequencies (from Table 1) of the single phenotypes [e.g. (frequency of Caf^+) × (frequency of Inr^-) = the expected frequency of the Caf^+ /Inr^- double phenotype]. The null hypothesis was tested by comparing the observed with the expected number of double phenotypes by means of a chi-square test, yielding probability (*P*) values with one degree of freedom (Table 2).

Ethylene treatment of the flowers

A random sample of 27 genetic lines from the ''Dagan'' population was selected, and 4–5 flowers of each genetic line were harvested at the petal-opening stage before pollination occurred. Flowers were immediately placed in a 30-ml vial containing tap water arranged in a multi-well box, and incubated under continuous fluorescent light at a controlled temperature of 21°C. Each box was wrapped in a sealed polyethylene bag, creating a closed volume of about 20 l. Ethylene was injected via a caulking material into the polyethylene bag to a final concentration of 2 ppm and this concentration was maintained for 6 h. The control treatment boxes were injected with fresh air. The ethylene concentration in each bag was analyzed by withdrawing a 2-ml gas sample with a hypodermic syringe and injecting it into a gas chromatograph (Varian, Palo Alto, Calif.) equipped with an activated-alumina column and a flame-ionization detector. After the 6th-h treatment the flowers were removed to fresh air and the ethylene effect was monitored by determining the wilting and the inrolling indices (see Fig. 2). The results are presented as the mean values of the indices of four or five flowers for each genetic line.

Results

Description of the flower phenotypes

Usually, carnation flowers inroll or wilt after pollination, concomitantly with ovary development (Halevy 1986; Woltering et al. 1994; Van Altvorst and Bovy 1995). Hence inrolling accompanies senescence and a developed ovary is associated with a wilted corolla. In the ''Dagan'' population we observed genetic lines that either exhibit inrolling (Inr^+) or did not inroll (Inr^-)

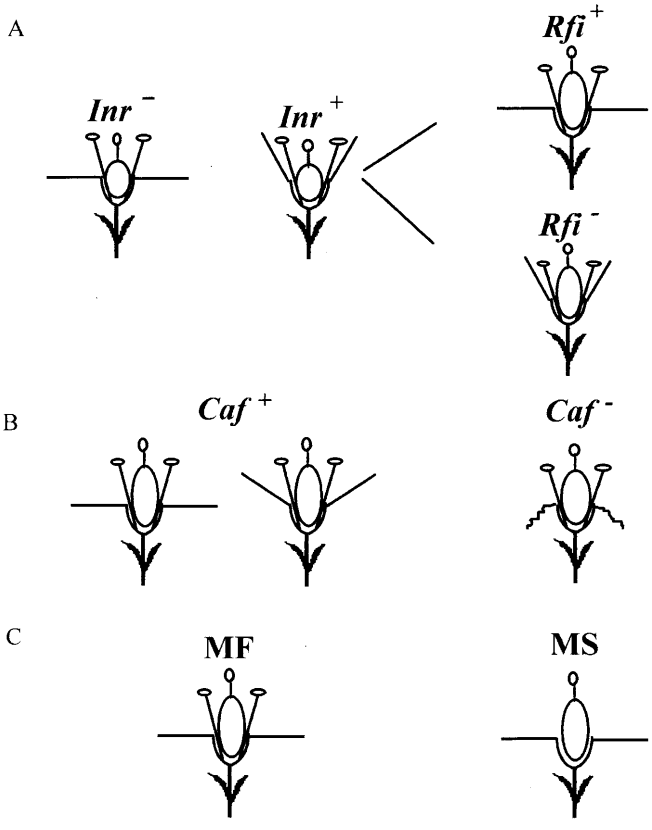

Fig. 1A–C Schematic representation of the various phenotypes. Inr^- (*Inr*olling) – the petals do not fold at all and are held in a horizontal position at all stages of flower development, Inr^+ – petals fold towards the centre of the flower, Rfi^- (*R*ecovery *f*rom *i*nrolling) – the petals remain inrolled at all stages of flower survival, Rfi^+ – the inrolling of the petals is followed by a recovery process that brings them back to a horizontal position, sometimes even folding towards the base of the flower (**A**). Caf^- (*C*orolla *a*ttached to *f*ruit) - the petals wilt concomitantly with ovary development, Caf^+ - fresh petals attached to a developed ovary with seeds (**B**). MS (*M*ale *S*terile) phenotype, characterized by flowers without anthers, and an MF (*M*ale *F*ertile) phenotype, characterized by an androgynous flower (**C**)

(Fig. 1A). In some genetic lines, the inrolling of the petals was followed by a recovery process that brought the petals back to a horizontal position (*Rfi*$^+$). The population also contains genetic lines in which the corolla wilted after pollination (*Caf*$^-$) and those that are characterized by fresh petals that do not senescence concomitantly with pollination and ovary development, but remain for several days fresh and attached to the developed ovary (*Caf*$^+$) (Fig. 1B). Each of the phenotypes of *Inr*$^+$ and *Caf*$^-$ comprised about 70% of the population (Table 1). Among genetic lines expressing the inrolling phenotype (n = 123) about 84% exhibited the *Rfi*$^+$ phenotype. In addition to these phenotypes, about 20% of the studied population comprised of genetic lines expressing MS (Male Sterile) and the rest were MF (Male Fertile) (Fig. 1C and Table 1).

Table 1 Description and frequency of various flower phenotypes in the *D. barbatus* cv ''Dagan'' population. The total number of genetic lines was 174. The frequency of *Rfi* $^+$/*Rfi* $^-$ was calculated only for the 123 genetic lines that expressed the *Inr*$^+$ phenotype

Phenotype	Total number	Frequency
Caf$^+$	56	0.322
Caf$^-$	118	0.678
Inr$^+$	134	0.770
Inr$^-$	40	0.230
Rfi$^+$	103	0.84
Rfi$^-$	20	0.16
MS	35	0.201
MF	139	0.799

Are the different phenotypes associated with each other and/or are they associated with the flower sex phenotype?

Since ethylene is involved both in petal senescence and inrolling, the traits *Caf*$^+$, *Inr*$^-$ and *Rfi*$^+$ might all be related to either low ethylene production or ethylene insensitivity. Therefore, it is possible that all these traits are associated with each other. Based on the assumption that the phenotypes appear randomly in the population (see Materials and methods), we determined the number of genetic lines exhibiting the double phenotype and calculated the number of genetic lines expected to exhibit these various phenotypes (Table 2). The analysis yielded a low chi-square value for *Caf* and *Inr* and a P value larger than 0.9 (Table 2A); therefore, these two traits are independent of /or not associated with each other. A similar analysis for *Caf* and *Rfi* in a sample of 123 genetic lines that exhibited the inrolling phenotype yielded a P value larger than 0.1 (Table 2B), leading to the conclusion that theses phenotypes are not associated with each other.

A similar analysis was performed to determine if each of the ethylene-related phenotypes was associated with the sterility phenotype, (Table 2C, D and E). The result-

Table 2 Distribution of the genetic lines expressing various paired phenotypes within a population of *D. barbatus* cv ''Dagan''. The data represent the observed and expected numbers of genetic lines, showing the indicated phenotypes, within a population of 174 genetic lines. The numbers in parentheses are the frequencies for each paired phenotype. Bold numbers emphasizes differences between the expected and observed numbers

A	*Caf*$^+$/*Inr*$^+$	*Caf*$^+$/*Inr*$^-$	*Caf*$^-$/*Inr*$^+$	*Caf*$^-$/*Inr*$^-$	Chi-square value[a]	*P* value
Observed	43 (0.24)	14 (0.08)	89 (0.52)	28 (0.16)		
Expected	43.5 (0.25)	13.9 (0.08)	88.8 (0.52)	27.8 (0.16)	0.007 n.s.	$P > 0.9$

B	*Caf*$^+$/*Rfi*$^+$	*caf*$^+$/*Rfi*$^-$	*Caf*$^-$/*Rfi*$^+$	*Caf*$^-$/*Rfi*$^-$	Chi-square value[a]	*P* value
Observed	37 (0.30)	4 (0.03)	66 (0.54)	16 (0.13)		
Expected	34.4 (0.28)	6.2 (0.05)	68.9 (0.56)	13.5 (0.11)	1.55 n.s.	$P > 0.1$

C	*Caf*$^+$/*MF*	*Caf*$^+$/*MS*	*Caf*$^-$/*MF*	*Caf*$^-$/*MS*	Chi-square value[a]	*P* value
Observed	35 (0.2)	23 **(0.13)**	104 (0.6)	12 **(0.07)**		
Expected	45.2 (0.26)	12.2 **(0.07)**	94.0 (0.54)	22.6 **(0.13)**	17.8**	$P < 0.005$

D	*Inr*$^+$/*MF*	*Inr*$^+$/*MS*	*Inr*$^-$/*MF*	*Inr*$^-$/*MS*	Chi-square value[a]	*P* value
Observed	100 (0.57)	34 (0.194)	39 (0.23)	1 **(0.006)**		
Expected	108 (0.62)	26 (0.15)	31.3 (0.18)	8.7 **(0.05)**	11.7**	$P < 0.005$

E[b]	*Rfi*$^+$/*MF*	*Rfi*$^+$/*MS*	*Rfi*$^-$/*MF*	*Rfi*$^-$/*MS*	Chi-square value[a]	*P* value
Observed	81 (0.66)	22 (0.18)	9 **(0.07)**	11 **(0.09)**		
Expected	75.1 (0.61)	28.3 (0.23)	14.7 **(0.12)**	4.9 **(0.04)**	11.56**	$P < 0.005$

[a] Chi-square value (left): n.s. = not significant, ** significant at the 1% level

[b] Genetic analysis for phenotype pairs including *Rfi* $^+$/*Rfi*$^-$ was performed on 123 genetic lines that were *Inr*$^+$

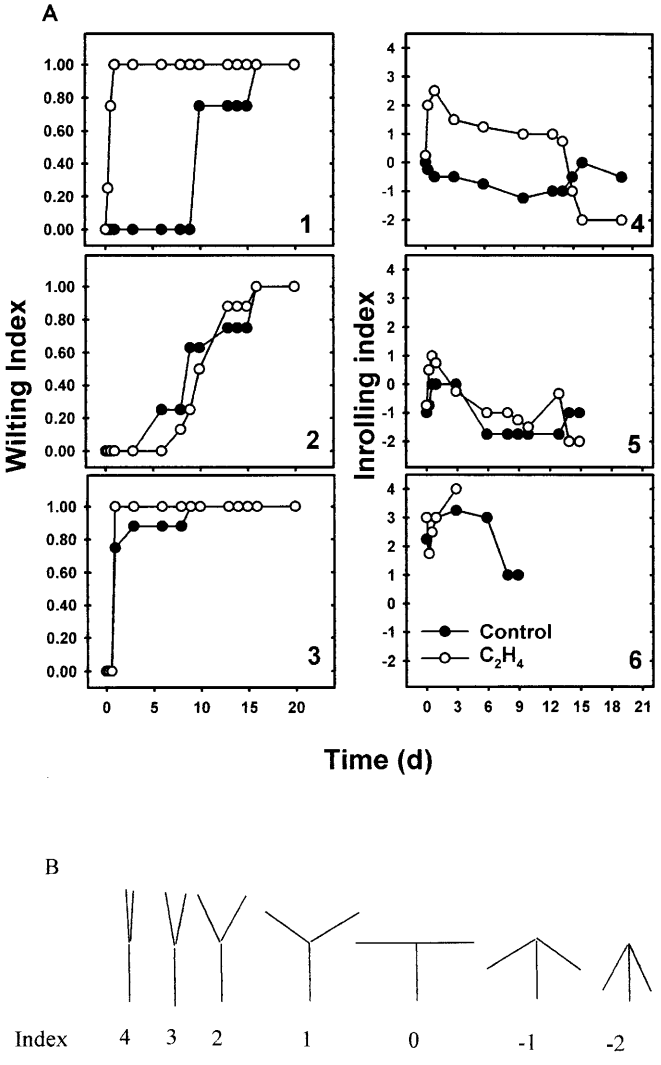

Fig. 2 Wilting and inrolling responses to exogenous ethylene in varoius genetic lines (**A**). Ethylene (2 ppm) and regular air as a control was supplied to flowers in a closed chamber for 6 h. The inrolling and wilting of five individual flowers from each genetic line was determined after exposure to fresh air. The wilting was determined by ascribing 0 – unwilted, 0.5 – partially wilted, 1 – wilted. The inrolling index was determined according to the schematic presentation in **B**. The SD of the experiment was about 1% of the presented value

ing chi-square values were significant, indicating that each of the traits *Inr*, *Caf* and *Rfi* was associated with the male sterility phenotype.

Characterizing the response of various genetic lines to exogenous ethylene

The phenotypes *Caf*$^+$ and *Inr*$^-$ could be related to ethylene insensitivity, and therefore the ethylene sensitivity to exogenous ethylene was determined. Flowers of 27 genetic lines randomly chosen from the studied population were either exposed to exogenous ethylene or to air as a control for 6 h and than removed to fresh air. The inrolling and wilting indices of the petals were then monitored for 20 days (see Fig. 2A). Various responses to exogenous ethylene are presented in Fig. 2B. Five different phenotypic groups were observed and the average flower life span for each of the groups without ethylene treatment and after ethylene treatment was determined (Table 3). In group A neither wilting (Fig. 2B-2) nor inrolling (Fig. 2B-5) were enhanced by exposure to ethylene. In group B ethylene enhanced wilting (Fig. 2B-1) but did not enhance inrolling (Fig. 2B-5). In group C ethylene did not affect wilting (Fig. 2B-2), but enhanced inrolling (Fig. 2B-4). In contrast to these groups, in group D both the wilting (Fig. 2B-1) and the inrolling (Fig. 2B-4) were enhanced by ethylene. The average flower life span of groups A,B,C and D without ethylene treatment was between 15 to 17 days, whereas, in groups B and D ethylene reduced the average life span to about 11 days (Table 3). In group E the average flower life span was only about 8 days, significantly shorter than those of the other groups, either treated or not-treated with ethylene; in this group, as in group A, ethylene did not enhance wilting (Fig. 2B-3) or inrolling (Fig. 2B-6).

Is insensitivity to exogenous ethylene related to the *Caf*$^+$ phenotype?

To test this hypothesis, the number of *Caf*$^+$ phenotypes within each of the five groups (A–E) was determined (Table 3). It can be seen that most lines of the *Caf*$^+$ phenotype (three genetic lines of group A, two genetic lines of group B and three genetic lines of group C) were among the three groups that expressed some form of ethylene insensitivity. Moreover, in group A, which

Table 3 Description of the flower life span and the distribution of the *Caf*$^+$/*Caf*$^-$ phenotype within the various exogenous ethylene response groups

Phenotype	Total number of plants	Flower life span (days)		Ethylene-enhanced inrolling	Number of plants	
		– Ethylene	+ Ethylene		*Caf*$^+$	*Caf*$^-$
A	3	16.9±3.6	16.9±3.6	–	3	0
B	2	17.4±4.9	10.9±2.8	–	2	0
C	5	15.0±3.3	15.0±3.3	+	3	2
D	7	15.1±2.0	11.7±3.2	+	1	6
E	10	8.1±1.5	8.1±1.5	–	2	8

expressed complete insensitivity to ethylene, all the genetic lines expressed the *Caf*$^+$ phenotype. Furthermore, in group D, where both the inrolling and the wilting were enhanced by ethylene, seven out of eight genetic lines expressed the *Caf*$^-$ phenotype. These results support the notion that the *Caf*$^+$ phenotype could result from insensitivity to ethylene. It is also noteworthy that the inrolling could be enhanced by ethylene in both the *Caf*$^+$ and the *Caf*$^-$ phenotypes, as seen in categories C and D.

Discussion

Carnation petals usually senesce after pollination and ethylene plays an essential role in this process (Halevy 1986; Woltering et al 1994; Van Altvorst and Bovy 1995). Wilting or inrolling of the flower immediately after pollination has an enormous evolutionary benefit to the species, by reducing redundant visits of the pollinators to the same flower, enabling more-efficient pollination of the individual flowers in a population (Woltering et al. 1994), while the wilting ensures a supply of metabolites to the developing ovary and seeds (Stead 1992).

In the ''Dagan'' population of *D. barbatus*, we have identified flower phenotypes *Caf*$^+$, *Inr*$^-$ and *Rfi*$^+$ that do not follow the normal pollination-induced senescence patterns. The *Caf*$^+$ phenotype (Fig. 1B) resembled the ethylene-insensitive flower phenotypes of tomato and *Arabidopsis* mutants (Lanahan et al. 1994; Friedman and Halevy, unpublished results), suggesting that it is a result of a non-functional gene(s) in the ethylene response pathway. The *Inr*$^-$ phenotype (Fig. 1A) has been described previously for other carnation cultivars and has been thought to be associated with the lack of ethylene production (Wu et al. 1991a, b; Mayak and Tirosh 1993). It is not clear yet if the *Inr*$^-$ phenotype in ''Dagan'' is elicited by lack of ethylene production or by inability to respond to ethylene. The *Rfi*$^+$ phenotype (Fig. 1A) has not been reported previously to the best of our knowledge, and the physiological processes underlying such response have yet to be elucidated.

Frequency and association of phenotypes in the population

The unusual phenotypes mentioned in the present paper have been observed in the population during the last 15 years. Although these phenotypes should not have any evolutionary benefits, their relatively high frequencies [0.32, 0.23 and 0.84 for *Caf*$^+$, *Inr*$^-$ and *Rfi*$^+$ respectively (Table 1)] probably result from years of continuous selection for flowers with open and long-lasting corollas. It is interesting that these phenotypes exist naturally in *D. barbatus* without the induction of mutations. The frequency of the MS phenotype in the population was about 20% (Fig. 1C). Male sterility or partial male sterility is a common trait in many natural populations and has been observed at non-negligible frequencies in some 200 independent populations of *Dianthus* species; it even exists among the commercial cultivars (Starshova 1996; Umiel et al., unpublished data).

Since both inrolling and wilting of the petals on plants are associated with the ethylene response (Halevy 1986; Woltering et al. 1994; Van Altvorst and Bovy 1995), it was possible to assume that both the phenotypes *Inr*$^-$ and *Caf*$^+$ could result from a common defective process. However, it was shown (Table 2) that the distribution of double-phenotype groups is expected from the independent segregation of the individual phenotypes in the population, indicating either that the two traits segregate independently and/or that they are not associated physiologically with each other (Table 2A). A *Caf*$^+$ phenotype, which is characterized by a fresh corolla associated with a developed ovary with seeds, does not necessarily lead to a lack of inrolling (*Inr* $^-$). The *Rfi*$^+$ (recovery from inrolling) and *Caf*$^+$ are also independent and traits (Table 2B), which either segregate independently or are not physiologically dependent.

A response to ethylene has been reported to be associated with male sterility (Rudich et al. 1972; Abeles et al. 1992; Dolan 1997). In contrast, our analysis showed that the ethylene-insensitive phenotypes *Caf*$^+$ and *Inr*$^-$ are somehow associated with male sterility (Table 2C–E). The nature of the male sterility in our population was not characterized and it is possible that it could have resulted from the dysfunction of several independent processes. Therefore, it is difficult to determine the nature of the association between the ethylene-insensitivite phenotypes and MS.

Response to exogenous ethylene

Exposure of flowers to exogenous ethylene is used to identify ethylene non-sensitive genetic lines (Woltering and Van Doorn 1988). This approach was used in the present study and the wilting and inrolling of the flowers were monitored. It is important to emphasize that the trait *Inr*$^-$, mentioned above, and the lack of inrolling in response to exogenous ethylene are not necessarily identical traits. Lack of inrolling (like *Inr*$^-$) has been reported for Sandrosa and Sandra cultivars of carnation, in which the exposure to exogenous ethylene causes inrolling of the flowers (Wu et al 1991a,b; Mayak and Tirosh 1993).

Genetic lines of carnations in which exogenous ethylene increased both the wilting and the inrolling has previously been described (Woltering and Van Doorn 1988). In our population most of these genetic lines showed a *Caf*$^-$ phenotype. On the other hand, we observed genetic lines in which both these responses were completely insensitive to exogenous ethylene, were of the *Caf*$^+$ phenotype and exhibited the longest vase life (Table 3, group A). Such genetic lines might harbor a mutation(s) in components for the perception of ethylene or for downstream components similar to the mutations that were identified in *Arabidopsis* and tomato (Lanahan et al.

1994; Ecker 1995; Wilkinson et al. 1995; Bleecker and Shaller 1996; Keiber 1997; McGrath and Ecker 1998). In a preliminary ''triple response'' test on seeds from the ''Dagan'' population (data not shown), we identified ethylene non-responsive seedlings, indicating the existence of genetic lines that are insensitive to ethylene and might be defective in components that function both in flowers and in seedlings.

In groups B and C (Table 3) ethylene enhanced either one process or the other (wilting/inrolling) which suggests that, although both processes can be activated by exogenous ethylene, their activation is not mutually dependent. In group C there are genetic lines of both Caf^{+} and Caf^{-} phenotypes, which shows that inrolling in response to exogenous ethylene is not related to the *Caf* phenotypes. Therefore, genetic lines of B and C could be defective in an unidentified petal-specific gene(s) related to the ethylene-response pathway.

The existence of genetic lines with very short life spans and insensitive to exogenous ethylene (Table 3, group E) could be due to the early production of high endogenous ethylene levels, rendering the petals insensitive to exogenous ethylene; or, alternatively, due to activation of a senescence pathway not associated with ethylene.

In summary, the present study showed the existence of genetic lines exhibiting various ethylene-related phenotypes and various responses to exogenous ethylene, which strengthens the notion that multiple response pathways are activated by ethylene and, possibly, that there are flower-specific components of this response pathway. Further future heritability studies will establish the genetic nature of the phenotypes, and the ''triple response'' screen would aid in determining whether some of these ethylene-insensitive traits are petal specific.

Acknowledgements We thank Dr. I. Levine for critical reading of the manuscript and Dr. R. Marcus for the advice on the statistical analysis. The experiments comply with the current laws of Israel. Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel. No.213/99.

References

Abeles FB, Morgan PW, Saltveit ME Jr (1992) Ethylene in plant biology. 2nd edn. Academic Press, San Diego

Bleecker AB, Schaller EG (1996) The mechanism of ethylene perception. Plant Physiol 111: 653–660

Bleecker AB, Estelle MA, Somerville C, Kende H (1988) Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. Science 241: 1086–1089

Chang C (1996) The ethylene signal transduction pathway in *Arabidopsis*: an emerging paradigm. Trends Biol Sci 21: 129–133

Chang C, Kwok SF, Bleecker AB, Meyerowitz EM (1993) *Arabidopsis* ethylene-response gene *ETR1*: similarity of product to two-component regulators. Science 262: 539–544

Deikman J (1997) Molecular mechanisms of ethylene regulation of gene transcription. Physiol Plant 100: 561–566

Dolan L (1997) The role of ethylene in the development of plant form. J Exp Bot 48: 201–210

Ecker JR (1995) The ethylene signal transduction pathway in plants. Science 268: 667–675

Fluhr R (1998) Ethylene perception: from two component signal transducers to gene induction. Trends Plant Sci 3: 141–147

Guzman P, Ecker JR (1990) Exploiting the triple response of *Arabidopsis* to identify ethylene-related mutants. Plant Cell 2: 513–523

Halevy AH (1986) Pollination-induced corolla senescence. Acta Hort 181: 25–32

Johnson PR, Ecker JR (1998) The ethylene gas signal transduction pathway: a molecular perspective. Annu Rev of Genet 32: 227–254

Kieber J (1997) The ethylene response pathway in *Arabidopsis*. Annu Rev Plant Physiol Plant Mol Biol 48: 227–296

Lanahan MB, Yen H-C, Giovannoni JJ, Klee HJ (1994) The *Never Ripe* mutation blocks ethylene perception in tomato. Plant Cell 6: 521–530

Mayak S, Tirosh T (1993) Unusual ethylene-related behavior in senescing flowers of the carnation Sandrosa. Physiol Plant 88: 420–426

McGrath RB, Ecker JR (1998) Ethylene signalling in *Arabidopsis*: events from the membrane to the nucleus. Plant Physiol Biochem 36: 103–113

Okamuro JK, Caster B, Villarroel R, Van Montagu M, Jofuku KD (1997) The AP2 domain of *APETALA 2* defines a large new family of DNA binding proteins in *Arabidopsis*. Proc Natl Acad Sci USA 94: 7076–7081

Rudich J, Halevy AH, Kende H (1972) Ethylene evolution from cucumber plants as related to sex determination. Plant Physiol 49: 998–999

Shor Y, Zohar B, Herman M, Sela R, Halevy A (1987) Clones of *Dianthus barbatus* (in Hebrew). Hassadeh 67: 2070–2071

Smalle J, Van Der Straeten D (1997) Ethylene and vegetative development. Physiol Plant 100: 593–605

Solano R, Ecker JR (1998) Ethylene gas: perception, signalling and response. Curr Opin in Plant Biol 1: 393–398

Starshova NP (1996) Partial male sterility in some members of the Caryophyllaceae. Bot Zhur 81: 64–74

Stead AD (1992) Pollination-induced flower senescence. Plant Growth Reg 11: 13–20

Van Altvorst VAC, Bovy AG (1995) The role of ethylene in senescence of carnation flowers. Plant Growth Reg 16: 43–53

Wilkinson JQ, Lanahan MB, Hsiao-Ching Y, Giovannoni JJ, Klee HJ (1995) An ethylene-inducible components of signal transduction encoded by *Never-ripe*. Science 270:1807–1809

Woltering EJ, Van Doorn WG (1988) Role of ethylene in senescence of petals – morphological and taxonomical relationships. J Exp Bot 39: 1605–1616

Woltering EJ, Ten Have A, Larsen PB, Woodson WR (1994) Ethylene biosynthetic genes and inter-organ signalling during flower senescence. In: Scott RJ, Stead AD (eds) Molecular and cellular aspects of plant reproduction, vol 55. Cambridge University Press, Cambridge, UK, pp 285–307

Wu MJ, Van Doorn WG, Reid MS (1991a) Variation in the senescence of carnation (*Dianthus caryophyllus* L.) cultivars. I. Comparison of flower life, respiration and ethylene biosynthesis. Scien Hort 48: 99–107

Wu M, Zacarias L, Reid MSH (1991b) Variation in the senescence of *carnation (Dianthus caryophyllus* L.) cultivars. I. Comparison of sensitivity to exogenous ethylene and of ethylene binding. Scien Hort 48: 109–116