This report serves as an update on some of the progress that has been made in my laboratory in on the project “Horticultural Performance of Ethylene Insensitive Petunias”, funded for the 1998 research year by The FNGA. As a result of experiments conducted with these funds, we made progress on two of the three goals set forth in the initial proposal.

Initially, the objectives of the project were:

1. **Genetically transform inbred lines of petunia with the dominant mutant ETR1-1 gene from *Arabidopsis thaliana*.**
2. **Breed the mutant ETR1-1 gene to homozygosity using traditional breeding methods.**
3. **Evaluate horticultural performance of ethylene insensitive progeny in relation to commercial success.**

RESULTS & DISCUSSION

Part 1 – Genetic transformation of inbred petunia lines with the dominant mutant ETR1-1 gene *from Arabidopsis thaliana*.

We started this research project by having one selected line of transgenic *ETR1-1* ethylene insensitive petunias in the inbred ‘Mitchell Diploid’ (MD) genetic background with which all of our preliminary work (Wilkinson *et al.,* 1997) was conducted. In order to verify all of the results we saw in this line, we produced more independent transgenic lines in the inbred ‘V26’ background using the procedure of Jorgensen *et al.* (1996). We produced several new transgenic lines, many of which are still currently being bred and evaluated. All of the transgenic plants produced so far in both genetic backgrounds have the *ETR1-1* gene under the control of the constitutive CaMV35S promoter – meaning the mutant gene is expressed in all tissues all the time. *As will be reported later, we feel that having ethylene insensitivity expressed in all tissues at all times throughout the development of a plant could be extremely detrimental to various aspects of the plant lifecycle.* We generated two new independent lines in the ‘V26’ background that have been verified to show the ethylene insensitive phenotype of delayed flower wilting in response to ethylene treatment and pollination induced ethylene production.

Part 2 - Breed the mutant gene to homozygosity using traditional breeding methods.

In order to conduct the appropriate experiments to determine the effects of ethylene insensitivity on other traits, we had to reliably know the correct genotype of plants, and show that it correlated to a delayed flower wilting phenotype. To conduct genetic studies we bred the mutant *ETR1-1* gene to homozygosity – in theory, that allowed us to produce ethylene insensitive F1 hybrid seed populations, which are normally the way commercial petunias are produced. It also allowed us to test for transmissibility of traits to offspring from maternal versus paternal sources and to determine if there is any incidence of gene silencing – i.e., we wanted to determine if the engineered gene in the plant is being consistently expressed to show a phenotype or visual effect. The theoretical breeding scheme for a single copy dominant mutant is as follows:

T0	→	T1	→	T2
	self⊕		self⊕	
		1 AA*	→	**AA***
Aa	→	**2 Aa**	→	3:1
	self⊕	1 aa	→	aa
			self⊕	

T0 – original transgenic plants – In a diploid plant with a single copy gene insertion, these are theoretically heterozygous (Aa).
T1 – self progeny of orignal transgenics – For a dominant gene in a diploid plant, this population will theoretically segregate **3**:1 for the **ethylene insensitive**:sensitive phentotype.
T2 – self progeny of T1 individuals – Lack of segregation reveals homozygosity of given T1 individuals, whereas segregation indicates heterozygosity of a T1 individual.

We developed two selection tools that we utilized for selecting ethylene insensitive plants containing the ETR1-1 gene. First, we utilized a PCR technique that allowed us to selectively amplify portions of the transgene that are not normally contained in the petunia genome – i.e., we detected the presence or absence of the engineered gene in a given plant by extracting DNA from a small leaf disk, and running PCR reactions on it to specifically amplify the foreign gene. We used this tool to screen large numbers of individuals in any given population at very early stages of development, thus saving much time, space and money. Based on these results, we were able to select individuals containing the transgene, then allow them to proceed to flowering and test for an ethylene insensitive flower phenotype. Once we identified the most ethylene insensitive individuals, we attempted to breed them to homozygosity using traditional breeding techniques based on dominant Mendelian genetics in the described breeding scheme. We evaluated both ethylene insensitive V26 *ETR1-1* lines at the T1 generation and produced the T2 progeny for evaluation in greenhouse trials. We bred MD *ETR1-1* plants to the T2 generation and isolated homozygous inbred plants.

CONCLUSIONS AND PLANS FOR THE UPCOMING YEAR

So far, we have been able to successfully engineer ethylene insensitivity into petunia plants and pass on the delayed flower wilting trait through sexual reproduction. The fact that a mutant ethylene receptor gene from *Arabidopsis* can be engineered into petunia to produce delayed wilting flowers has extremely important commercial significance for the floriculture industry. It is hoped that information gained through the experiments with plants generated in these experiments will help pave the way for commercialization of this promising biotechnology. At the current time, results from the experiments described in this report have been written up for publication, and are in the final stages of review before submission to either *Plant Physiology* or the *ASHS Journal*. We plan to continue our work on the ethylene insensitive lines we have engineered so far, in an attempt to breed ethylene insensitive plants to homozygosity for further genetic and physiological studies. This work will ultimately lead to new insights on the role of ethylene in a number of physiological responses pertaining to various points in the plant lifecycle. While conducting research to date, we have also discovered that there are some problems that arise from making a plant totally insensitive to ethylene. These problems could have serious implications for how this new technology will be used in the floriculture industry, and will likely necessitate directed gene expression. After observing plants containing the *ETR1-1* gene from *Arabidopsis* driven by the constitutive CaMV35S promoter (in all tissues all the time), we feel that there is a serious need to engineer ethylene insensitivity only in specific parts of the plant – i.e., ethylene insensitivity in the flower only. Although we have observed significantly lower rates of seed germination in ethylene insensitive plants, we have been able to devise a simple treatment (GA_3) that will allow seed germination at normal levels. However, we have not been able to overcome inhibition of adventitious root formation in ethylene insensitive plants using traditional rooting hormones. This is perhaps the most limiting aspect of this technology for commercial use in other ethylene insensitive floriculture crops such as geranium, carnation and hibiscus. Flowers can be engineered to be ethylene sensitive, but if cuttings cannot be propagated normally there will be little potential for the technology.