Factors Affecting Seed Production in Transgenic Ethylene-insensitive Petunias

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ABSTRACT. Pollen viability, in-vivo pollen tube growth, fruit ripening, seed germination, seed weight, whole plant vigor, and natural flower senescence were investigated in homozygous and heterozygous transgenic ethylene-insensitive CaMV35S::etr1-1 petunias (*Petunia xhybrida* ‘Mitchell Diploid’). Homozygous or heterozygous plants were used to determine any maternal and/or paternal effects of the CaMV35S::etr1-1 transgene. All experiments except for those used to determine natural flower senescence characteristics were conducted in both high and low temperature greenhouses to determine the effect of temperature stress on transgenic plants when compared to wild-type. Results indicated that ethylene-insensitive plants had a decrease in pollen viability, root dry mass, seed weight, and seed germination. Fruit ripening, seed germination, and seed weight were maternally regulated. In contrast, the CaMV35S::etr1-1 transgene is completely dominant in its effect on natural flower senescence.

The plant hormone ethylene is involved in many physiological processes in plants including fruit ripening, petal senescence, abscission, and seed germination (Abeles et al., 1992). The senescence of many flowers is accompanied by a dramatic increase in ethylene production resulting in petal wilting, abscission or color fading. Treatment of ethylene-sensitive flowers with chemical inhibitors of ethylene biosynthesis or perception reduces this burst of ethylene production and delays visual symptoms of corolla senescence (Jones and Woodson, 1997; Serek et al., 1995; Whitehead et al., 1984). Genetic modification of ethylene biosynthetic and signal transduction pathways has provided further evidence of ethylene’s role in regulating flower senescence (Bovy et al., 1999; Savin et al., 1995; Wilkinson et al., 1997). Since many economically important floral crops are grown for their display, the control of ethylene perception is essential for increasing vase life and enhancing quality.

Since one of the major concerns in the floriculture industry is flower longevity, research has been conducted to use genetic engineering on floriculture crops that are insensitive to ethylene. Wilkinson et al. (1997) transformed petunia with a dominant mutant *Arabidopsis* ethylene receptor, *etr1-1*, under the control of a constitutive Cauliflower Mosaic Virus 35S (CaMV35S) promoter to produce ethylene-insensitivity throughout the whole plant. These petunias (44568) had an increase in natural and pollination-induced flower longevity compared to wild type ‘Mitchell Diploid’ (MD) plants, but had physiological side effects that limit their commercial use (Gubrium et al., 2000; Wilkinson et al., 1997). Ethylene-insensitive petunias and tomatoes both showed a significant reduction in adventitious root formation, and even exogenous treatments with auxin did not increase adventitious root formation to the level of untreated wild-type controls (Clark

et al., 1999). Since many horticultural species are often propagated through vegetative cuttings this characteristic would severely limit the commercial utility of ethylene-insensitive plants.

Previous experiments showed that during the conventional breeding of ethylene-insensitive petunias to homozygosity, low germination rates of seeds were encountered (Gubrium, 1998). This was not surprising because it is well known that ethylene plays a role in seed germination and subsequent hypocotyl elongation in many plants species (Arshad and Frankenburger, 1988; Goeschl et al., 1966; Goeschl and Kays, 1975; Harpham et al., 1991; Koch and Moore, 1990). As a result of these observations we conducted experiments to characterize some of the physiological processes required for the production of seeds in *Petunia*, and to determine if these processes are affected by ethylene-insensitivity. We focused on pollen viability, pollen tube growth, seed yield, seed germination, and whole plant vigor in transgenic ethylene-insensitive 44568 and ‘Mitchell Diploid’ (MD) petunias. Particular focus was given to determining the basis of factors that could influence the phenotype of subsequent progeny that are independent of the dominant CaMV35S::etr1-1 transgene. Since ethylene is a stress-related hormone, most of our experiments were conducted in high and low temperature greenhouses to determine if temperature would affect these processes differently in ethylene-insensitive plants.

Materials and Methods

PLANT MATERIALS AND CULTURAL CONDITIONS. Inbred *Petunia xhybrida* Vilm.-Andr. ‘Mitchell Diploid’ (MD), and transgenic plants homozygous or heterozygous for CaMV35S::etr1-1 (referred to as line 44568) (Wilkinson et al., 1997) were used in all experiments. Plants used for physiological studies were grown in a commercial potting substrate (Fafard No. 2, Conrad Fafard, Agawam, Mass.) in 15-cm, 1.5-L pots and fertilized weekly with 300 mg N/L 20N–4.8P–16K Peter’s soluble fertilizer (Excel Cal-Mg, Scotts-Sierra Horticultural Products Co., Marysville, Ohio). Otherwise, plants were irrigated as needed. Plants used for the

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pollen performance, fruit ripening, and seed weight experiments included MD and homozygous 44568 plants grown under two greenhouse temperature regimes. Plants of each genotype were grown in air-conditioned glass greenhouses maintained at either a high (29/24 °C) or a low (24/18 °C) day/night temperature regime. Different growing temperatures were used to determine if ethylene sensitivity influenced temperature effects on various aspects of plant development investigated. Each experiment was replicated during this same period in two additional greenhouses that were maintained at identical temperature regimes as previously described. Plants used for the whole plant vigor and natural flower senescence experiments and seed production included MD, homozygous 44568, MD x 44568 (female x male), and 44568 x MD plants. These plants were grown under identical cultural conditions with a day/night temperature regime of 24/18 °C.

POLLEN PERFORMANCE. To determine if ethylene sensitivity is required for proper pollen viability and in vivo pollen tube growth through the style, four blocks of MD and homozygous 44568 plants were arranged in a randomized complete-block design and grown for 14 weeks in a greenhouse. Each block consisted of 12 plants of each genetic line. Once weekly for 3 weeks, pollen from flowers of both genotypes was collected one day after anthesis. Pollen from two anthers of each flower was stained for viability using an Alexander stain (Alexander, 1969). Due to variability in flower production from week to week, the total number of samples taken from both experiments was 32 MD and 22 44568 from the 29/24 °C greenhouses and 36 MD and 26 44568 from the 24/18 °C greenhouses. Self (⊗) and reciprocal cross-pollinations were completed once weekly on flowers at the same stage of development for 2 weeks. Stigma/styles were collected at 8 or 12 h after pollination and fixed in 3 ethanol : 1 acetic acid by volume. Following rinsing with 1 M phosphate buffer (pH 7) and clearing with 1 N NaOH for 12 h, styles were stained with 0.1% aniline blue for visualization of pollen tubes using a fluorescence microscope (Leitz Wetzlar, Germany) at a wavelength of 450 nm. The length from the beginning of the style to the farthest point of pollen tube growth was then measured with an ocular micrometer. The two experiments resulted in the visualization of pollen tubes from 47 MD ⊗, 30 44568 ⊗, 32 MD x 44568 (female x male), and 36 44568 x MD styles from the 29/24 °C greenhouse, and 55 MD ⊗, 25 44568 ⊗, 33 MD x 44568, and 29 44568 x MD styles from 24/18 °C greenhouse. Data were analyzed using SAS (v6.12, SAS institute, Cary, N.C.).

FRUIT RIPENING. To determine the maternal and paternal factors involved in fruit set, ripening, and seed production, MD and homozygous 44568 petunia plants from the above experiment were used. Four self (⊗) and two reciprocal cross-pollinations were performed on each of 12 plants in the high and low temperature greenhouses over the course of 6 weeks using flowers at the same developmental stage (one day after anthesis). Plants were then checked for fruit set 15 d after pollination and then daily after for fruit ripening. Fruit was considered ripe when totally brown in color, but not yet dehiscent. This experiment was also replicated in separate greenhouses with similar environmental conditions at the same time. Data were analyzed using SAS (v6.12, SAS institute, Cary, N.C.).

SEED GERMINATION. Since previous results have shown that ethylene-insensitivity in both petunia (Gubrium, 1998) and *Arabidopsis* (Bleecker et al., 1988) resulted in decreased seed germination, a germination experiment was performed using homozygous and heterozygous CaMV35S::etrI-1 seeds to determine if maternal and/or paternal factors were associated with germination. Seeds

used were produced in the above experiments and were grouped together by genotype and the temperature at which the seeds were produced. This resulted in four groups of seeds for 29/24 °C and four for 24/18 °C temperature conditions. Before sowing, seeds were stored at room temperature for ≈2 months to overcome the one-month dormancy period required for petunia seeds (Sink, 1984). Eight lots of 100 seeds of each MD, homozygous 44568, MD x 44568 (female x male), and 44568 x MD were weighed, then germinated on Whatman no. 1 filter paper in 100 x 15 mm petri plates. Filter paper was moistened with sterile tap water and excess water drained off daily after sowing seeds. Each lot of seeds was represented by a total of five plates, with 20 seeds per plate per genotype. Plates were placed in a germination chamber using a completely randomized design with a constant temperature of 24 °C with 12-h light of 3–4 μmol·m⁻²·s⁻¹. Data were collected on days 3, 5, 7, 9, 11, and 13 for percent seed germination (radicle emergence). A second replication was conducted one week after the completion of the first replication using similar procedures. Data for seed weight and germination percentage were analyzed using SAS (v6.12, SAS institute, Cary, N.C.).

WHOLE PLANT VIGOR. To determine if ethylene-insensitivity in the maternal and/or paternal parent affected plant vigor, MD, homozygous 44568, MD x 44568 (female x male), and 44568 x MD plants were grown for 12 weeks at 24/18 °C day/night for destructive analysis. To determine the shoot and root dry mass (g), plants were excised at substrate level, roots washed with water to remove substrate, and shoots and roots placed into separate paper bags. Individual plant roots and shoots were weighed after drying at 70 °C for 3 d. The experimental design consisted of 24 plants of each genotype arranged into three randomized complete blocks. Data were analyzed using SAS (v6.12, SAS institute, Cary, N.C.).

NATURAL FLOWER SENESCENCE. To determine if natural flower senescence was affected differently by the presence of the CaMV35S::etrI-1 transgene in the homozygous versus the heterozygous state, two replications of 10 plants each of MD, homozygous 44568, MD x 44568 (female x male), and 44568 x MD plants were grown under identical conditions to those used for whole plant vigor experiments previously. Seven flowers from the first flush of flowers produced by each plant were emasculated the day before anthesis to prevent self-pollination, and then checked 10 d later to assure that there was no fruit set. Flowers were tagged the day they opened and then checked daily for visual senescence (petal wilting). Data were analyzed using SAS (v6.12, SAS institute, Cary, N.C.).

Results and Discussion

POLLEN PERFORMANCE. Results of the pollen viability experiments showed that the main effects of genotype and greenhouse temperature were significant ($P = 0.0001$, ANOVA), but the interaction of both variables was not significant ($P > 0.05$). MD and 44568 pollen viabilities in the high temperature greenhouse were ≈13% to 14% lower than their counterparts in the low temperature greenhouse (Table 1). This decrease in pollen viability due to high temperature was not unexpected since similar results have also been observed in other species (Stanley and Poostachi, 1962). Both MD and 44568 pollen were affected similarly by temperature, but ethylene-insensitive pollen had an overall 10% reduction in pollen viability compared to wild-type pollen at both production temperatures. How ethylene insensitivity resulted in this decrease in pollen viability is not known, but it is likely not

a major factor affecting commercial production since a large percentage of the 44568 pollen was viable.

The main effect of in vivo pollen tube growth showed that production temperature and genotype did not affect pollen tube growth ($P > 0.05$) measured at both eight and 12 h after pollination (Table 2). Thus data for both eight and 12 h after pollination were pooled over both production temperatures and show no differences between genotypes (Table 2). These results indicate that ethylene is not a major requirement for pollen tube growth in petunia and that self and cross-pollinations result in a similar rate of tube growth. Although the viability of 44568 pollen was slightly less than MD pollen there was no noticeable difference in the number of pollen tubes growing through the style between these two types of pollen (data not shown). This observation suggests that this difference in pollen viability is probably small enough that it should not cause a significant reduction in the number of seeds produced.

FRUIT RIPENING. Genotype, temperature, and the interaction of both of these factors were significant for data collected for fruit ripening ($P < 0.0001$), thus results are shown separated by genotype and temperature (Table 3). Fruit on 44568 and MD maternal parents were affected by temperature similarly with an approximate 9- to 10-d delay in fruit ripening in the 24/18 °C greenhouse when compared to the 29/24 °C greenhouse. These results indicate that temperature accelerates the fruit ripening process independent of the effects of ethylene. Results also showed that regardless of greenhouse temperature, fruit produced from all pollinations on a 44568 maternal parent took approximately 9 to 10 d longer to ripen than fruit produced on a wild-type maternal parent (Table 3). These results agree with previous reports on delayed fruit ripening with 44568 (Gubrium et al., 2000), leading us to conclude that the maternal parent's ability to respond to ethylene is a determining factor in fruit ripening regardless of the seed genotype, and ethylene acts to accelerate the ripening process. Since fruit development on an ethylene-insensitive maternal parent is delayed by 33% to 50% it is possible that other processes occurring during seed development affecting subsequent germination might be altered.

Similar results of delayed fruit ripening have been observed in transgenic tomato that produce less ethylene (Hamilton et al., 1990; Klee et al., 1991; Oeller et al., 1991) or are ethylene-insensitive (Lanahan et al., 1994; Wilkinson et al., 1997). Although the *Never-ripe* ethylene-insensitive mutant of tomato fails to produce fruit that fully ripen (Lanahan et al., 1994) this mutant is able to produce a large percentage of viable seeds (H.J. Klee, unpublished). Experiments using oilseed rape, which ripens similarly to petunia by drying down and dehiscing, show that fruit ripening is associated with an increase in ethylene production (Child et al., 1998). Also, further experiments using oilseed rape show that treatment with an inhibitor of ACC synthesis resulted in a 3- to 4-d delay in ripening (Child et al., 1998). Since the *etr1-1* mutant and transgene are dominant to the wild-type allele, the production of F_1 hybrid seeds using a wild-type maternal parent will be a viable alternate to producing ethylene-insensitive seeds without encountering a delay in the time required to harvest the seeds.

SEED WEIGHT. Significant main effects on seed weight resulted from seed genotype ($P < 0.0001$) and greenhouse temperature ($P < 0.0001$). The interaction of both of these factors was also significant ($P = 0.0055$) (Table 4). Results of average seed weight (g)/100 seeds indicated that greenhouse temperature affected seed weight, with lower greenhouse temperature producing slightly heavier seeds when compared to the same genotype in a higher

temperature greenhouse (Table 4). Maternal genotype also affected seed weight, with seeds produced on an ethylene-insensitive maternal parent weighing less than seeds produced on a MD maternal parent. When combined, these results clearly indicate that the maternal genotype determines seed weight. The effect of maternal genotype on determining seed weight regardless of paternal genotype has been observed in plants such as morning glory and radish (Nakamura and Stanton, 1989; Mojonner, 1998). In *Petunia*, fruit and seed development after pollination begins with cell differentiation for both developing ovules and fruiting

Table 1. Percent viable pollen produced on wild-type 'MD' and ethylene-insensitive 44568 petunia plants grown at 29/24 °C or 24/18 °C.

Genotype	Viable pollen (%)	
	Temp (°C)	
	29/24	24/18
MD	69.4 A ^z	82.3 A
44568	59.0 B	73.0 B

^zMeans within columns followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey HSD)

Table 2. Mean length (mm) of wild-type 'MD' and ethylene-insensitive 44568 pollen tubes assayed in-vivo at 8 and 12 h after self and reciprocal cross-pollinations (female x male).

Pollination type	Pollen tube length (mm)	
	Time (h)	
	8	12
MD self	7.0 A ^z	16.0 A
44568 self	7.0 A	17.0 A
MD x 44568	7.5 A	16.5 A
44568 x MD	8.5 A	19.0 A

^zMeans within columns for the same hours after pollination followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey HSD).

Table 3. Mean number of days from pollination to fruit ripening of fruit grown on wild-type 'MD' and ethylene insensitive 44568 plants at 29/24 °C and 24/18 °C after self or reciprocal pollination (female x male).

Pollination type	Days to fruit ripening	
	Temp (°C)	
	29/24	24/18
MD self	20.6 B ^z	30.3 B
44568 self	31.9 A	39.3 A
MD x 44568	20.8 B	29.9 B
44568 x MD	29.8 A	40.4 A

^zMeans within columns followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey HSD).

Table 4. Mean weight (g) of 100 seeds produced by fruit grown on wild-type 'MD' and ethylene insensitive 44568 plants at 29/24 °C and 24/18 °C after self or reciprocal pollination (female x male).

Pollination type	Wt (g)/100 seeds	
	Temp (°C)	
	29/24	24/18
MD self	0.0103 B ^z	0.0112 A
44568 self	0.0081 C	0.0097 B
MD x 44568	0.0108 A	0.0112 A
44568 x MD	0.0081 C	0.0098 B

^zMeans within columns followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey HSD).

tissues, followed by cell expansion and seed filling. Once seeds are filled with carbohydrates, the fruit begin to ripen and senesce, and the seeds begin to dry down and dehisce within the fruit (Sink, 1984). If the seed filling process is completed at the same time in seeds produced on both MD and 44568, but the ripening and dehiscence processes are delayed in 44568, it is possible that respiratory enzymes and processes could still be active in the 44568 fruit that would not be active in MD fruit due to normal dehydration during dehiscence. Maintenance of even a low level of respiration during an extended ripening period in 44568 fruit and seeds could cause catabolism of carbohydrates that would have normally been intended for storage, thereby reducing the dry weight of the seeds. In desiccation-tolerant species such as pea,

normal rapid dry down of seeds leads to normal seed viability, whereas slower rates of desiccation lead to reduced viability, presumably due to stress associated with ageing (Walters et al., 2001). Alternatively, it is possible that seeds produced on 44568 plants may simply proceed through development more slowly than seeds produced on MD plants. Since it is known that tolerance of desiccation increases as embryos approach maturity (Farrant and Walters, 1998), any delay in embryo maturity due to slower development could make these seeds less tolerant to desiccation, thus reducing viability. Further studies focused on gaining a better understanding of the entire seed development process in 44568 plants should shed light on these factors.

SEED GERMINATION. Two general trends in seed germination were observed (Fig. 1A and B): 1) Regardless of the pollen source, seeds produced on MD plants germinated faster compared to seeds produced on 44568 plants and 2) The overall percentage of seeds germinated was greater for seeds produced on MD plants compared to seeds produced on 44568 plants. Since smaller seeds have been observed to have lower germination rates than larger seeds in many species (Castro, 1999; Khan et al., 1999), this

reduction in seed size may be the cause of reduced germination. The fact that heterozygous seeds produced on MD germinate better than heterozygous seeds produced on 44568 raises several interesting issues. First, petunia seed germination may not be dependent on ethylene perception in the seed itself, but may be dependent on the maternal parent's ability to respond to ethylene. This observation is likely related to the slower ripening time and lower seed weight of fruit produced on 44568 maternal plants when compared to MD. As seeds dry down their metabolism slows to conserve energy reserves that the embryo will need for survival. Since seeds ripened on an 44568 maternal parent take 33-50% longer to dry down it is possible that respiration may be continuing much longer than with seeds ripened on a MD maternal parent. This may account for the lower seed weight of these seeds, thus leading to less vigorous embryos because they have less stored reserves. Additionally, there may be processes in the seedcoat that may be dependent on ethylene during the seed germination process. Ethylene produced during seed germination is known to induce cellulases and other enzymes associated with the seed coat that facilitate the breakdown of macromolecules to help support the growing embryo (Abeles, 1992). If these breakdown processes depend on ethylene, they would likely not occur as rapidly in seeds produced on 44568. Other factors such as the carbohydrate or hormone status could potentially interact with ethylene in seeds during germination. Experiments with *gin-1*, an *Arabidopsis* glucose-insensitive mutant, which is impaired in glucose signaling show that glucose signaling and ethylene interact to influence seed germination (Zhou et al., 1998). The *gin-1* mutant produces seeds that germinate faster than wild-type seeds by antagonizing a branch

Fig. 1. (A) Mean percent germination ($\pm$ SE) of homozygous wild type 'MD' and ethylene-insensitive 44568 or hemizygous ethylene-insensitive seeds produced from reciprocal cross-pollinations (female $\times$ male). All seeds were produced on plants grown at 29/24 °C. (B) Mean percent germination ($\pm$ SE) of homozygous wild type 'MD' and ethylene-insensitive 44568 or hemizygous ethylene-insensitive seeds produced from reciprocal cross-pollinations (female $\times$ male). All seeds were produced on plants grown at 24/18 °C.

Table 5. Mean shoot and root dry mass and days from anthesis to natural flower senescence of homozygous wild type 'MD' and ethylene-insensitive 44568 or hemizygous ethylene-insensitive plants produced from reciprocal cross-pollinations (female x male). All plants were grown for 12 weeks at 24/18 °C.

Genotype	Dry mass (g)		Days to senescence
	Shoot	Root	
MD	20.30 A ^z	6.21 A	7.4 A
44568	19.92 A	4.59 B	17.7 C
MD x 44568	20.13 A	5.09 B	16.4 B
44568 x MD	20.30 A	5.07 B	17.1 BC

^zMeans within columns followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey HSD).

of the ethylene signal transduction pathway leading to seed germination (Zhou et al., 1998). Another possible factor contributing to reduced germination of seeds produced by 44568 maternal plants could be related to a possible interaction between ethylene and other phytohormones such as abscisic acid (ABA). It is well known that a programmed decrease in both ABA content and sensitivity occurs as seeds dry down during maturation. Since ethylene is a negative regulator of ABA during seed germination (Ghassemian et al., 2000) it stands to reason that an altered fruit ripening program due to conferred ethylene insensitivity could affect the ABA status in developing and ripening petunia seeds. Future research directed toward elucidating the interaction between ethylene and ABA or other hormones should provide insight on the interplay of plant hormones during seed development.

WHOLE PLANT VIGOR. At 12 weeks of age shoot dry mass was not different ($P > 0.05$) between genotypes, while root dry mass was different ($P = 0.0001$) (Table 5). All ethylene-insensitive genotypes had an 18% to 26% reduction in root dry mass compared to wild-type, thus suggesting that ethylene is involved in the normal growth of the root system in petunia. An earlier experiment using the *Nr* tomato mutant showed that 35-d-old seedlings produced approximately the same root mass as wild type (Clark et al., 1999). Since root mass data were not collected on older plants in previous experiments, it is possible that a reduction in root mass may become more apparent as the plant ages. The greater root mass observed in MD plants may be due to the roots of 44568 being unable to respond properly to a physical obstruction in the soil. *Nr* mutants of tomato that are also ethylene insensitive have been shown to be unable to penetrate a physically dense soil medium (Clark et al., 1999). At 12 weeks of age, the root system of *Petunia* plants in these experiments reached the edge of the containers, and had become pot-bound. If ethylene is the signal for root swelling and decreased elongation normally observed when roots encounter an obstruction, 44568 plants may not be able to respond and explore the media as well as MD plants, resulting in reduced root growth. Further experiments need to be conducted on ethylene-insensitive plants to better determine how ethylene influences various aspects of root growth. These experiments would need to focus on both ethylene-insensitive and wild type plants at various stages of development, and studies should be conducted on root impedance of ethylene-insensitive and wild type plants that focus at the cellular level.

NATURAL FLOWER SENESCENCE. Natural flower senescence was affected by plant genotype ($P < 0.001$), with all ethylene-insensitive genotypes having flowers staying open and turgid for ≈ 10 d longer than MD (Table 5). There was only a slight difference in flower longevity between plants that were heterozygous or

homozygous for the CaMV35S::*etr1-1* transgene, indicating that it confers dominance in *Petunia*. These results are in agreement with Chang et al. (1993), who showed that dominant ethylene insensitivity could be achieved through transformation of the CaMV35S::*etr1-1* transgene in *Arabidopsis*.

Conclusions

The experiments presented here confirm that constitutively expressed ethylene-insensitivity in petunia creates some detrimental physiological effects that would limit their commercial use, while providing evidence that some of these effects could potentially be overcome. Although ethylene insensitive plants produce viable pollen that appears to travel normally through the stigma/style after pollination, many subsequent developmental events are dramatically affected in ethylene-insensitive reproductive organs compared to wild-type plants. Transgenic ethylene-insensitive plants show delayed fruit ripening, which leads to reduced weight of seeds produced on these plants. Subsequently, seed germination and early seedling vigor of seeds produced on these plants is greatly reduced. Interestingly, seeds produced as a result of crossing pollen from homozygous ethylene-insensitive plants onto wild-type maternal plants do not have reduced seed weight, and do not have reduced germination and early seedling vigor. Since the CaMV35S::*etr1-1* transgene confers dominant ethylene insensitivity, these observations suggest that fruit and seed development, as well as seed coat factors during subsequent seed germination may be greatly affected by the presence of this transgene. Although Bleecker et al. (1988) mentioned that seed germination in the original *etr1-1* mutant in *Arabidopsis* had reduced seed germination, there has been no research to date focused on determining the factors involved in this phenomenon. Since the CaMV35S::*etr1-1* petunia system offers a useful experimental system by which to study the effects of ethylene on a number of physiological processes in plants, future research on seed development and subsequent germination should use this system to focus on determining the carbohydrate status of seeds, and the potential interactions of ethylene with other hormones such as ABA, auxin and gibberellic acid. Regardless of the scientific importance of this system in studying the role of ethylene in seed development these results suggest that the use of flower specific gene promoters to drive ethylene-insensitivity combined with the production of F₁ hybrid seeds using a wild-type maternal parent will be necessary to overcome these limitations and enable the commercial production of long-lasting ethylene-insensitive flowers with few negative physiological side effects.

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