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INHERITANCE OF MALE STERILITY AND INFLUENCE OF
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Jaemin Lee

has been accepted towards fulfillment
of the requirements for

M.S. degree in Horticulture

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Major professor

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**INHERITANCE OF MALE STERILITY AND INFLUENCE OF ENVIRONMENTAL
FACTORS ON MALE STERILITY IN *Impatiens wallerana* Hook. f.**

By

Jaemin Lee

A THESIS

**Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of**

MASTER OF SCIENCE

Department of Horticulture

1998

ABSTRACT

INHERITANCE OF MALE STERILITY AND INFLUENCE OF ENVIRONMENTAL FACTORS ON MALE STERILITY IN *Impatiens wallerana* Hook. f.

By

Jaemin Lee

The investigation of male sterility (MS) in the germplasm used in this experiment showed that male sterile plants did not produce exuded pollen grains and that a genic cytoplasmic male sterility (GCMS) model may control the inheritance of MS in *Impatiens wallerana* Hook. f.. The genotype of male sterile inbreds in this germplasm was hypothesized as *Smsms*, and the genotype of male fertile inbreds was hypothesized as *SMsMs* or *NMsMs*.

The results for one of commercial F1 hybrids, 'Super Elfin Red Velvet' (SERV), showed that MS of this population was not controlled entirely by genetic inheritance pattern, but was influenced by relative humidity (RH). Normal pollen grains like normal male fertile plants were observed at RH of $80 \pm 5\%$. Further investigation of environmental influences on MS in SERV showed that temperature and photoperiod affected flower number and flower size, but had no influence on the expression of MS.

To my wife, son, and parents

ACKNOWLEDGEMENTS

I would like to express my deepest and sincerest appreciation to my major professor, Dr. Lowell C. Ewart, for his guidance, advice, and innumerable assistance throughout this study and thesis. I would also like to appreciate the other committee members, Dr. John F. Kelly, who gave me tremendous advice and help, and Dr. James D. Kelly, whose suggestions were meaningful for my research.

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INTRODUCTION

Impatiens L.(*Balsaminaceae*) is a world-wide genus that includes 400-1000 species (Grey-Wilson,1980). The genus *Impatiens* (*I*) contains many popular cultivars both in the garden balsam (*Impatiens balsamina* L.) and the patient plant group (*Impatiens wallerana* Hook. f.) (Zinoveva-Stahevitch and Grant 1985). *Impatiens* have been described as being annual or perennial plants. In the genus *I*, *I. wallerana* (IW) is included in an aggregate of several other species groups that are phylogeographically restricted to East Africa, mainly central and southern Kenya and the mountains of northern and eastern Tanzania, but reaching as far south as Malawi (Grey-Wilson, 1980). According to Grey-Wilson (1980), this group includes nine species; *I. usambarensis*, *I. wallerana*, *I. hamata*, *I. pseudohamata*, *I. serpens*, *I. thamnoidea*, *I. cinnabarina*, *I. messumbaensis*, and *I. pseudoviola*.

The members of this aggregate form a fairly close group in which the lateral united petals are positively or negatively regular with a central mid-vein, the upper and lower petals of each pair are fused together only near the base. Among those species, IW commonly known as “patience plant” and “impatiens”, has become an extremely popular bedding plant due to its ability to bloom profusely in shady locations, and its diverse colors (Merlin and Grant, 1986). The species is generally recognized by its normally two-flowered inflorescences and rather bright green, somewhat translucent leaves. The

inflorescences shows one flower to rarely five flowers according to species. *Impatiens* has a very characteristic flower, rather flat, with a large upper petal, and lateral petals of almost equal size and shape and fused together only at the base. The stems are typically rather thick, fleshy, and semi-translucent. The leaves are also generally rather fleshy and thin, becoming membranous, and transparent when dried. After pollination, abundant seeds are set, being expelled from the seed pod when matured. This characteristic also gave the plant a nickname “Touch-me-nots”. The commercial variation in IW is due to leaf shape and flower color. This variability has been known to flower breeders and growers for many years, and numerous forms have been selected for cultivation (Grey-Wilson, 1980).

Most *impatiens* today that are produced as bedding plants are autogamous, and propagated by seed, but they can be propagated by cuttings. Morphologically *impatiens* have markedly proponderous flowers that are well adapted to pollination by insects or birds, and the filaments are fused with the anthers held closely together by the fusion of the anther walls. The anthers project downwards so that they act as a brush against the pollinator’s body for ease of self-pollination.

Hybridization studies of the genus *Impatiens* have been conducted by Grey-Wilson (1980), Merlin and Grant (1985), and Arisumi (1987). Cytogenetic studies of *Impatiens* have shown a range of chromosome numbers of $n = 3, 6, 7, 8, 9, 10, 13, 16, 17, 18, 20$, and 24, and the aggregate species of IW from East Africa have been reported to be $n = 8$ (Zinoveva-Stahevitch and Grant, 1984). Merlin and Grant (1985) reported interspecific hybridization among the East African aggregates. They investigated the biosystematic relationships of IW and other selected species such as *I. gordonii* and *I. usamberensis* by

means of hybridization experiments and cytological studies. Arisumi (1987) also used several *I.* species in his hybridization studies. He obtained hybrids between IW and *I. thomasetti*, and used ovule culture to make hybrids between species.

Impatiens (IW) has currently been the most popular species of *Impatiens*, and has been ranked as the top bedding plant in the United States since 1985 (Armitage, 1994). According to the summary of floriculture crops in 1996, the wholesale value of IW was \$108,050,000 in 1996 (National Agricultural Statistics Service Bulletin, 1997). This popularity has generated studies about flower production, flower size, seed germination, and flower colors to develop new and more popular cultivars.

The purpose of this study was to identify and determine the inheritance of male sterility (MS), and to investigate environmental conditions that might influence MS in IW. It is hoped that the results will help with F1 hybrid seed production, and also provide an expanded information base for the study of MS in IW. This study was divided into two sections, the first covering the inheritance of MS and the second covering the environmental conditions that might affect MS in IW.

INHERITANCE OF MALE STERILITY IN *Impatiens wallerana* Hook. f.

MALE STERILITY IN *Impatiens wallerana* Hook.f.

Literature review

New cultivars in plants produced through seed are developed by inbreeding or hybridization of self-pollinating species, and selective intermating in out-crossing species. The process varies according to the type of flower and pollination mechanisms. The vast majority of IW cultivars produced and sold today are F1 hybrids. The production of F1 hybrids in autogamous species such as the *impatiens* requires effective sexual isolation to prevent self-pollination. Commercially, this objective has been achieved in many crops by hand-emasculature, mechanical emasculature, genetic or cytoplasmic male sterility, or the use of chemicals. Hand emasculature is rather tedious, time-consuming, and costly, and chemical treatments can cause undesirable effects in plant growth.

The use of MS in flower breeding as well as crop breeding has proven to be an effective means of obviating hand-emasculature and chemical treatments. Several MS systems such as genic male sterility (GMS), cytoplasmic male sterility (CMS), genic-cytoplasmic male sterility (GCMS) have been used to prevent self-pollination and to permit the production of F1 hybrids in many flower crops (Kaul, 1988; Rao et al., 1990). Self-incompatibility (SI) is one of the systems used to prevent self-pollination and to control outbreeding genetically (Rao et al., 1990). It has been reported to be unreliable in eliminating male gametes from normally monoecious plants, and the level of

heterozygosity realized is not as high as that of pure hybrid breeding (Rao et al.1990). Therefore, MS systems have received more attention than SI in breeding and hybrid seed production of autogamous species.

Male sterility systems in higher plants are classified into genic or non-genic MS. They arise either spontaneously or can be induced by chemical treatments, environmental manipulation or biochemical alteration (Kaul, 1988). Genic male sterility also is divided on phenotypic and genotypic base. Phenotypic systems are categorized into three types. First is structural MS, which is due to structural anomalies in the male sex organs, so that the male reproductive organs are either absent or deformed. The second is sporogeneous MS, in which stamens develop, but sporogeneous tissue is either mis- or mal-formed, or it develops normally. However, microsporogenesis or gametogenesis is impaired so that pollen is malformed or its premature abortion occurs. The third is functional MS, which produces viable pollen, but it fails to fertilize due to barriers preventing pollens grains from reaching a stigma. The main barriers of functional male sterility are indehiscent anthers, faulty or non-exine formation, and inability of pollen to migrate to a stigma or influence fertilization (Kaul, 1988; Phatak et al. 1991). Under functional MS, seed companies have proprietarily mentioned that mechanical MS as occurring in IW where the anther cones fall off before the stigma is receptive.

The three major genotypic systems of MS are GMS, CMS, and GCMS. Genic male sterility is controlled by nuclear genes, and CMS is controlled by specific MS-inducing cytoplasm known as S cytoplasm (Frankel and Galun, 1977; Kaul, 1988; Sawhney and Shukla, 1994). Genic-cytoplasmic male sterility, which results from a combined effect of

male sterile nuclear genes (*rf*) and sterile cytoplasm, is also known (Sawhney and Shukla, 1994) (Fig. 1).

Male sterility systems have been reported for many crops since the first discovery of MS in 1905. In maize, Beadle and McClintock (1928) reported MS from over 30 unrelated maize cultures. Singleton and Jones (1930) demonstrated the MS was controlled by a recessive gene designated as *ms*. Following this report, many *ms* genes inducing complete or partial MS were detected. Rhodes (1933) described the first case of GCMS in maize, and found MS was due to a cytoplasmic factor contributed by the female parent. Following the research of Rhodes (1933), Texas sterile cytoplasm (T-cytoplasm)(Rogers and Edwardson, 1952), USDA sterile cytoplasm (S-cytoplasm)(Jones et al., 1957), and C-cytoplasm (Beckett, 1971) were discovered.

The research of MS in onion demonstrated early recognition of GCMS and immediate utilization for hybrid production. Jones and Clarke (1943) reported that male sterile plants had S-cytoplasm and *frfr* genes. The results confirmed that *Sfrfr* plants were male sterile, and others, like *SFrFr*, *SFrfr*, *NFrFr*, *NFrfr*, and *Nfrfr* were male fertile.

In tomato, the first report of a male sterile mutant was from Crane (1915). The MS was found to be controlled by a recessive gene. Further investigations revealed that the tomato genome had a large number of *ms* genes (Rick, 1944, 1966, 1980; Kaul, 1988).

There have been several reports related to MS in floriculture crops. In petunia, Frankel (1962, 1971) reported the existence of a single recessive gene controlling MS. Perkins and Ewart (1990) found a GMS system that is controlled by a single recessive gene.

Ewart and Walker (1958) reported that hybrid petunia cultivars produced through the use of CMS were always inferior to the normal counterpart in regard to flower production. Edwardson and Warmke (1967) reported a genetic-cytoplasmic interaction in petunia, in which two recessive nuclear genes (*fr1* and *fr2*) interacted with S-cytoplasm derived from the line P-266 which led to MS.

Kaul (1988) demonstrated GMS in carnation (*Dianthus caryophyllus* L.). He mentioned that genetic control of the MS is not clearly defined, but segregations indicated a monogenic recessive control. In *Dianthus chinensis*, a monogenic recessive spontaneous mutant was found in a plant having rose-lilac petals (Kaul, 1988).

In *Zinnia*, since Metcalf et al. (1971) discovered a male sterile inflorescence, Cowen and Ewart (1990) reported this MS to be controlled by a three-gene recessive model, and that the apetalous and male sterile characteristics are very likely pleiotropic. Kaul(1988) also mentioned that MS in zinnia is controlled by male sterile genes, *ms1*, *ms2*, and *ms3*. All the male sterile flower heads had aborted stamens and non-viable pollen grains.

Kaul (1988) also reported spontaneous MS in pansy (*Viola tricolor* L.) in which the anthers were nonhairy and indehiscent. The plants showing MS had normal meiosis, but the maturing microspores degenerated along with the tapetum. This anomaly was controlled by one recessive male sterile gene.

Research for any MS system in impatiens has not been documented even though seed companies have recognized the application of MS in the production of F1 hybrid. According to several seed companies, the information of MS in impatiens breeding is considered to be proprietary, and current speculation is that one or more MS systems may

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be used in impatiens breeding. General information indicates that male sterile plants are simply selected, and then, propagated vegetatively (Grazzani, 1995).

This research was, therefore, undertaken to try and determine the system(s) and inheritance pattern(s) that may be controlling MS in IW.

Materials and Methods

Germplasm used

The majority of the germplasm for this study consisted of eighteen inbreds and five experimental hybrids provided from a commercial seed company (Grime Seeds). Some initial observations came from screening nine other commercial hybrids from the Michigan State University (MSU) flower seed trials. The nine commercial hybrids included cultivars developed by several seed companies (Pan American, Sluis&Groot, Bodger, and Goldsmith) (Table 1).

Additional observations were recorded on this germplasm to make an initial assessment of the best material to use in the study (Table 2).

Seed handling

All impatiens seed used in this experiment were sown in 20-row plastic germination trays containing a peat-lite mix. The seeds were covered with fine vermiculite. After sowing the seeds, transparent plastic covers were placed over the trays to maintain proper moisture and temperature. Temperature for germination was maintained at 25 °C in a greenhouse. Radicle emergence took place five days after sowing. The germination rate of inbreds and hybrids was determined as the ratio of germinated seeds to the number of seeds planted, and any abnormal seedlings were eliminated. Three weeks after sowing, the healthy seedlings were transplanted to flats with 48 cells. Standard greenhouse procedures were followed for normal plant development, disease and pest control.

The research was conducted in two phases: 1) assessment of fertility / sterility of inbred and hybrid lines in IW 2) determination of inheritance of MS in IW.

Table 1. Germplasm lines used for the assessment of male sterility in *Impatiens wallerana* Hook.f.

Plant ID	Pedigree	Cultivar	Flower color
GA1	GSG1	Inbred	Red
GA2	GSG44	Inbred	Red
GA3	GSG15	Inbred	White
GA4	GSG49	Inbred	White
GA5	GSG24	Inbred	Deep rose
GA6	GSG46	Inbred	Rose
GA7	GSG30	Inbred	Scarlet
GA8	GSG37	Inbred	Scarlet
GA9	GSG39	Inbred	Orange
GA10	GSG47	Inbred	Orange
GA11	GSG17	Inbred	Red
GA12	GSG6	Inbred	Rose
GA13	GSG8	Inbred	Orange
GA14	GSG18	Inbred	Deep orange
GA15	GSG19	Inbred	Light orange
GA16	GSG20	Inbred	Light orange
GA17	GSG22	Inbred	Purple
GA18	GSG23	Inbred	Purple
GH1	GSG1XGSG44	Hybrid	Red
GH2	GSG15XGSG49	Hybrid	White
GH3	GSG46XGSG24	Hybrid	Rose
GH4	GSG30XGSG37	Hybrid	Scarlet
GH5	GSG47XGSG39	Hybrid	Orange
CH1	Super Elfin Red velvet	Hybrid	Red velvet
CH2	Impulse Deep pink	Hybrid	Deep pink
CH3	Super Elfin Red	Hybrid	Red
CH4	Impulse Rose	Hybrid	Rose
CH5	Impulse Lilac-blue	Hybrid	Lilac-blue
CH6	Tempo Scarlet	Hybrid	Scarlet
CH7	Tempo White	Hybrid	White
CH8	Cajun Red	Hybrid	Red
CH9	Cajun Violet	Hybrid	Violet

1. Assessment of male sterility in *Impatiens wallerana* Hook.f.

All plants were grown under standard greenhouse conditions, and evaluated by visual and microscopic observation for presence or absence of pollen grains. Visual determination of MS was based on the judgment of several observations for a month after the onset of flowering in each generation. The visual observations were conducted using a magnifying lens (5x). Samples of anthers from fertile and sterile plants were examined microscopically for the presence of normal pollen grains, using a cotton blue solution. The cotton blue solution (Darlington and LaCour, 1976) was prepared by adding equal parts of water, 85% liquified lactic acid, 88% liquified phenol, and U. S. P. glycerine, in that order, and mixing well before each addition (Ewart, 1963). Anther cones were squashed and mixed with a drop of cotton blue solution on a glass slide, and the excess debris was removed. A cover slip then was placed over the drop of cotton blue solution. The slide then was observed under an binocular microscope at 100X, 200X, and 400X magnification. Pollen grains from fertile plants stained dark blue and exhibited spherical uniformity, whereas the pollen grains from male sterile plants stained light blue and were elliptical and shrunken (Fig. 1).

The assessment of MS was based not only on the aspects of pollen fertility, but also on the number of populations that could be handled, and the assessment of other horticultural characteristics such as branching, earliness, height, flower size, and germination rate (Fig. 2, and Table 2).

To determine if functional MS might be involved in this germplasm, seed set through natural self-pollination was investigated in selected MF plants (GA2, 5, 9, GH1,

3, 5, CH2, 3, 4, and 5) among inbred and hybrid populations. Observations were done to see if the anther cones of this material would fall off early before the stigma was receptive. Five plants with abundant pollen in each population were investigated for five weeks after flowering to see if each plant was successful in setting seeds by natural selfing.

2. Inheritance of male sterility in *Impatiens wallerana* Hook.f.

Selected plants from each population were planted directly into six-inch diameter plastic pots. The inbred and hybrid male sterile populations (GA1, 6, 10, and CH1) selected were test-crossed to male fertile inbred (GA2) and hybrid populations (GH1, 3, and 5) to determine if either GMS, CMS, or GCMS might be involved. The inbred male fertile populations (GA2, 5, and 10) were selfed to determine if the inbreds were true breeding, and the hybrid male fertile populations (GH 1, 3, 5, CH2, and 3) were selfed to determine if they would segregate for MS. Pollinations among male sterile plants and male fertile plants were performed in a screened greenhouse. Three to four weeks passed before seeds were harvested. Harvested seeds were placed in a storage room controlled at 5 °C, 35% RH for at least two weeks before sowing. In order to investigate the relationship between MS and plant characteristics, flower color, leaf color and shoot color also were examined.

Chi-square and goodness-of-fit analyses for MS were performed to test the segregation ratio on each population.

Table 2. Characteristics of *Impatiens wallerana* Hook f. germplasm used for the assessment of male sterility

Plant ID	Branching <i>z</i>	Earliness <i>y</i>	Height <i>x</i>	Flower size <i>w</i>	Germination rate <i>v</i>
GA1	4	3	5	3	A
GA2	4	5	5	3	A
GA3	4	4	5	3	A
GA4	4	5	5	3	A
GA5	4	3	5	5	B
GA6	3	5	5	3	A
GA7	4	4	3	3	B
GA8	4	4	5	3	C
GA9	4	5	4	5	A
GA10	3	5	5	3	A
GA11	4	4	1	3	D
GA12	4	3	5	3	D
GA13	5	4	1	3	C
GA14	4	4	1	3	D
GA15	5	4	3	3	D
GA16	5	4	1	3	C
GA17	5	4	1	3	D
GA18	5	4	1	3	D
GH1	4	4	5	3	B
GH2	4	4	5	3	A
GH3	4	5	5	4	B
GH4	4	4	4	3	C
GH5	4	5	4	5	B
CH1	5	4	5	5	A
CH2	5	4	5	5	A
CH3	5	4	5	5	A
CH4	5	5	5	5	A
CH5	5	5	5	5	A
CH6	5	4	5	5	A
CH7	5	4	5	5	A
CH8	5	4	5	5	A
CH9	5	4	5	5	A

z: represents branching (1=least, 5=most)

y : represents earliness (1=latest, 5=earliest)

x : represents height (1=dwarf, 5=tall)

w : represents flower size (1=small, 5=large)

v : represents the degree of germination rate (%) : A=100%,B=75%,C=50%, and D=below 50%

Results and Discussion

Assessment of male sterility in *Impatiens wallerana* Hook.f.

The assessment of MS for the IW germplasm used in this study was mainly determined by abnormalities of the anther cone, absence or presence of pollen, and the success of seed set after pollination. The identification of male fertile and male sterile plants was accomplished by visual or microscopic observation (Table 3). Male fertile plants produced plenty of visually evident exuded pollen grains (Fig. 1). The pollen grains from male fertile plants, when stained by cotton blue solution and observed through a microscope, were plump and spherical (Fig. 2).

Male sterile plants did not produce any visually evident, exuded pollen grains. The anther cones of male sterile plants were smaller than that of the male fertile plants, and did not become plump. Under microscopic observation, elliptical and deformed pollen grains within the anther cone in male sterile plants did not stained completely with the use of cotton blue solution (Fig. 2).

The observed abnormal pollen shape in the male sterile plants concurs with the abnormal pollen shape in wheat (*Triticum aestivum*). The anther anomalies and pollen abortion in wheat are conditioned by two different genes or sets of genes (Kaul, 1988). The abnormal structure of male sterile pollen in this germplasm also concurs with the observation of pollen grains in IW by Van Went (1981). According to his cytological observations of pollen development, abnormal development in the sterile anther begins at the time of release of microspores from the tetrads, resulting from a slight delay in callose dissolution. The tapetal cells begin to enlarge and become vacuolated, but fail to develop

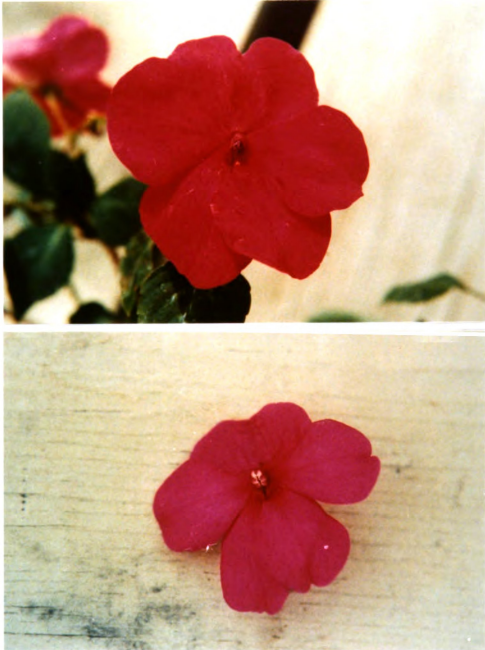


Fig. 1 Male fertile (top) and male sterile flower (bottom) in *Impatiens wallerana*
Hook f.

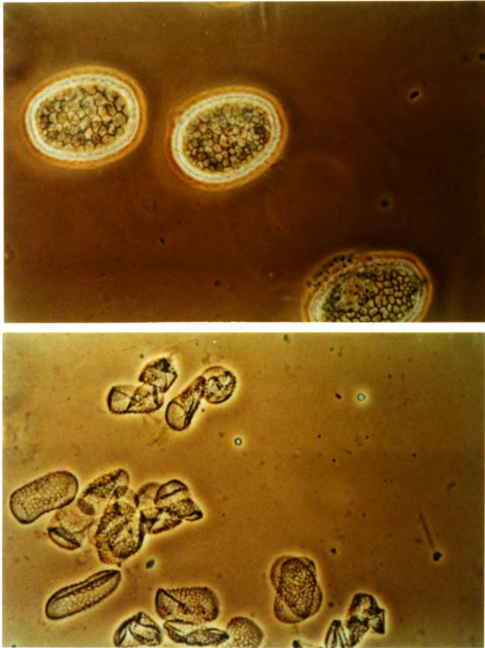


Fig. 2 Male fertile (top) and male sterile (bottom) pollen grains in *Impatiens wallerana* Hook f.

the specific structure of the fertile anther, and the cytoplasm becomes highly diluted.

In the flower-bud stage, the anther cones of the flowers of male sterile plants and male fertile plants appeared to be similar, but the anther cones of male sterile plants later did not exude pollen as the flowers opened. These results were similar to a male sterile mutant in mercury weed (*Mercurialis annua* L.) whose anthers are reduced in size, and the pollen grains are deformed and shrunken (Louis and Durand, 1978).

For the determination of MS, six inbreds (GA1, 2, 5, 6, 9, and 10), three experimental hybrids (GH1, 3, and 5), and two commercial hybrids (CH1 and 3) were selected from the observed germplasm lines. The rest of the germplasm lines were not used, because of the lack of population number and low seed germination. It is interesting to note from the results shown in Table 2 how uniform the commercial hybrids (CH1 through CH9) are for the characteristics observed compared to the rest of the material which is very important to the flower seed industry.

As a result of the assessment for MS, the plants of three inbreds (GA2, 5, and 9) and three experimental hybrids (GH1, 3, and 5) were male fertile, whereas, the plants of three inbreds (GA2, 9, and 15) were male sterile. The three male fertile inbreds, and the three experimental hybrids all produced seeds by natural self-pollination, and there was little evidence of early anther-cone shedding. In the assessment of the commercial hybrids (Table 3), the plants of hybrid CH1 were all male sterile, and the rest of the commercial hybrids were male fertile. The commercial male fertile hybrids also produced seeds by natural self-pollination, and functional MS or early anther-cone shedding was not in evidence.

There has been some mention of the possible use of functional MS or early anther-cone shedding in IW on a rare occasion for hybrid seed production (personal communication by Ewart, 1996). It was stated that the functional MS might be controlled genetically. However, the successful seed-setting by natural self-pollination of the MF germplasm studied in this research suggests that functional MS or early shedding of the anther cone might be difficult to manage in IW.

Table 3. Assessment of male sterility and male fertility in *Impatiens wallerana***Hook.f.**

Plant ID	Pedigree	Phenotype of germplasm (MS _z / MF _y)
GA1	GSG1	48 / 0
GA2	GSG44	0 / 48
GA5	GSG24	0 / 35
GA6	GSG46	42 / 0
GA9	GSG39	0 / 48
GA10	GSG47	48 / 0
GH1	GSG1XGSG44	0 / 48
GH3	GSG46XGSG24	0 / 48
GH5	GSG47XGSG39	0 / 48
CH1	Super Elfin Red velvet	48 / 0
CH3	Super Elfin Red	0 / 48

_z Male sterility_y Male fertility

Identification of male sterility in *Impatiens wallerana* Hook f.

The germplasm for this part of research was selected through the initial assessment of MS and the assessment of horticultural characteristics (Tables 2 and 3). The results in Table 3 for GA1, 2, 5, 6, 9, 10, GH1, 3, and 5 were derived from seeds provided directly by Grime seed company. The commercial hybrids CH1 and 2 were derived from seed obtained from Pan American seed company.

As a result of the visual and microscopic assessment, lack of seed volume for GA3, 4, 7 and GH2 would make proper population assessment of MS difficult, so they were eliminated from the study. Also, GA8, 11, 12, 13, 14, 15, 16, 17, 18, and GH4 were not selected due to low seed germination. The selection of desirable horticultural traits in the germplasm was considered to be important, because such traits are useful and valuable for inbred and hybrid development.

Six inbred lines (GA 1, 2, 5, 6, 9, and 10), three experimental hybrids (GH1, 3, and 5), and the two commercial hybrids (CH1 and CH3) were selected for identification of the inheritance pattern. All the plant populations from selfing the male fertile inbred lines (GA2, 5, and 9) were male fertile. This suggested that these male fertile inbreds were homozygous for MF. The segregation ratios of sterile and fertile plants in the F₂ population of GH3 and GH5 produced a very good fit to a 3:1 ratio ($P=.78$ and $P=.47$) (Table 4). The F₂ population of GH1 ($P=.33$) did not show a real good fit for a 3:1 ratio

with less male sterile plants than expected. This could have come from improper classification or reduced population size.

Test-crosses among the plants of male sterile inbreds (GA1, 6 and 10) and the plants of experimental male fertile hybrids (GH1, 3 and 5), however, showed a good fit to a 1:1 ratio ($P=.77$, $.63$ and $.50$)(Table 5). The results from the test-crosses and F₂ populations suggest that the inheritance of MS in GA1, 6 and GA10 is probably controlled by a single recessive *ms* gene. This result suggests that MS could be controlled by GMS in this group of germplasm lines, and controlled by a single recessive gene.

Further evaluation of MS in this germplasm, however, indicates that the inheritance of MS may not involve a straight GMS model. As shown in the results from Table 3, GA1, 6, and 10 was male sterile. If a straight GMS was involved with MS in this germplasm, the MS should be maintained by heterozygous (*Msms*) male fertile plants, and the phenotype of three inbreds (GA1, 6, and 9) should have segregated in a ratio of 1 male sterile (*msms*) : 1 male fertile (*Msms*) plants in case of GMS.

In addition, the results from the experimental hybrids (GH1, 3, and 5) indicated that true CMS was not controlling this germplasm. Segregation of the testcross and F₂ populations suggests that this germplasm is not affected by true CMS which is controlled solely by the male sterile (S) cytoplasm of the female parent.

These results, therefore, suggest that the inheritance of MS in this particular germplasm could be controlled by GCMS as in onion (Jones and Clarke, 1943). Here, there is an interaction between the sterile cytoplasm and male sterile genes. To maintain GCMS, sterile plants (*Smsms*) must be backcrossed with normal cytoplasm plants homozygous for the male sterile gene (*Nmsms*). Otherwise, the pure sterile plants can not

be maintained, and only fertile plants will be obtained by crosses with the genotypes *SMsMs*, and *NMsMs*, or 50% fertile plants, like in the *GMS* pattern, will be obtained from crosses with *SMsms* and *NMsms* plants. The crossing scheme as shown in Fig. 3 was used to help identify the genetic system operating in the germplasm studied in this research.

Two explanations for the inheritance pattern are possible. The genotype of the male sterile inbreds (GA1, 6, and 10) would be *Smsms*, and the genotype of the male fertile inbreds (GA2, 5, and 9) would be *SMsMs* or *NMsMs*. The crossing between these male sterile and male fertile inbreds could produce F1 hybrids having the genotype of *SMsms*. The segregation ratios from F2 and test-cross populations of a genotype *SMsms* would be the same as those for a recessive *GMS* model. Even though *Nmsms* maintainer lines in this particular germplasm were not identified, a personal communication (Grazzini, 1995) suggests the existence of such lines in these populations.

The results from the F2 populations of CH1xGA2 population did not produce a good fit for a 3:1 ratio (Table 6). The results from the populations from the CH1 crosses (Table 6) were completely different from expected. The segregation ratio varied from 2:1, 3:1 to 9:1 (male sterile plants : male fertile plants), and suggests that a different MS system such as the control by multiple male sterile genes might be operating in CH1, or some environmental condition is affecting the MS response. The possibility of another inheritance pattern was not pursued due to time limitation for this research, but would make for an interesting future research project.

On contacting Pan American seed company, the producer of CH1 (Super Elfin Red Velvet) concerning the unexpected results, it was explained that one parent of CH1 is sterile at same geographic locations, but fertile at other locations for seed production. The

explanation supports that this particular germplasm CH1 could be conditioned by some environmental factors such as temperature, photoperiod, or humidity. Since there was still time, though limited, to complete this research, a quick environmental study was performed, and the results from the environmental experiment was reported in the second section of this thesis.

Flower color, leaf color, and shoot color in all the crosses also were studied to see if there was any pleiotropy or linkage with MS. No relationship, however, between MS and these characteristics could be identified.

In summary, the results from the segregation data from most of the germplasm lines used in this study indicate that at least a single recessive gene is involved with possible cytoplasmic interaction as in onion. The results, however, from the CH1 material indicates another response system, possibly a different genic system or some environmental interaction. Additional research is needed on this particular material to reach a more definitive conclusion. The second section of this thesis addresses some environmental observations.

Table 4. Chi-square determination for a 3:1 ratio of male fertile to male sterile plants resulting from selfing F1 hybrid plants in *Impatiens wallerana* Hook f..

Line	No. of male fertile plants	No. of male sterile plants	Chi-square	P
F2(GH1⊗)	35	8	1.16	.33
F2(GH3⊗)	53	19	0.07	.79
F2(GH5⊗)	55	22	0.63	.43

Table 5. Chi-square determination for a 1:1 ratio of male fertile to male sterile plants resulting from test-crosses among male sterile inbreds and of experimental hybrid plants in *Impatiens wallerana* Hook f..

Line	No. of male fertile plants	No. of male sterile plants	Chi-square	P
GA1xGH1	23	25	0.08	.77
GA6xGH3	34	36	0.06	.63
GA6x(GA6xGH3)	27	23	0.32	.57
GA10xGH5	20	16	0.44	.50

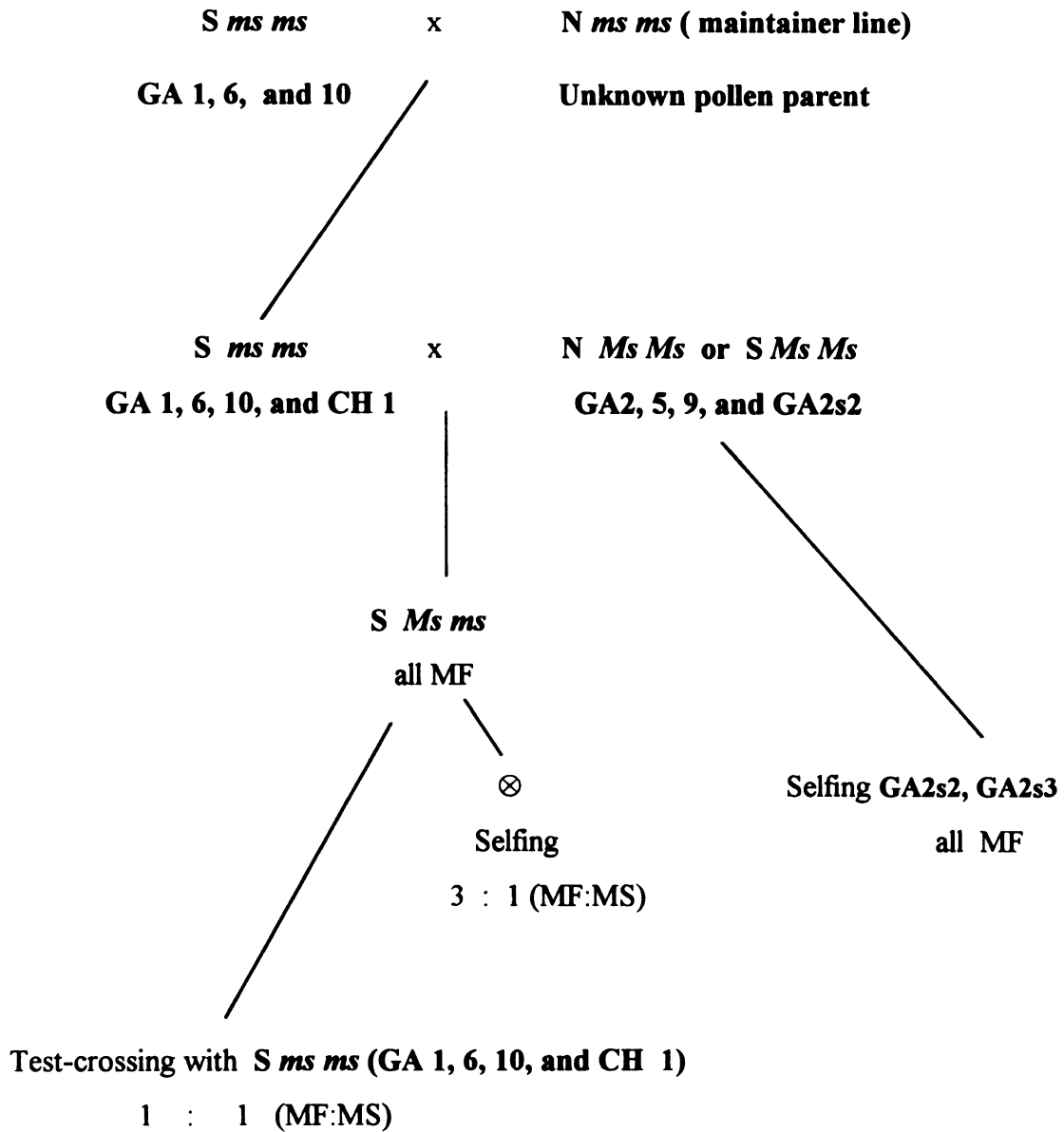


Fig. 3 Crossing scheme to identify genetic model for inheritance of male sterility in *Impatiens wallerana* Hook f.

Table 6. Chi-square determination for segregation ratios of male fertile and male sterile plants resulting from crosses between cv. ‘Super Elfin Red Velvet’ and other germplasms (GA2 and GH1)

Line	Observed male fertile / male sterile (no.)	Expected male fertile / male sterile (no.)	Chi-square	P
CH1xGH1	27/14	20.5/20.5	4.12	.042
CH1xGA2	35/13	48/0	—	—
CH1x(CH1xGA2)	17/6	11.5/11.5	5.26	.022
(CH1xGA2) ⊗	84/8	69/23	22.83	.00003

Summary

An inheritance study for male sterility (MS) was conducted on a particular germplasm collection of *Impatiens wallerana* Hook f. (IW). The assessment for MS showed that male sterile plants did not produce any pollen grains that were exuded, plump or spherical under microscopic investigation.

Three inbreds (GA2, 5, and 9) and three experimental hybrids (GH1, 3, and 5) were determined to be male fertile, whereas the plants of three inbreds (GA1, 6, and 10) were male sterile. All commercial F1 hybrids except for 'Super Elfin Red Velvet'(CH1) were male fertile.

The results from F1, F2 and testcross, populations suggest that a genic cytoplasmic male sterility (GCMS) model probably controls the inheritance of MS in this particular germplasm. The genotype of three male sterile inbreds (GA1, 6, and 10) is probably *Smsms*, and the genotype of the male fertile inbreds (GA2, 5, and 9) is probably *SMsMs* or *NMsMs*.

The results of the segregating populations from CH1 crosses were not a good fit for a GMS or a GCMS model, indicating that MS of these populations was controlled by either a different genic inheritance pattern or influenced by environmental conditions.

There was no relationship between MS and plant characteristics of flower color, leaf color, and shoot color.

INFLUENCE OF ENVIRONMENTAL FACTORS ON MALE STERILITY IN

Impatiens wallerana Hook f.

THE INFLUENCE OF ENVIRONMENTAL FACTORS ON MALE STERILITY IN *Impatiens wallerana* Hook f.

Literature review

The use of male sterility (MS) to produce F1 hybrids can make the hybridization process less labor-intensive and economically viable. The stable expression of MS is important for the hybridization process in many crops such as onion, corn, and wheat. Male sterility in *Impatiens wallerana* Hook f. (IW) appears to be controlled by genic and cytoplasmic factors.

The stabilization of MS in IW for some germplasm selections may be influenced by environmental factors (Pan American Seed Inc., 1997). Any environmental influence caused by light intensity, photoperiod, temperature, or relative humidity (RH) can make the identification of MS difficult.

The sensitivity of MS to the environment has been shown in many previous studies. In *Capsicum*, *Daucus*, *Sorgham*, and *Vicia*, temperature was an environmental factor to influence the expression of MS (Kaul, 1988). Martin and Crawford (1951) reported that one strain (No. 4558) in *Capsicum frutescense* was fully male sterile under greenhouse conditions and male fertile in field conditions. Fisher (1972) showed the effect of photoperiod on MS in wheat. Subjecting wheat at the stage of main shoot elongation to a 10-hour photoperiod treatment led to the transformation of stamens into ovaries. Ahokas

and Hockett (1977) demonstrated sensitivity of MS to photoperiod and physiography in barley. In *Cosmos* and *Oryza sativa*, pollen sterility was increased by subjecting the plants to a long photoperiod of 14 hours after floral induction by short-day cycles (Kaul, 1988). Lisci et al. (1994) found that anther dehiscence in *Mercurialis annua* was related to RH as well as temperature. Flower anthesis was delayed at low temperature (16-20 °C), whereas it occurred earlier at high temperature (27-29 °C). At high RH and low temperature (50-80%, 16-20 °C), anther dehiscence was retarded, whereas it occurred sooner at low RH and high temperatures (30-44%, 27-29 °C). On the contrary, Carter and McNeilly (1975) reported the influence of humidity on pollen germination and pollen tube growth in *Brassica oleraceae* var. *gemmifera*. They found that high humidity conditions created by placing polythene bags over the flowers, promoted the rate of pollen germination over the control treatment. Plant breeders agree that optimum conditions for anthesis and pollen formation vary from species to species and even among cultivars of a species.

According to results from the previous investigation of MS in the available IW germplasm, the F1 hybrid cultivar 'Super Elfin Red Velvet' (SERV) exhibited MS, but did not show the same consistent results for MS segregation as those observed in the other germplasms. In general, the commercial impatiens cultivars observed except for SERV were male fertile. The results as shown in Table 6 of part 1 in the thesis showed that the segregation ratios from crosses among SERV, and an inbred (GA2) or experimental hybrid (GH1) were not a good fit to expected ratios. Possibly in this case, there was some environmental influence that affected the expression for MS. Proprietary

information (Pan American Seed Inc.) also supported the possibility that some environmental factor could influence the expression for MS in the F1 hybrid cultivar SERV. The information indicated that the male sterile line would be male sterile in one geographic location, and to a degree, male fertile in another location. Therefore, this research was conducted to identify if any environmental factors could be identified that could affect MS in SERV and subsequent populations.

Materials and Methods

This experiment was carried out in two phases using four walk-in growth chambers (GC), and a temperature- regulated greenhouse. The F1 hybrid impatiens ‘Super Elfin Red Velvet’ (SERV) was used for this research. Previous research showed SERV to be a male sterile cultivar that was not controlled by a genetic inheritance pattern in populations segregating for MS.

Seeds used in this research were sown in two white styrofoam containers (7.5cm x 15 cm) containing a peat-lite mix, and covered with fine vermiculite. The germination containers were placed in a greenhouse which was maintained at $25(\pm 1) ^\circ\text{C}$. Three weeks after sowing, seedlings were transplanted to three flats containing 48 cells. Two weeks after transplanting to flats, 100 healthy plants were transplanted into 4 inch diameter pots. Sixty plants with ten fully expanded leaves were selected for exposure to the interaction of temperature and photoperiod for phase I, and twenty plants were selected to receive the RH conditions set up for phase II.

The phase I day/night temperature treatments were 24/20 and $18/16 \pm 0.5 ^\circ\text{C}$. With each temperature treatment (GC), there were two different photoperiod treatments (day/night) of 14/10 and 10/14. Fifteen pots were used in each temperature x photoperiod combination. Relative humidity (RH) in each GC was controlled at 50 to 55%.

The phase II RH treatments were $80 \pm 1\%$ in the GC, and $50 \pm 5\%$ in the greenhouse. The temperature and photoperiod in the GC were maintained at $18/16 \pm 1 ^\circ\text{C}$

day/night, and 10/14 day/night respectively. The temperature and photoperiod in the greenhouse were $18/16 \pm 2$ °C day/night, and $10/14 \pm 1$ hours day/night, respectively. Each treatment consisted of nine pots.

Flower number, and flower diameter were determined in both phase I and II experiments (Tables 1 and 2). The presence of pollen was investigated by visual and microscopic observation. Microscopic observation was conducted by the same procedure used in the assessment of MS. The initiation of flowering also was investigated in phase I treatment (Fig. 1). These experiments were conducted on a completely randomized design (CRD). Analysis of variance (ANOVA) was used to compare flower number and flower diameter in each treatment.

Results and Discussion

Phase I experiment

All plants grown under high temperature (24/20 °C) and long photoperiod (14/10 hours) had smaller flowers compared to those grown under a lower temperature (18/16 °C) and short photoperiod (10/14 hours) (Table 1). These results concurred with previous observations in several reports related to the effect of temperature on flower size. Lee et al. (1991) also mentioned that high temperature (30/24 °C day/night) produced a smaller flower size than a low temperature (24/18 °C) in nineteen cultivars in IW. Sawheny (1983) reported that high temperature produced smaller flower, in tomato species. Dinar and Rudich (1985) explained that the smaller flower could be due to the inhibition of assimilate by high temperature.

The observation for flower initiation showed that the flowering at high temperatures (24/20 °C) was faster than at lower temperatures (18/16 °C) (Fig. 1). The number of flowers was less at low temperature of 18/16 °C compared to the temperature treatment of 24/20 °C. This was due to late initiation of flowers at the lower temperature treatment. Figure 1 shows that the earlier flower initiation was responsible for the increase of flower number at the higher temperatures. According to Lee et al. (1991), the higher temperature of 30/24 °C increased the rate of flower bud development, hence the number of flowers

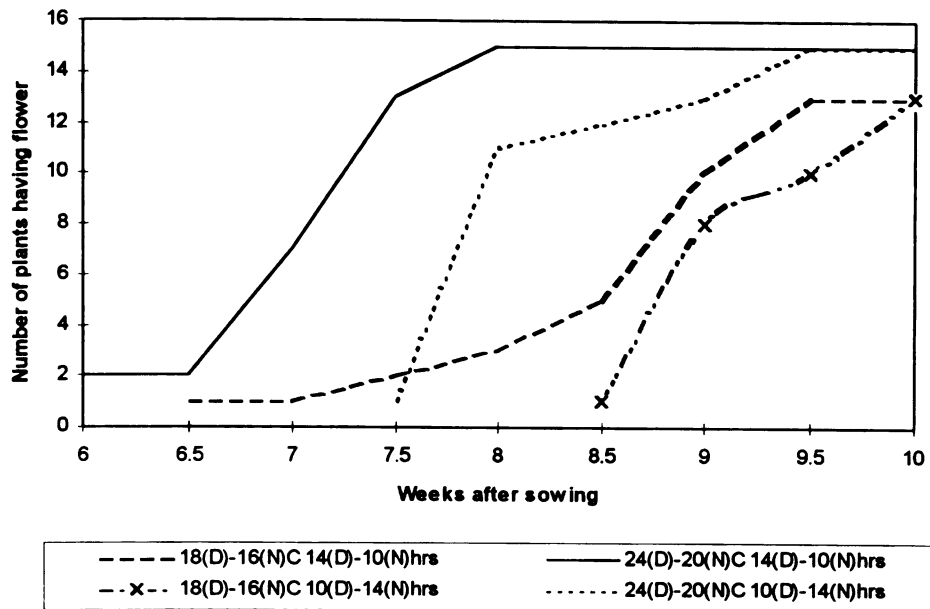


Fig. 1 The change of flower initiation in *Impatiens wallerana* Hook f. cv. Super Elfin Red Velvet as affected by temperature and photoperiod

2

Fig. 1

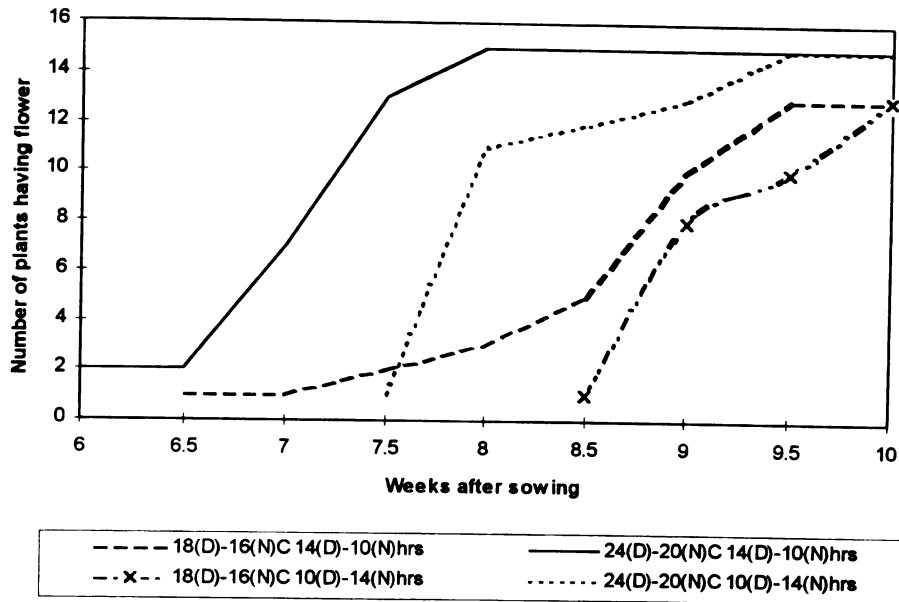


Fig. 1 The change of flower initiation in *Impatiens wallerana* Hook f. cv. Super Elfin Red Velvet as affected by temperature and photoperiod

Table 1. The effect of temperature and photoperiod on flower development in***Impatiens wallerana* Hook f. cv. Super Elfin Red Velvet**

Temperature (°C)	Photoperiod (h)	Number of flower	Diameter of flower (cm)
^z 18(D)/16(N)	14 (D)/10 (N)	4.5	4.9
	10 (D)/14 (N)	7.9	4.9
^y 24 (D)/20 (N)	14 (D)/10 (N)	27.7	3.8
	10 (D)/14 (N)	39.0	4.4
ANOVA			
Temperature		**	**
Photoperiod		**	**
Temperature x Photoperiod		**	**

^z

Day time

^y

Night time

** significant at $P=0.01$, respectively

was greater. Armitage et al. (1981) reported that the number of days needed from bud formation to flower initiation decreased as air temperature increased from 15 to 32 °C in geranium.

Even though the effect of high temperature and long photoperiod on growth of the flowers was highly significant (Table 1), male fertile flowers were not observed in any of temperature and photoperiod treatments.

At a lower temperature (18/16 °C) and short photoperiod (10/14 hours), however, the appearance of the anther cones was similar to that of a normal anther cone, but no male fertile flowers were found. Figure 2 shows the difference between anther cones at a higher temperature (24/20 °C) with long photoperiod (14/10 hours), and at a lower temperature (18/16 °C) with short photoperiod (10/14 hours). These results showed that unlike MS in barley (Ahokas and Hockett, 1981), pepper (Martin and Craford, 1951), and cabbage (Dickson, 1971), the environmental changes of temperature and photoperiod did not alter the MS condition in this particular germplasm.

Proprietary information (Pan American Seed Inc., 1997) that developed SERV indicated that the environmental response of MS in SERV was different at two locations. This difference of response might be due to difference of RH at the two locations. Relative humidity (RH) was, therefore, examined to see what effect it would have on this material under study.

Phase II experiment

In experiment phase I, even though temperature and photoperiod effect were significant for flower development, temperature and photoperiod did not alter the male



sterile status in SERV. The anther cones subjected to the lower temperature (18/16 °C) and short photoperiod (10/14 hours), however, did have a normal morphological appearance, similar to that of male fertile plants even though they did not produce normal fertile pollen. Under the conditions of a lower temperature (18/16 °C) and short photoperiod (10/14 hours), the effect of two different RH ($50 \pm 5\%$ and $80 \pm 5\%$) on MS was investigated.

Under these two different RH conditions, the flowers were bigger under the higher RH ($80 \pm 5\%$) (Table 2). The visual and microscopic investigation of the anther cone under higher RH ($80 \pm 5\%$) gave a different result in phase II (Fig. 2, 3, and 4). Under the higher RH ($80 \pm 5\%$), the anther cone was very similar to that of male fertile plants. Even though the anther cone did not produce a large quantity of pollen, the pollen grains found were plump and spherical under microscopic observation (Fig. 4). The appearance of pollen under the lower RH ($50 \pm 5\%$) through microscopic investigation was sparse, deformed, and similar to that of male sterile plants.

This result may help to explain why the male sterile inbred parent of SERV has shown different responses at different locations, and also influenced the expression of MS in the hybrid SERV. Lisci et al. (1994) demonstrated with *M. annua* that anther dehiscence and the volume of pollen were dependent on RH differences. Yates (1993) also reported the effect of RH on anther dehiscence in pecan.

Based on the results from the studies related to RH, it indicates that RH could influence anther dehiscence and pollen formation, and could be a factor influencing MS the SERV germplasm.

The phase I results showed the influence of temperature and photoperiodic factors on flower development and number over time, but having with no influence on MS in SERV. The phase II results indicated that under environmental conditions of a lower growing temperature sequence (18/16 °C), short photoperiod (10/14), and higher humidity of $80 \pm 5\%$ could change the expression of MS in SERV where the anther cones had normal appearing pollen grains.

This indicates that the variable expression of MS in this SERV germplasm could be problematic to manage for hybrid seed production.

**Table 2. The effect of relative humidity (RH) on flower development in
Impatiens wallerana Hook f. cv. Super Elfin Red Velvet**

Relative humidity (RH) (%)		Number of flower	Diameter of flower (cm)
Greenhouse	(50 $\pm$ 5)	35.0	4.0
Growth chamber (80 $\pm$ 5)		41.6	4.4



Fig. 2 A flower under a higher relative humidity ($80 \pm 5\%$) in *Impatiens wallerana* Hook.f. cv. 'Super Elfin Red Velvet'



Fig. 3 Anther cones under a higher relative humidity ($80 \pm 5\%$) (left) and under a lower relative humidity ($50 \pm 5\%$) (right) in *Impatiens wallerana* Hook.f. cv. 'Super Elfin Red Velvet'

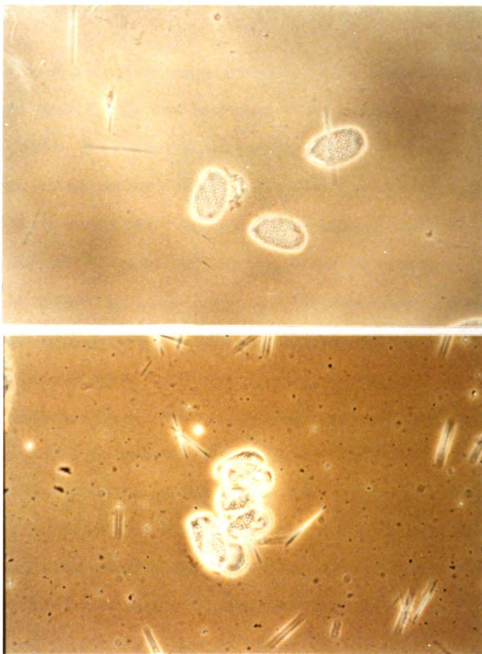


Fig. 4 Pollen grains under a higher relative humidity ($80 \pm 5\%$)(top) and under a lower relative humidity ($50 \pm 5\%$)(bottom) in *Impatiens wallerana* Hook f. cv. 'Super Elfin Red Velvet'

Summary

The effect of environmental factors on flower development and MS in *Impatiens wallerana* Hook f. (IW) were investigated in the cultivar 'Super Elfin Red Velvet'(SERV). The treatment of high temperatures (24/20 °C day/night) and long photoperiods (14/10 day/night) was effective for earlier flower initiation and produced greater flower numbers when compared to lower temperatures (18/16 °C) and short photoperiods (10/14) treatment. Flower size was smaller at a higher temperature sequence (24/20 °C day/night) and a long photoperiod (14/10 day/night) compared to a lower temperature sequence (18/16 °C) and a short photoperiod (10/14). The influence of temperature and photoperiod on MS was not identified in any treatment.

Relative humidity (RH) of $80 \pm 5\%$ was found to produce a larger flower than that of a RH of $50 \pm 5\%$. However, a higher RH($80 \pm 5\%$) treatment versus a lower RH ($50 \pm 5\%$) treatment did have an effect on MS in SERV. Anther cones of plants grown under a higher RH ($80 \pm 5\%$) were very similar to those of male fertile plants. Pollen grains within these anther cones were plump and spherical, similar to normal pollen grains but less in number. This indicates that RH may influence male fertility in SERV.

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